

PhD Thesis in Biotechnology

## Regulation of the nitric oxide synthesis and signaling by posttranslational modifications and N-end rule pathway-mediated proteolysis in **A**rabidopsis thaliana

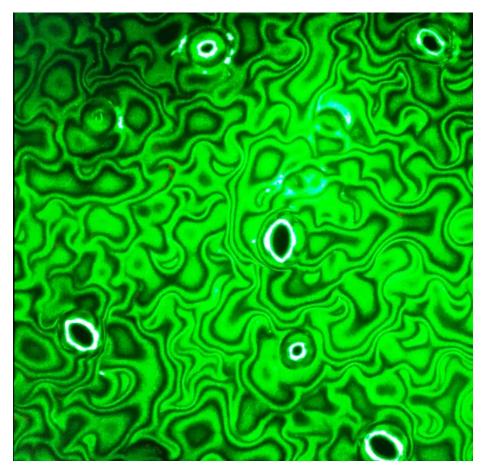
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Cover art by Álvaro Costa Broseta. Z stack image of a DAF-FM DA-stained cotyledon obtained by confocal microscopy.





El Dr. José León Ramos, doctor en Ciencias Químicas e Investigador Científico del Consejo Superior de Investigaciones Científicas (CSIC).

CERTIFICA:

Que la presente memoria titulada "Regulation of the nitric oxide synthesis and signaling by post-translational modifications and N-end rule pathway-mediated proteolysis in Arabidopsis thaliana", ha sido realizada por Álvaro Costa Broseta bajo mi dirección y constituye su Memoria de Tesis para optar al grado de Doctor en Biotecnología.

Para que conste a todos los efectos oportunos, firma el presente certificado en Valencia, noviembre del 2018.

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#### **SUMMARY**

Nitric oxide (NO) is a highly reactive gaseous molecule that regulates plant growth and development as well as defense responses. NO is mainly produced from nitrite by nitrate reductases (NRs) in balance with nitrite reductases (NiRs), and is sensed through a mechanism involving the N-end rule pathway-mediated proteolysis of the group VII of ERF transcription factors (ERFVIIs). NO especially exerts its signaling function by triggering post-translational modifications in proteins and altering their function, structure and/or stability. By these means and in collaboration with different phytohormone signaling pathways, NO is capable of regulating a wide array of cell processes in plants, including those related to the acquirement of freezing tolerance.

By using Arabidopsis thaliana as model plant, during the development of this work it was found that NO can regulate its own biosynthesis, as NRs and NiR enzymes were regulated by three main factors: nitrate-induced signaling controlled by the function of the NIN-like protein 7 (NLP7) transcription factor, N-end rule proteolytic pathway, and proteasome-mediated degradation, likely triggered by NO-related post-translational modifications. In addition, the ERFVII transcription factor RAP2.3 was found to negatively regulate both the NO biosynthesis and their triggered responses through a rheostat-like mechanism that involves specific NO-related branches of jasmonate and abscisic acid signaling pathways. On the other hand, a combined metabolomic and transcriptomic characterization of NO-deficient nia1,2noa1-2 mutant plants and NO-fumigated plants allowed to unravel a number of mechanisms that are controlled by NO. First, NO perception in hypocotyls would require various hormones to be fulfilled as it was confirmed by NO-triggered hypocotyl shortening screenings with hormone-related mutants and the TRANSPLANTA collection of transgenic lines conditionally expressing Arabidopsis transcription factors. Second, high NO doses caused a massive but transient reprogramming of primary and secondary metabolism, including alteration of the cellular redox status, alteration of the permeability of lipidic structures or turnover of proteins and nucleic acids. Lastly, NO was found to prevent the development of freezing tolerance under non-stress temperature conditions, while being essential for the low temperature stress-triggered cold acclimation that leads to enhanced freezing tolerance. NO would achieve this fine-tuned modulation of the activation of the cold-related responses by coordinating the accumulation of different metabolites and hormones. Altogether, this work sheds light on the mechanisms by which, by interacting with various signaling and metabolic pathways, NO can regulate several key processes of plant physiology.

#### **RESUMEN**

El óxido nítrico (NO) es una molécula gaseosa altamente reactiva que regula el crecimiento y el desarrollo de las plantas así como sus respuestas de defensa. El NO se produce principalmente a partir de nitrito por las nitrato reductasas (NRs) en balance con las nitrito reductasas (NiRs), y es percibido a través de un mecanismo en el que está involucrada la proteólisis dirigida por la secuencia aminoterminal del grupo VII de los factores de transcripción ERF (ERFVIIs). El NO ejerce especialmente su función señalizadora al causar modificaciones postraduccionales en las proteínas y alterar su función, estructura y/o estabilidad. Por estos medios y en colaboración con distintas rutas de señalización fitohormonales, el NO es capaz de regular un amplio abanico de procesos celulares en plantas, incluyendo aquellos relacionados con la adquisición de tolerancia a la congelación.

Utilizando Arabidopsis thaliana como planta modelo, en este trabajo se descubrió que el NO puede regular su propia biosíntesis, puesto que las enzimas NRs y NiRs fueron reguladas por tres factores principales: señalización inducida por nitrato y controlada por la función del factor de transcripción NIN-like protein 7 (NLP7), la proteólisis dirigida por la secuencia aminoterminal, y la degradación mediada por el proteasoma, probablemente ocasionada por modificaciones postraduccionales relacionadas con el NO. Adicionalmente, se descubrió que el factor de transcripción ERFVII RAP2.3 regula negativamente tanto la biosíntesis de NO como las respuestas que desencadena a través de un mecanismo similar a un reóstato en el que están involucradas ramas específicas relacionadas con el NO de las rutas de señalización de jasmonato y ácido abscísico. Por otro lado, una caracterización metabolómica y transcriptómica combinada de plantas mutantes nia1,2noa1-2 deficientes en NO y plantas fumigadas con NO permitió desentrañar una serie de mecanismos que están controlados por NO. En primer lugar, la percepción de NO en los hipocotilos requeriría varias hormonas para ser completada, como fue confirmado por los rastreos de acortamiento de hipocotilo por NO con mutantes relacionados con hormonas y la colección TRANSPLANTA de líneas transgénicas que expresan condicionalmente factores de transcripción de Arabidopsis. En segundo lugar, dosis elevadas de NO causan una reprogramación masiva aunque transitoria de los metabolismos primario y secundario, incluyendo la alteración del estado redox celular, la alteración de la permeabilidad de estructuras lipídicas y el recambio de proteínas y ácidos nucleicos. Por último, se descubrió que el NO previene el desarrollo de la tolerancia a congelación bajo condiciones no estresantes de temperatura, mientras que resulta esencial para la aclimatación a frío desencadenada por bajas temperaturas que conduce a una tolerancia mejorada a congelación. El NO conseguiría esta modulación afinada de la activación de respuestas relacionadas con frío al coordinar la acumulación de diferentes metabolitos y hormonas. En conjunto, este trabajo arroja luz sobre los mecanismos mediante los cuales, al interactuar con varias rutas señalizadoras y metabólicas, el NO puede regular distintos procesos clave de la fisiología vegetal.

#### <u>RESUM</u>

L'òxid nítric (NO) és una molècula gasosa altament reactiva que regula el creixement i desenvolupament de les plantes així com les seves respostes de defensa. El NO es produeix principalment a partir de nitrit per les nitrat reductases (NRs) en balanç amb les nitrit reductases (NiRs), i és percebut a traves d'un mecanisme que inclou la proteòlisi dirigida per la seqüència aminoterminal del grup VII dels factors de transcripció ERF (ERFVII). El NO exerceix la seva funció senyalitzadora majoritàriament al provocar modificacions postraduccionals en les proteïnes i alterar la seva funció, estructura i/o estabilitat. Mitjançant aquestes modificacions i en col·laboració amb distintes rutes de senyalització fitohormonals, el NO es capaç de regular un ampli espectre de processos cel·lulars en plantes, inclosos aquells relacionats amb l'adquisició de tolerància a la congelació.

Emprant Arabidopsis thaliana com a planta model, en aquest treball es va descobrir que el NO regula la seva pròpia biosíntesi, donat que els enzims NRs i NiRs foren regulades per tres factors principals: senvalització induïda per nitrat i controlada per la funció del factor de transcripció NIN-like protein 7 (NLP7), la proteòlisi dirigida per la següència aminoterminal, i la degradació mitjançant el proteasoma, probablement а causa de modificacions postraduccionals relacionades amb el NO. A més, es va descobrir que el factor de transcripció ERFVII RAP2.3 regula negativament tant la biosíntesi de NO com les respostes que desencadena aquest a través d'un mecanisme similar a un reòstat en el que estan involucrades branques específiques de les rutes de senyalització de jasmonat i àcid abscísic relacionades amb el NO. Per altre costat, una caracterització metabolòmica i transcriptòmica combinada de plantes mutants nia1,2noa1-2 deficients en NO i plantes fumigades amb NO va permetre desentranyar una sèrie de mecanismes que estan controlats per NO. En primer lloc, la percepció de NO en els hipocòtils requeriria de varies hormones, com fou confirmat pels rastrejos d'acurtament d'hipocòtil per NO amb mutants relacionats amb hormones i la col·lecció TRANSPLANTA de línies transgèniques d'expressió condicional de factors de transcripció d'Arabidopsis. En segon lloc, dosis elevades de NO causen una reprogramació massiva, encara que transitòria, dels metabolismes primari i secundari, incloent l'alteració de l'estat redox cel·lular, canvis en la permeabilitat de estructures lipídiques i el recanvi de proteïnes i àcids nucleics. Per últim, es va descobrir que el NO prevé el desenvolupament de la tolerància a congelació en condicions no estressants de temperatura, mentre que resulta essencial per a l'aclimatació a fred induïda per baixes temperatures que condueix a una tolerància millorada a congelació. El NO aconseguiria aguesta modulació minuciosa de l'activació de les respostes relacionades amb fred al coordinar l'acumulació de diferents metabòlits i hormones. En conjunt, aquest treball clarifica els mecanismes pels quals el NO pot regular distints processos clau de la fisiologia vegetal al interactuar amb varies rutes senvalitzadores i metabòliques.

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#### 1. INTRODUCTION

#### 1.1. Arabidopsis thaliana as model organism

The plant *Arabidopsis thaliana* has been used as model organism in the present work. This experimental system has numerous advantages such as: its short life cycle and its manageable size, which allows the analysis of a big number of individuals; its completely sequenced and well annotated genome; the availability of a huge amount of mutants and transgenic lines, organized in stocks of public access, and finally, the availability of a large number of -omic tools. Much of the obtained information in Arabidopsis may be extrapolated to other angiosperms, however those data must be carefully used as a start point for the reconfirmation in other plant species.

#### 1.2. Biosynthesis and metabolism of nitric oxide

Plants are sessile organisms so their development can be compromised by biotic or abiotic stresses coming from an environment they cannot run away from. Plants have acquired plasticity in their development as an adaptation mechanism, therefore the processes related to growth or defense may be enhanced depending on the situation. This distinctive dilemma is controlled on multiple levels through the regulation of transcription, post-transcrition, translation and protein posttranslational modifications, in such a way that the development of plants is the outcome of a highly complex network of finely regulated metabolic processes. The control over the metabolism is done via the modulation of the different cell processes in plants, which are determined by the numerous sensed stimuli and signaling pathways. It has been known for a long time that most of these processes are under direct control of regulatory molecules, particularly phytohormones, each of them affecting a wide spectrum of cell events. Nevertheless, even nowadays the details of the mechanisms underlying this complex regulation are not fully understood. Therefore, the characterization of secondary regulators represents the key in deciphering the way the cell machinery is modulated to coordinate plant development and responses to environmental stimuli.

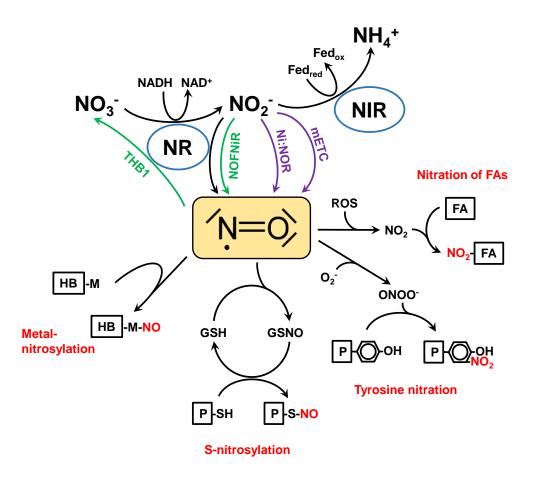
Nitric oxide (NO) is a small gaseous molecule and a free radical that plays numerous key roles in the physiology of living organisms, being particularly important in animal processes like neurotransmission or inflammation response, among others (Schmidt & Walter, 1994). However, it has been in the last twenty years when the function of NO has started to be properly analyzed in plants. Currently, NO is considered a signaling molecule regulating multiple plant physiological processes in collaboration with phytohormones and other growth regulators. However, neither its biosynthesis nor its regulatory involvement in cell processes are fully understood yet (Astier *et al.*, 2017). NO synthesis in plants is enhanced by stress and two main routes have been described for it: one reductive and one oxidative.

#### Introduction

The most studied and confirmed biosynthesis pathway of NO in plants is the reduction from nitrite (NO<sub>2</sub>) (Fig. 1.1). Rarely, this reaction can take place through non-enzymatic means in specific conditions such as low pH or highly reducing environments, or spontaneously in a light-mediated manner (Cooney et al., 1994; Durner et al., 1998; Caro & Puntarulo, 1999; Bethke, 2004). However, various proteins have been described to catalyze this reaction. One of them is the Nitrate Reductase (NR), for which exist two isoforms in A. thaliana: NIA1 and NIA2. This protein is a multifunctional cytosolic enzyme that is key for the nitrogen (N) assimilation and metabolism in plants, since it catalyzes the nitrate (NO<sub>3</sub>) reduction to nitrite by using NADH as an electron donor (Fig. 1.1). In this reaction, the homodimeric complex can only work with molybdopterin, heme and FAD as cofactors (Campbell, 2001). As nitrite is toxic for plants (Wang et al., 2007), usually the next step in the metabolic pathway of nitrogen assimilation is the further reduction of nitrite to ammonium (NH<sub>4</sub><sup>+</sup>) by chloroplastic Ferredoxin-Nitrite Reductases (NiR) (Joy & Hageman, 1966; Mikami & Ida, 1984) of which there is only one isoform in A. thaliana : NIR1 (Fig. 1.1). Nevertheless, NR has also been described to catalyze the conversion of nitrite to NO (Ni-NR activity) (Fig. 1.1), a marginal reaction that occurs only in specific situations such as high concentration of nitrite and low concentration of nitrate or anoxic or acidic environments (Yamasaki et al., 1999; Yamasaki & Sakihama, 2000; Rockel et al., 2002). Even if NO production by NR is just a secondary role of the enzyme, several pharmacological and genetic approaches have pointed out the importance of this activity for various NO-regulated processes in plant physiology (Astier et al., 2017).

Recently, another NO-producing mechanism has been described. It has been discovered that in the alga *Chlamydomonas reinhardtii*, NO can be produced from nitrite through the interaction of NR with NOFNiR (Nitric Oxide-Forming Nitrite Reductase) (Fig. 1.1). NOFNiR belongs to the Amidoxine Reducing Component (ARC) protein family, whose physiological function is not fully understood yet (Havemeyer *et al.*, 2006). Two genes coding for ARC proteins can be found in the *A. thaliana* genome, but to date the NR:NOFNiR system has not been confirmed in higher plants (Chamizo-Ampudia *et al.*, 2016). Interestingly, it has been described in the same alga that NR can supply electrons from NADPH through its diaphorase/dehydrogenase domain to the Truncated Hemoglobin 1 (THB1), which oxidizes NO to nitrate by its dioxygenase activity in the presence of oxygen (Fig. 1.1) (Sanz-Luque *et al.*, 2015). These two contradictory roles of NR in NO signaling fit the complex modulation of the nitrate cycle of the algae (Calatrava *et al.*, 2017), and highlight also how NR may play a key function in the regulation of the NO homeostasis in plants (Chamizo-Ampudia *et al.*, 2017).

Both NRs and ARCs present a molybdenum cofactor (Moco) in their structure. Other Moco-containing enzymes has been described in plants, such as Xanthine Oxidases (XOs), Aldehyde Oxidases (AOs), and Sulfite Oxidases (SOs), and are suspected to be able to reduce nitrite to NO. XO is a highly conserved enzyme responsible for purine catabolism, and hydroxylating hypoxanthine to xanthine and xanthine to urea. Two isoforms in plants have been reported to support the reactive oxygen species (ROS) homeostasis during biotic stress (Yesbergenova *et al.*, 2005), but the implication of XOs in NO production is still unclear, with very little evidence (Planchet *et al.*, 2005). AOs are cytoplasmic enzymes with a very similar structure to that of the XOs, which mainly catalyze the oxidation of aldehydes to carboxylates, producing superoxide anions. In plants, AOs play important roles in several defense and development processes, but its implication in the production of NO has not been described yet (Astier *et al.*, 2017). Lastly, SO is a conserved peroxysomal enzyme that catalyzes the oxidation of sulfite to sulfate, but as it occurs with AOs, despite some research with mammalian isoforms, its implication in NO synthesis in plants hasn't been addressed yet (Astier *et al.*, 2017).



**Figure 1.1. Scheme of the main biosynthetic and metabolic pathways of NO.** Pathways only confirmed in algae are shown in green; hypoxia-dependent pathways are shown in purple, and post-translational modifications are shown in red. NAD, Nicotinamide adenine dinucleotide; Fed, ferredoxin; NR, Nitrate Reductase; NIR, Nitrite Reductase; THB1, Truncated Hemoglobin 1; NOFNiR, Nitric Oxide-Forming Nitrite Reductase; Ni:NOR, Nitrite-Nitric Oxide Reductase; mETC, mitochondrial Electron Transport Chain; ROS, Reactive Oxygen Species; HB, Hemoglobin; M, transition metal; P, protein; FA, unsaturated fatty acid.

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Besides the cytosolic NR, a putative plasma membrane-bound Nitrite-Nitric Oxide Reductase (Ni:NOR) has been described to produce NO in plants. This unidentified protein would produce NO from the nitrite provided by an apoplastic membrane-bound NR, using NADPH as an electron donor, but only in roots and under a very low oxygen pressure (Fig. 1.1) (Stöhr et al., 2001; Stöhr & Ullrich, 2002). In addition, nitrite can be reduced to NO in plants by the mitochondrial electron transport chain (mETC) (Fig. 1.1). This process involves mainly complex III and IV, and is dependent on the concentration of nitrite and oxygen, requiring anaerobic conditions (Gupta et al., 2005). Therefore, it is a process that takes place only in tissues exposed to hypoxia such as roots, and its existence could be a way to preserve respiration when oxygen is scarce, by using nitrite as an electron acceptor (Gupta & Igamberdiev, 2011). Hence, not only the balance between NR and NiR activities but also the levels of available O<sub>2</sub> determine the degree of reduction that nitrate undergoes and the amount of NO synthesized. It has been recently reported that NO production under hypoxia seems to be partially mediated by alternative oxidase (AOX) activity (Vishwakarma et al., 2018).

Similar to the main pathway of NO production in animals via the Nitric Oxide Synthases (NOSs), there is evidence that plants possess an oxidative pathway of NO synthesis in addition to the reductive one. In the search for a NOS homolog in plants, an enzyme was identified in Arabidopsis that seemed to be implicated in NO synthesis (Guo et al., 2003). Initially named AtNOS1, the protein was renamed as Nitric Oxide Associated 1 (AtNOA1) and turned out to be a small GTPase (Moreau et al., 2008). Although the way this protein contributes to the NO production is still unclear, the *noa1* mutant is widely used as a genetic tool due to its low endogenous NO content (Astier et al., 2017). To date, the only NOSs from the plant kingdom that have been described belong to algal species (Foresi et al., 2015; Jeandroz et al., 2016), suggesting that is not likely that canonical NOSs exist in land plants. Because of this, efforts have been lately focused on finding the responsible for the NOS-like activity that for a long time has been described in plants (Astier et al., 2017). Several groups have reported measurements of NOSlike activity strictly dependent on arginine, NADPH and various NOS co-factors in plant tissues, thus supporting the potential existence of a NOS homolog in plants (Durner et al., 1998; Barroso et al., 1999; Corpas & Barroso, 2014). Moreover, the use of NOS inhibitors in pharmacological approaches as well as the heterologous expression of NOS in plants, which have demonstrated that the cofactors and conditions needed for NOS activity indeed occur in plants have been used to support the existence of plant NOS-like enzymes (Frungillo et al., 2014; Astier et al., 2017). Taken together these results, the existence of an oxidative NO synthesis route in higher plants remains controversial.

Currently, the reductive pathway of nitrite to NO by NRs is considered the most relevant route of NO production in higher plants. This pathway is directly integrated in the inorganic nitrogen assimilatory metabolism, as NRs and NiRs compete for the reduction of nitrite, generated upon nitrate reduction (Fig. 1.1). Nitrate and

ammonium are the most available inorganic sources for nitrogen acquisition, without considering atmospheric dinitrogen (N<sub>2</sub>) which can only be used by some prokaryotic organisms (Bloom, 2015). However nitrate is both a nutrient and a signal for plants: its signaling is crucial to regulate nitrate assimilation, the expression and activity of assimilatory enzymes and the whole N status of the plant (Armijo & Gutiérrez, 2017; Gent & Forde, 2017; Xuan *et al.*, 2017). The transcription factor NIN-Like Protein7 (NLP7) is the master regulator of nitrate signaling and assimilation (Castaings *et al.*, 2009; Marchive *et al.*, 2013; Yu *et al.*, 2016; Cao *et al.*, 2017; Zhao *et al.*, 2018).

As usual in signaling, the NO pathway in plants is strongly controlled by the action of several specific enzymes. Levels of cytosolic NO are the result of the balance between its synthesis and its turnover. NO can react with glutathione (GSH), thus producing S-nitrosylated glutathione (GSNO), considered a reservoir of NO and a signal for protein nitrosylation (Fig. 1.1). GSNO is metabolized to glutathione disulfide (GSSG) and ammonia (NH<sub>3</sub>) by GSNO Reductase (GSNOR) (Wilson et al., 2008). In addition, NO can be scavenged by reacting with ROS. The reaction of NO and superoxide anion  $(O_2)$  generates peroxynitrite (ONOO<sup>-</sup>), which is a powerful nitrating agent able to cause tyrosine nitration of some proteins (Fig. 1.1) (Gaupels et al., 2011; Begara-Morales et al., 2014). NO can react chemically with oxygen and generate nitrite and nitrate (Hancock, 2012) and through a mechanism still unknown, can react with ROS and lipid peroxyl radical (LOO) to produce nitro-fatty acids (NO<sub>2</sub>-FAs) (Fig. 1.1) (Rubbo, 2013). Finally, the homeostasis of NO can also be regulated through its oxidization to nitrate by nonsymbiotic and truncated hemoglobins (HB) (Fig. 1.1). HBs must be reduced to Fe(II)HB to dioxygenate NO (Chamizo-Ampudia et al., 2017). Nitrate has been proposed to regulate coordinately HB expression and NO homeostasis (Trevisan et al., 2011).

#### 1.3. NO as a regulator

Plants may be affected by both exogenous (air and ground) and endogenous (biosynthesized) NO. The major sources of NO in the atmosphere are those derived from industrial activity and car engines (Skalska *et al.*, 2010) as well as the microbial-related release from soils (Pilegaard, 2013). Due to the spontaneous conversion of NO to NO<sub>2</sub> under aerobic conditions, it is frequent to use the term NOx when NO is supplied in an oxygenated environment (Kasten *et al.*, 2017). Levels of NO have been increasing continuously in the Earth atmosphere since industrial revolution started (Jaeglé *et al.*, 2005). Considering this tendency, plants may be exposed in the future to relatively high NO concentrations thus potentially altering their physiology.

NO is a gasotransmitter-diffusible multitasked messenger in a wide range of organisms that belongs to the group of reactive nitrogen species (RNS). Its nature as a free radical is in the basis of its regulatory activity, which is often exerted through interaction with the signaling pathway of most of the phytohormones,

including the five so-called classical plant hormones (gibberellins, auxins, abscisic acid, cytokinins and ethylene) and some of the most recently characterized (brassinosteroids, salicylic acid, jasmonates and strigolactones) (Durbak *et al.*, 2012; Freschi, 2013; Simontacchi *et al.*, 2013). The NO-hormone functional interaction has been reported to regulate numerous physiological processes in plants such as seed dormancy and germination, skotomorphogenic and photomorphogenic vegetative development, root growth, stomatal closure, pollination, flowering, fructification or leaf senescence (Beligni & Lamattina, 2000; He *et al.*, 2004; Bethke *et al.*, 2006; Tsai *et al.*, 2007; Qiao & Fan, 2008; Prado *et al.*, 2008; De Michele *et al.*, 2009; Manjunatha *et al.*, 2010; Lozano-Juste & Leon, 2011; Arc *et al.*, 2013; Liu & Guo, 2013; Du *et al.*, 2014).

NO is able to act as a regulator at two different levels: regulating gene expression and triggering post-translational modifications (PTMs) in proteins. This is because NO is able to react with proteins and alter their activity, subcellular localization, function, structure or stability, and when the affected protein is a transcription factor likely induces transcriptome changes (Bruckdorfer, 2005; Grün et al., 2006; Palmieri et al., 2008). Thus, NO can regulate the expression of numerous genes involved in hormonal signaling, primary metabolism or stress responses (Grün et al., 2006; Besson-Bard et al., 2009) and usually it impacts on the signaling of many phytohormones involved in developmental or defense processes against biotic and abiotic stresses (Freschi, 2013; Fancy et al., 2017). Some examples of transcription factor regulation by NO include the repression of **Phytochrome** Interacting Factors (PIF) aene familv controlling skotomorphogenenic growth (Lozano-Juste & Leon, 2011), the promotion of the TGA1 action in salicylic acid-dependent defense processes (Lindermayr et al., 2010; Gupta, 2011) or the inhibition of AtMYB2 function in ABA signaling pathway (Serpa et al., 2007).

NO can induce PTMs such as the nitrosylation of thiols and amines; the nitration of tyrosine, tryptophan and phenylalanine, the ubiquitylation of lysine, the phosphorylation of serine, threonine and tyrosine or the oxidation of tyrosine and thiols (Gow *et al.*, 2004; Hess & Stamler, 2012). However, due to its potential signaling effect in the plant physiology, the three main specific PTMs caused by NO are the S-nitrosylation of cysteines, the tyrosine nitration and the binding to metallic centers of proteins (Fig. 1.1) (Astier & Lindermayr, 2012). Alternatively, acting as a molecular messenger, NO can also regulate gene expression through the interaction and modulation of secondary messengers of signaling pathways dependent on cGMP, cADP-ribose, Ca<sub>2</sub><sup>+</sup>, and notably with reactive oxygen species (ROS) (Durner *et al.*, 1998; Lamotte *et al.*, 2006; Astier *et al.*, 2010; Mur *et al.*, 2013).

S-nitrosylation (or S-nitrosation) is a redox modification consisting in the reversible covalent binding of an NO moiety to the thiol group of a cysteinyl residue (Cys) of a target protein, leading to the formation of an S-nitrosothiol (SNO) (Fig.

1.1) (Astier et al., 2011). This modification is restricted to specific Cys residues and it is completely dependent on the nature of the surrounding amino acids (Seth & Stamler, 2011). GSNO seems to be one of the main donors for the transnitrosylase activity in plants, modulating the total SNO content (Wang et al., 2006; Yu et al., 2012). Tyrosine (Tyr) nitration is the mainly irreversible reaction of a nitrating agent with a tyrosine residue of a protein. It results in the addition of a nitro group (NO<sub>2</sub>) in the ortho position of the phenolic hydroxyl group, leading to the formation of 3nitrotyrosine (3-NY) (Fig. 1.1) (Schopfer et al., 2003). The NO<sub>2</sub> group originates mainly from peroxynitrite (ONOO<sup>-</sup>). Like in S-nitrosylation, Tyr nitration is also restricted to specific target tyrosine residues (Bayden et al., 2011) and can trigger conformational changes that lead to the activation or the inhibition of the target proteins. These conformational changes seem to promote the polyubiquitylation and subsequent proteasomal degradation of the affected proteins (Castillo et al., 2015). Because of its chemical properties, NO can also interact reversibly with the transition metals (iron, zinc or copper) at the heme center of metalloproteins to form metal-nitrosyl complexes through coordination chemistry (Fig. 1.1) (Ford, 2010; Astier & Lindermayr, 2012). The bound NO group is then susceptible to further nucleophilic or less frequently electrophilic attacks, depending on the protein bounded (Astier & Lindermayr, 2012; Toledo & Augusto, 2012). The formation of the metal-nitrosyl complex can induce conformational changes that compromise the proper functioning of the affected protein (Ford, 2010; Toledo & Augusto, 2012). The best characterized plant proteins undergoing metal nitrosylation are hemoglobins (HB), which are separated in three groups based on their structural properties: class 1, 2 and truncated hemoglobin class 3 (Gupta et al., 2011). The oxidation of HBs by NO produces nitrate, thus scavenging the NO in what is considered a general mechanism modulating NO bioavailability, participating in the regulation and detoxification of NO in plants (Gupta et al., 2011; Igamberdiev et al., 2011). Recently, the nitration of fatty acids by NO has also been demonstrated to be an important part of NO signaling in plants (Mata-Pérez et al., 2017). This reaction would consist in the nitration of unsaturated fatty acids through a redox reaction to form electrophilic nitro-fatty acids (NO<sub>2</sub>-FAs) (Fig. 1.1), although the exact molecule responsible for the modification is still unknown (Rubbo, 2013; Mata-Pérez et al., 2017).

In contrast to the increasing knowledge of the effect of NO on protein function, our current knowledge on the NO impact on global metabolome of plants is scarce, being limited to a small number of stress-related processes. Both ROS and NO are produced in stressed plants, where NO seems to be able to alleviate the oxidative status by enhancing the antioxidant capacity and thus contributing to the redox homeostasis (Correa-Aragunde *et al.*, 2015). However, extensive evidence suggest that NO is involved in somehow paradoxical processes exerting sometimes opposing regulatory functions. For instance, NO has been described to enhance or reduce the redox status of the plants depending on either acting in a chronic or acute mode (Groß *et al.*, 2013). This paradox could be due to multiple

factors such as the relative NO cellular concentration, the location where it is produced or the complex interacting microenvironment.

#### 1.4. NO sensing

If the biosynthesis of NO still remains controversial, the way plants sense NO is even less known. NO perception in animals is performed through NO-inducible Guanylate Cyclases (GC) that synthesize the second messenger 3',5'-cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) (Friebe & Koesling, 2003; Russwurm & Koesling, 2004). Although a flavin monoxygenase called NO-dependent Guanylate Cyclase 1 (NOGC1), with higher affinity for NO than for molecular oxygen, was identified in Arabidopsis (Mulaudzi et al., 2011), it is not clear yet if this enzyme produces enough cGMP to work as a truly NO receptor (Gross & Durner, 2016). Moreover, it is also still unknown whether enzymes involved in cGMP degradation and downstream signaling, such as phosphodiesterases, are functional in plants (Gross & Durner, 2016), which makes the functionality of a NO-cGMP signaling pathway in plants even more uncertain. In the absence of a GC receptor for NO in plants, plants seem to sense NO mostly through chemical interaction with cofactor metals or with specific amino acid residues of proteins that often undergo NO-triggered post-translational modifications (Astier & Lindermayr, 2012). Nevertheless, alternative NO sensing mechanisms have been searched for and it was recently reported one involving the so called Cys-Arg/N-end rule proteolytic pathway (Gibbs et al., 2014a). It consists in the specific oxidation of the C2 residue of transcription factors of the group VII of Ethylene Response Factors or ERF/AP2 family (ERFVIIs), which is strictly dependent on NO and molecular oxygen, and allows further arginylation, polyubiquitylation and proteasome-mediated degradation of ERFVIIs through the N-end rule proteolytic pathway (Fig. 1.2) (Gibbs et al., 2014a). This pathway acts as a sensor that integrates both NO and O<sub>2</sub> (Gibbs et al., 2014a) and it has been proposed to function as a general sensor of abiotic stress (Vicente et al., 2017) besides being essential for responses to anaerobiosis (Gibbs et al., 2014b; Pucciariello & Perata, 2017). The turnover of regulatory proteins through focused proteolysis by an ATP-dependent proteasome machinery is a conserved mechanism in eukaryotes (Gibbs et al., 2014b).

ERFVIIs can undergo degradation through the cysteine (Cys) subdivision of the Arg/N-end rule pathway (Cys-Arg/N-end rule pathway) (Gibbs *et al.*, 2014a). The beginning of this proteolytic pathway consists in the removal of the methionine by a Methionine Aminopeptidase (MAP), thus exposing the tertiary destabilizing cysteine residue (which constitutes an N-degron) to direct oxidation (Fig. 1.2). The specificity of this reaction depends on the Plant Cysteine Oxidase 1 (PCO1) and PCO2 enzymes, which catalyze the oxidation of the thiol group in the N-terminal Cys of the substrate to sulphinic acid using molecular O<sub>2</sub> and NO as co-substrates (Fig. 1.2) (Gibbs *et al.*, 2014a; Weits *et al.*, 2014). Once it is oxidized, the Cys acts as a secondary destabilizing residue that becomes the substrate for arginylation

by Arg-tRNA Transferase (ATE) ATE1 and ATE2 enzymes (Fig. 1.2). This transfer of an Arg to the N-terminal residue of the target protein constitutes a signal for the N-recognin E3 ubiquitin ligase Proteolysis6 (PRT6) for polyubiquitinylation and further degradation by the proteasome (Fig. 1.2) (Gibbs *et al.*, 2014b). The N-end rule proteolysis of ERFVIIs takes place only under O<sub>2</sub>-rich conditions, being deactivated under low O<sub>2</sub> (Pucciariello & Perata, 2017).

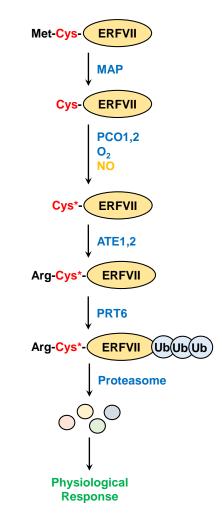


Figure 1.2. Scheme of the Cys-Arg/N-end rule pathway-mediated degradation of ERFVII transcription factors as sensor mechanism of NO in plants. MAP, Methionine Aminopeptidase; PCO1,2, Plant Cysteine Oxidase 1 and 2; ATE1,2, Arginyl tRNA Transferase 1 and 2; PRT6, Proteolysis6 E3 ligase; Cys\*, oxidized cysteine.

The ERFVIIs comprise five proteins. Three of them are constitutively expressed transcription factors: Related to AP 2.12 (RAP2.12), RAP2.2 and RAP2.3/EBP. The other two are hypoxia-inducible factors: Hypoxia Responsive ERF 1 (HRE1) and HRE2. All five contain a cysteine residue right after the initial methionine and have been demonstrated to be substrates of the N-end rule pathway (Gibbs *et al.*, 2015). Initially, RAP2.3 was isolated as a suppressor of Bax-induced cell death by functional screening in yeast, and further confirmed its cell death suppressing activity and its capacity to induce resistance to hydrogen peroxide and heat stress

(Ogawa *et al.*, 2005). Moreover, RAP2.3 seems to regulate also defense against pathogens likely through interaction with acyl-CoA binding proteins ACBP2 and 4 (Li *et al.*, 2008), and also with bZIP transcription factors (Buttner & Singh, 1997). Despite their stress-related functions, RAP2.3 has also development-related regulatory functions. It has been identified as a protein interacting with the Gibberellin Insensitive (GAI) DELLA protein in such a way that the interaction impaired the activity of RAP2.3 on target promoters, thus controlling the differential growth during apical hook development (Marín-de la Rosa *et al.*, 2014; Abbas *et al.*, 2015). On the other hand, RAP2.3 together with the other members of ERFVII group have been extensively characterized as key regulators in the expression of hypoxia-responsive anaerobic metabolism-related genes involved in different abiotic stresses (Bui *et al.*, 2015; Gasch *et al.*, 2015; Papdi *et al.*, 2015). However, despite the relevance of ERFVIIs in sensing NO and regulating all this aspects of plant physiology, other still not deciphered components should be likely involved in mediating NO sensitivity and responsiveness.

#### 1.5. <u>NO and stress-triggered responses. The role on plant tolerance to</u> <u>freezing</u>

Plants face two different environmental stresses: those caused by other living organisms or biotic stresses, and those caused by non-living factors or abiotic stresses. Nowadays, abiotic stresses constitute the bigger impact (up to 70%) on crop growth and yield (Mantri et al., 2012) and it is estimated that 90% of cultivable land is exposed to salinity, drought, low or high temperature, heavy metal exposure or high light, among other abiotic stresses (dos Reis et al., 2012). Plants display an array of adaptation responses to ensure their survival against abiotic stress (Nakashima et al., 2009), leading to two possible outcomes: programmed cell death or stress acclimation (Kreps et al., 2002). The stress-triggered production of redox active molecules like ROS or RNS (including NO) is a common trait of plant responses to abiotic stress as a mechanism to ameliorate the resultant oxidative stress (Mittler, 2006). On the other hand, besides its function as regulator of several developmental processes (including seed germination, seed dormancy, and closure of stomata), major phytohormone abscisic acid (ABA) plays an essential role in the plant response against a wide range of biotic and abiotic stresses (Vishwakarma et al., 2017). In several of these processes, NO is a downstream regulator of ABA signaling (Hancock et al., 2011) and an increment of the ABA content correlates with the induction of NO production (Neill et al., 2002; Lozano-Juste & León, 2010). However, NO can also exert a negative effect on ABA signaling by reducing the hormone perception (Castillo et al., 2015), as seen in the NO-deficient nia1,2noa1-2 triple mutant, which is hypersensitive to ABA (Lozano-Juste & León, 2010). This complex relation between NO and ABA would be another scenario in which NO regulatory effect would depend on factors such as the nature of the process and the site and timing of production of the both regulators (León et al., 2014).

One of the main abiotic stresses whose response is regulated by ABA is cold. This stress factor triggers the accumulation of endogenous ABA in many plants (Mantyla et al., 1995) while exogenous ABA treatment leads to enhanced cold resistance (Kumar et al., 2008; Kim et al., 2016). Among abiotic stresses, stress by low temperatures is one of the most important barriers for productivity in agriculture along with drought, salinity and heat (Calanca, 2017). Unlike the stress caused by cold (5-15 °C, chilling stress), very few crops can face freezing stress (below 0 ° C), which interestingly is a kind of stress more similar to drought stress due to the formation of intracellular ice crystals. When facing these temperatures, plants respond by reprogramming metabolism in order to attenuate the cell damage caused by freezing (Fowler & Thomashow, 2002; Cook et al., 2004; Hannah et al., 2005; Lee et al., 2005; Chinnusamy et al., 2007; Kaplan et al., 2007; Guy et al., 2008). The tolerance to freezing is achieved through endogenous components of the plant and inducible environmental factors. The main endogenous plant components that favor constitutive freezing tolerance seem to be the accumulation of metabolites with osmoprotective activities that limit freeze-induced dehydration and avoid ice nucleation inside the cells (Janská et al., 2010), hormones (Eremina et al., 2016a) and antioxidants (Winkel-Shirley, 2002). Other responses include changes in membrane structure and function, and growth arrest (Renaut et al., 2004; Eremina et al., 2016a). On the other hand, among the environmental factors that enhance freezing tolerance, likely the most efficient is the cold acclimation, consisting in the previous exposure of plants to low non-freezing temperatures (Thomashow, 1999; Knight & Knight, 2012). This is an adaptive process that allows plants to survive the freezing winters. Cold acclimation and freezing tolerance are mainly controlled by changes in genes expression, which, in turn, are regulated at the transcriptional and post-transcriptional levels (Barrero-Gil & Salinas, 2013; Miura & Furumoto, 2013).

Among the cold-induced signaling pathways, the one mediated by the Crepeat/dehydration-responsive element Binding Factors (CBFs/DREB1s) is probably the most important and the best characterized (Gilmour *et al.*, 2004; Medina *et al.*, 2011). This pathway is controlled by Inducer of CBF Expression1 (ICE1), a MYC-type transcriptional activator that enhances freezing tolerance upon binding to the promoters of *CBF* genes (Fig. 1.3) (Chinnusamy *et al.*, 2003). The so-called CBF regulon is not only regulated by the ICE1-CBF pathway but by other transcription factors that are rapidly induced by cold and work as a complex regulatory network only partially dependent on CBFs (Fowler & Thomashow, 2002; Park *et al.*, 2015). Both CBF-dependent and –independent pathways converge into the activation of *Cold-Responsive* (*COR*) genes (Fig. 1.3) (Gilmour *et al.*, 1998), which are key for cold acclimation-induced freezing tolerance. *COR* genes code for dehydrins, heat shock proteins, glucanases and chitinases that are important to prevent membrane alterations, protein aggregation and the formation of inter- and intra-cellular ice crystals (Griffith *et al.*, 2005).

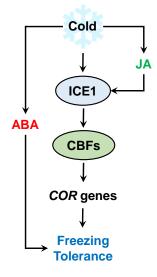


Figure 1.3. Scheme summarizing the CBF-dependent pathway of cold acclimationinduced freezing tolerance. JA, jasmonate; ABA, abscisic acid; ICE1, Inducer of CBF Expression1; CBFs, C-repeat/dehydration-responsive element Binding Factors; COR, Cold-Responsive.

Cold acclimation is also regulated by plant hormones. Besides ABA, whose quantitative and qualitative contribution is decisive (Nakashima *et al.*, 2014), other phytohormones, including jasmonates, salicylates, auxins, cytokinins, gibberellins, and brassinosteroids, have important functions in cold acclimation (Fig. 1.3) (Jeon *et al.*, 2010; Miura & Ohta, 2010; Rahman, 2013; Richter *et al.*, 2013; Eremina *et al.*, 2016b; Sharma & Laxmi, 2016). Moreover, other non-hormone regulatory molecules such as polyamines, lipids, reactive oxygen species and NO have been described to be involved in freezing tolerance and cold acclimation (Cuevas *et al.*, 2008; Zhao *et al.*, 2009; Puyaubert & Baudouin, 2014; Chen & Thelen, 2016; Takahashi *et al.*, 2016; van Buer *et al.*, 2016).

In the last years, several studies have reported an increased production of NO in cold-stressed plants using many different species and detection methods (Shimoda *et al.*, 2005; Corpas *et al.*, 2008; Zhao *et al.*, 2009; Liu *et al.*, 2010; Cantrel *et al.*, 2011; Bai *et al.*, 2012; Majláth *et al.*, 2012; Xu *et al.*, 2012; Puyaubert & Baudouin, 2014). Therefore, cold-induced NO production appears as a general response taking place in diverse plant species and organs such as leaves, fruits or seeds. However, most of the details behind this process are still unknown and further investigation would now require a more specific analysis of the respective functions of short- and long-term NO signaling during the cold response and acclimation (Puyaubert & Baudouin, 2014). Also, the mechanisms used by NO to control cold acclimation could be part of the discrimination between tolerant and sensitive species to freezing stress (Puyaubert & Baudouin, 2014).

Objectives

Objectives

# 2. OBJECTIVES

The present work aims to unravel the mechanisms underlying the regulation of the nitric oxide synthesis as well as its signaling by post-translational modifications and N-end rule pathway-mediated proteolysis in *Arabidopsis thaliana*. Therefore, the following objectives were proposed:

- 1) Characterization of the effect of NO and post-translational modifications in proteins on the NO homeostasis.
- 2) Characterization of the genome-wide NO-responsive transcriptome and metabolome.
- 3) Functional characterization of the N-end rule pathway-mediated control of NO responses by ERFVIIs.
- 4) Evaluation of the role of NO as a regulator of plant responses to abiotic stress. The case of responses to low temperatures.

Objectives

# **Materials and Methods**

Materials and methods

#### 3. MATERIALS AND METHODS

# 3.1. Biological material

#### 3.1.1. Plant material

#### 3.1.1.1. Plant species used

Arabidopsis thaliana Columbia-0 (Col-0) ecotype was the wild-type genetic background used in this work. The following knockout mutant lines were obtained from the Nottingham Arabidopsis Stock Centre (NASC) seed bank: nlp7-1 (SALK\_026134C), prt6-1 (SAIL\_1278\_H11), ein2-5 (N16707), etr1-3 (N3070), pyl6,7, sid2-1eds5-3nahG, det2-1 (N65989), bes1-d (N65988), nia1nia2 (N2356) and noa1-2 (SAIL\_507\_E11). Other mutants used include: jaz10, jaz1,3,5,9,10 or giaz, myc2, myc2,3,4 (donated by Roberto Solano, CNB, Madrid, Spain), hai1,2,3 (donated by Paul E. Verslues, Academia Sinica, Taipei, Taiwan), max1, max2, max4 (donated by Javier Agustí, IBMCP, Valencia, Spain), quintuple erfvii or gerfvii (rap2.12rap2.2rap2.3hre1hre2) and gerfviiprt6-1 (donated by Michael Holdsworth, University of Nottingham, UK). The double *snrk2.3,2.9* mutant was generated by crossing snrk2.3 and snrk2.9 (donated by Hiroaki Fujii, Center for Plant Cell Biology, Riverside, USA). The double *nlp7-1prt6-1*, 35S::NLP7nlp7-1prt6-1 and pNLP7::NLP7nlp7-1prt6-1 plants were generated by crossing nlp7-1, 35S::NLP7nlp7-1 and pNLP7::NLP7nlp7-1 plants (Marchive et al., 2013) with prt6-1 plants and screening for homozygous mutations and transgenes in the F3 generation by PCR with specific gene and T-DNA primers (Table A3). The triple nia1,2noa1-2 mutant seeds were obtained by crossing nia1nia2 and noa1-2 as previously reported (Lozano-Juste & León, 2010). Genotyping by PCR and Cleaved Amplified Polymorphic Sequences (CAPS) with specific primers (Table A3) were used to select triple homozygous mutant plants and to ensure that triple homozygous mutant plants were used from every seed stock obtained. A knockout mutant *nir1-1* was obtained by using the CRISPR/Cas9 technology.

Plants over-expressing HA-tagged versions of NR1, NR2 and NiR1 were generated by subcloning the full-length cDNAs in pAlligator 2 vector (Bensmihen *et al.*, 2004) (Fig. A2), and further transformation of *Agrobacterium tumefaciens* with the corresponding constructs. Plants were then genetically transformed by dipping floral organs in a suspension of transformed Agrobacterium (Clough & Bent, 1998) and selected (via a GFP reporter gene expressed in the seed) for homozygotic transgenes (Fig. 4.3). The transgenic plants *35S::MC-RAP2.3-HA* and *35S::MA-RAP2.3-HA* were obtained from Michael J. Holdsworth's lab (University of Nottingham, UK). Around 1000 different transgenic lines from the TRANSPLANTA (TPT) project expressing Arabidopsis transcription factors under the control of a  $\beta$ -estradiol-inducible promoter (Coego *et al.*, 2014) were also used (Supplementary Table S4 in Castillo *et al.*, 2018).

# 3.1.1.2. Plant growth

# 3.1.1.2.1. Growing media for plants

For propagation purposes and when indicated, plants were grown in soil mixture (50% blond Kekkilä peat, 25% perlite, 25% vermiculite) irrigated with a nutritive solution (34.95 mg/l gFe, 9.76 mg/l ZnSO<sub>4</sub>·7H<sub>2</sub>O, 28.95 mg/l MnSO<sub>4</sub>· H<sub>2</sub>O, 2.23 mg/l Cu<sub>2</sub>SO<sub>4</sub>, 32.55 mg/l H<sub>3</sub>BO<sub>3</sub>, 1.51 mg/l (NH<sub>4</sub>)6Mo<sub>7</sub>O<sub>24</sub>\*4H<sub>2</sub>O,  $Ca(NO_3)_2 \cdot 4H_2O_1$ 381 mg/l KNO<sub>3</sub>, 102 mg/l KH<sub>2</sub>PO<sub>4</sub>, 367.5 mg/l 885 mg/l MgSO<sub>4</sub>·7H<sub>2</sub>O, pH 6.5, CE 1.6 mS). For the propagation of *nir1-1* plants (unable to grow with nitrate), only vermiculite was used, irrigated with a modified version of the MS (Murashige & Skoog, 1962) medium containing no nitrogen (Bioworld) and supplemented with 0.5% (w/v) sucrose, 10 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> (pH 6.5), 2.5 mM (NH<sub>4</sub>)<sub>2</sub>-succinate and 2.35 mM MES buffer. For most of the experiments, plants were grown in agar-supplemented MS medium (Duchefa) supplemented with 1% (w/v) sucrose, 2.35 mM MES buffer and vitamins, adjusted to a pH 5,7. For the selection of homozygous plants of nlp7-1prt6-1, 35S::NLP7nlp7-1prt6-1 and pNLP7::NLP7nlp7-1prt6-1 the MS medium was also supplemented with 10 µg/ml glufosinate ammonium (BASTA) and/or 20 µg/ml Hygromycin. The selection of pU6::gNIR1 (CRISPR-Cas9 transgenic lines for the generation of nir1 mutants) T1 transformants was carried out in a medium composed by 4.3 g/l of MS medium salts, 8 g/l phytoagar, 25 µg/mL Hygromycin and 100 ug/ml cefotaxime. The induction of the expression of the transcription factors in the TPT lines was performed by supplementing the MS-MES medium with 10  $\mu$ M  $\beta$ -estradiol. For the experiments with different nitrogen sources, seedlings were grown in MS media depleted of N (-N) (Bioworld), or supplemented with 5 mM KNO<sub>3</sub>, 5 mM NaNO<sub>2</sub> or 2.5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> as the only nitrogen source. These media were supplemented also with agar (or none, as indicated in each experiment), 0.5% (w/v) sucrose, 10 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> pH 6.5 (5 mM in nitrate medium) and 2.35 mM MES buffer. Except when indicated, DAF-FM DA staining was performed on seedlings grown in MS-MES medium with or without agar (depending on the experiment) and without sucrose.

#### 3.1.1.2.2. Growing conditions for plants

Seeds were surface sterilized with chlorine gas (4h incubation) and aerated in laminar flow cabinet for 30 min before sowing in MS media plates. Alternatively, seeds were sterilized by incubating with 70% ethanol and 0.1% Triton X-100 for 2 min, 30% bleach solution with 0.02% Tween-20 for 10 min and 5 washes of sterile water, before sowing in liquid media. After sowing, seeds were stratified at 4°C under darkness for 4 days. Plants were grown under photoperiodic cycles of 16 h day and 8 h night (long days, at 22 °C and 20 °C, respectively), under 150  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> cool-white fluorescent lamps and 60% relative humidity.

# 3.1.2. Microbiological material

# 3.1.2.1. Bacterial strains used

For cloning purposes, different strains of *Escherichia coli* were used: One Shot TOP10, DH5 $\alpha$ , 5 $\alpha$  C2987 and DB3.1. Plant transformation was performed using *Agrobacterium tumefaciens* C58pMP90.

# 3.1.2.2. Bacterial growth

# 3.1.2.2.1. Growing media for bacteria

All bacteria were grown in LB medium (Bertani, 1951) with or without agar, supplemented with 100  $\mu$ g/ml spectinomycin, 100  $\mu$ g/ml kanamycin or 10  $\mu$ g/ml gentamicin for the selection of transformants depending on the plasmid used. Agrobacterium growth media were always supplemented with 100  $\mu$ g/ml rifampicin.

# 3.1.2.2.2. Growing conditions for bacteria

*E.coli* was grown at 37 °C while *A. tumefaciens* was grown at 28 °C, both kept at 200 rpm shaking if grown on liquid medium.

# 3.2. Manipulation of the biological material

# 3.2.1. Plant treatments

# 3.2.1.1. Plant transformation by floral dipping

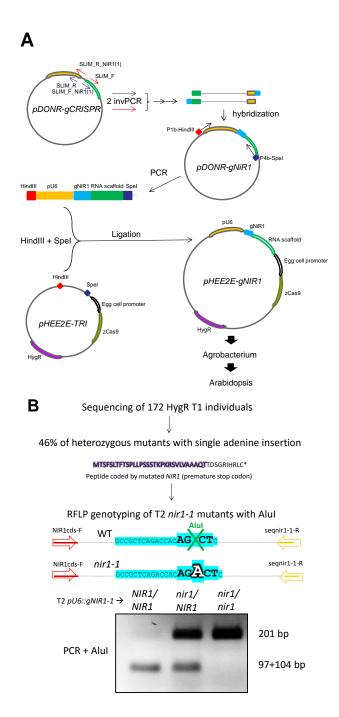
For the generation of transgenic plants, 1 month soil-grown plants (T0 generation) were transformed by floral dipping (Clough & Bent, 1998). Shoot apexes were cut after bolting and plants were transformed three days after. Two days before the plant transformation, the Agrobacterium transformed with the desired transgene was grown for 24 h at 28 °C in 10 ml LB medium with the corresponding antibiotics for selection. This culture was used to inoculate 200 ml of medium, which was again incubated for 24 h. Before plant transformation, bacteria were precipitated by centrifuging at 7000 rpm for 10 min and resuspended in 250 ml of transformation solution (5% (w/v) sucrose, 0.02% (v/v) Silwet L-77). Shoot apexes were submerged in this solution for 30-60 s and kept covered with plastic for two days to maintain humidity. After one month, T1 transgenic seeds were harvested.

3.2.1.2. Generation of a *nir1* mutant by CRISPR-Cas9 technology

A 19 bp target sequence (GCCGCTCAGACCACAGCTC) close to the Nterminus of the *NIR1* locus was chosen followed by the PAM sequence NGG. This sequence was used as guide and subcloned into the entry vector pMTN2982 (a modified pDONR207) (Fig. A3) by Site-directed, Ligase-Independent Mutagenesis (Chiu et al., 2004), which consists of two separate inverse PCR (SLIM) amplifications with tailed primers: (F+R\_tailed) and (F\_tailed+R) (Table A3, Fig. 3.1A). Both PCRs were performed with Phusion polymerase on an Applied Byosystems 96-well thermocycler, using 10 ng of template (pMTN2982). The program was 3 min at 98 °C initialization, 35 cycles of denaturation (15 s at 98 °C), annealing (30 s at 65°C) and extension (2.5 min at 72 °C), and 10 min at 72 °C of final elongation. After verifying the amplification, both PCR products were mixed and digested with DpnI (to remove template) in a mix consisting of 12.5 µl of each PCR product, 3 µl of commercial 10x buffer and 2 µl of Dpnl. Restriction mix was incubated at 37 °C for 12 h and inactivated by incubating at 80° C for 20 min. The hybridization was performed by mixing 30 µl of the digestion and 30 µl of H-buffer (300 mM NaCl, 50 mM Tris-HCl pH 9, 20 mM EDTA) and incubating at 99 °C for 3 min (melting) and two cycles of annealing of 65 °C for 5 min and 30 °C for 15 min. The resultant linear vector was transformed into E. coli 5a C2987, in which the 19mer sticky ends were ligated and the vector was circularized. The vector contained the guide (named gNIR1), following the structure: pDONR207 flanks; A.t. U6 promoter; *NIR1* target sequence; Guide RNA scaffold; Pol III terminator; pDONR207 flanks (Fig. 3.1A). The guide clone was verified with the primers pDNOR\_F and pDONR\_R (Table A3).

The pHEE2E-TRI plasmid (Fig. A4) was chosen as destination vector, which carries egg cell-specific promoter-controlled CRISPR/Cas9 technology. Thus, homozygous mutant plants can be obtained in the T1 generation (Wang et al., 2015a). In order to mobilize it, the guide was amplified by PCR using the primers P1b-HindIII and P4b-Spel (Table A3) and the Phusion polymerase in a 50 µl reaction. The product was checked by agarose electrophoresis and the amplicon was purified from the 500 bp sliced band. The clean amplicon was digested with HindIII and Spel for 3 h at 37 °C and cleaned with a kit. 5 µg of pHEE2E-TRI were digested with HindIII and Spel for 3 h at 37 °C and additional 30 min with alkalinephosphatase at 37°C, and cleaned with a PCR Clean-Up Kit. The ligation of 90 ng of digested vector with 9 ng of digested insert was carried out with the T4 DNA ligase (Thermo Scientific), at 16 °C for 24 h (Fig. 3.1A). A 1:5 dilution of this solution was used to transform E. coli 5a C2987 and the sequence was verified with the primers pU6seqF and pU6seqR (Table A3). The gNIR1-containing pHEE2E-TRI vector was used for transformation of Agrobacterium cells and further plant transformation.

T1 seeds were sown on Hygromycin selection plates (4.3 g/l of MS medium salts, 8 g/l phytoagar, 25  $\mu$ g/mL Hygromycin and 100  $\mu$ g/ml cefotaxime), stratified for 3 days and transferred to dark for additional 3 days after a 6 h white light pulse. When returned to long day conditions, transformants were selected by checking hypocotyl length (hygromycin resistant seedlings display larger hypocotyls) and transferred to soil. Genomic DNA was extracted from samples taken from these



**Figure 3.1. Generation of** *nir1* **mutant plants by CRISPR-Cas9 technology.** A, Scheme showing the process for the generation of a transgenic plant expressing both Cas9 and a guide with a scaffold to induce a mutation in the *NIR1* locus. The NiR1 specific guide was subcloned in the pDONR207-Cas9 vector via SLIM (two separate inverse PCRs with tailed primers) and hybridization. The construction was amplified and ligated to pHEE2E-TRI vector that carries with the CRISPR/Cas9 technology components under the control of an egg cell-specific promoter. The names of the primers used for the PCRs are included. B, Scheme showing the process for the generation of a homozygous *nir1* mutant plant. The region of the peptide sequence conserved from the wild type NIR1 protein is highlighted in purple. \* represents the stop codon. The region of the *NIR1* gene corresponding to the designed specific NIR1 guide is highlighted in light blue. Bottom panel shows a 2% agarose gel electrophoresis of the RFLP genotyping (amplifying by PCR and cutting with *Alul*) of a wild type, heterozygous and

homozygous *nir1* plant, all three from a second generation of transgenic pU6::gNIR1-1 plants. The size of the bands is shown at the right side of panel.

seedlings and used for the amplification of the target sequence of *gNIR1* and its flanking regions with the primers NIR1cdsF and NIR1midR (Table A3) by HF-PCR (to avoid false positive mutations). PCR products were cleaned and checked for mutations by sequencing with the primer seqnir1-1-R (Table A3). The selected *nir1-1* mutation consisted in a single A base insertion in position 98 causing a frame shift leading to a premature stop codon (Fig. 3.1B, Table A5) and a Restriction Fragment Length Polymorphism (RFLP). Genotyping of the RFLP was performed by PCR amplification with primers NIR1cdsF and seqnir1-1-R (Table A3) and subsequent digestion with the restriction enzyme *Alu*I, which cuts the wild type but not the mutant amplicon (Fig. 3.1B).

In order to segregate-out the CRISPR/Cas9 transgene, T2 seeds were sown on Hygromycin inverse selection plates (2.7 g/l of MS medium salts, 8 g/l phytoagar, 20 µg/mL Hygromycin and 0.5% sucrose) and after hypocotyl elongation, Hygromycin sensitive seedlings were selected and transferred to recovery medium plates (2.7 g/l of MS medium salts, 8 g/l phytoagar and 0.5% sucrose). Transgene-free plants were confirmed by PCR with the primers Hyg-F and Hyg-R (Table A3).

#### 3.2.1.3. NO treatments

Pulses of NO were performed by incubating plants for 5 min in a tightly sealed transparent box after injection of 300 ppm of pure NO gas (Linde AG, Germany). For the assays of NO-triggered inhibition of hypocotyl elongation, surface-sterilized seeds were sown in MS-MES media and germination program was activated by exposure to light for 6 h after stratification, and then, incubated in tight-sealed boxes under air supplemented with 300 ppm pure NO gas under darkness for four days. Control seedlings were incubated under the same conditions in air with no supplemented NO. The screenings of TPT transgenic lines (Coego *et al.*, 2014) were performed by using MS-MES media supplemented or not with 10  $\mu$ M  $\beta$ -estradiol and treated or not with NO. Hypocotyl length was measured for every seedling of genotype and condition tested by using Image J. The experiments were repeated three times with at least 20 individuals per genotype, condition and experiment.

# 3.2.1.4. Freezing tolerance assays

Seeds from the different genotypes were sown in soil-containing pots and allowed to develop for 7 days. Then, several plants for each pot were removed in order to leave a similar number (25-30) of plants, homogenously distributed in all pots. Before being subjected to freezing temperatures, plants were exposed for 1 h to 4 °C in the freezing chamber. Then, temperature was progressively decreased (-1 °C/30 min) until reaching the indicated freezing temperatures. After exposing

plants to the appropriate freezing temperature for 6 h, temperature was gradually increased to 4 °C (+1 °C/30 min). One hour later, plants were transferred to 20 °C under long-day light regime for recovering and subsequent survival evaluation 7 days later. For cold acclimation assays, the 7 days old plants were acclimated at 4°C for 7 days prior to the freezing tolerance assay. Control plants were the non-acclimated 14-day old plants grown in standard conditions of temperature.

3.2.1.5. Staining methods

# 3.2.1.5.1. NO detection by fluorescence and confocal microscopy

The endogenous levels of NO in shoots and roots were determined by staining with 10 µM 4-Amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA) as described (Guo et al., 2003) with some modifications. 4-day old seedlings grown on vertical MS plates (without sucrose) were transferred to the staining buffer (5 mM MES-KOH, pH 5.7; 0.25 mM KCl, 1 mM CaCl<sub>2</sub>) and incubated on liquid for 24 h at room temperature. For the staining of shoots, DAF-FM DA was added to the buffer used for this 24 h incubation, while for the staining of roots, the dye was added to buffer only during the last hour of incubation. In both cases, plants were kept in dark during the first hour of incubation with the dye and after the 24 h incubation, plants were washed three times for 10 min with staining buffer to remove the excess of DAF. Fluorescence was detected by confocal microscopy with a Zeiss LSM 780 (with excitation at 488 nm and emission at 500-527 nm range) or by fluorescence microscopy with a Leica DM 5000B (with a barrier filter for chlorophyll auto fluorescence), using unchanged parameters for every measurement. The specificity of NO-related fluorescence detection was assessed by a pre-incubation treatment with 0.5 mM of the NO scavenger 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) (Sigma, USA) and/or with 0.5 mM of the NO inducer salicylic acid (SA). The DAF-FM DA fluorescence intensities were analyzed using Adobe Photoshop by quantifying green pixels in 3 to 6 replicate images of every genotype and condition from three independent experiments.

For the determination of the NO content of cold acclimated plants, seedlings were grown in soil under standard long day photoperiod light conditions and 21°C for 6 d. Then, plants were either maintained at standard non-acclimated growing conditions or cold acclimated at 4°C for 7 days. At day 13, plants were sprayed with DAF-FM DA to run-off and kept under darkness for 1 h and later, after extensive washings, transferred to growing conditions at either 4°C or 21°C for additional 23 h. Fluorescence was detected by confocal microscopy with a CLSM LEICA TCS SP5.

# 3.2.1.5.2. Cuticle permeability tests

The toluidine blue test was carried out by placing 10  $\mu$ l droplets of a 0.025% (w/v) toluidine blue solution in potato dextrose broth (PDB) on the upper side

surface of undetached leaves. After 2 h leaves were washed gently with distilled water to remove excess of the toluidine blue solution, and then leaves were excised and photographed. For Calcofluor white staining, leaves were bleached in absolute ethanol overnight, equilibrated in 0.2 M phosphate buffer (pH 9) for 1 h, and incubated for 1 min in 0.5% (w/v) Calcofluor white in 0.2 M phosphate buffer (pH 9). Leaves were rinsed in phosphate buffer to remove excess of Calcofluor white and viewed under UV light.

3.2.1.5.3. Staining for cell permeability, cell death, superoxide and starch

To assess cell permeability, roots of 10 d-old seedlings were stained with propidium iodide by dipping into a 10  $\mu$ g/ml solution for 10 min. Stained roots were visualized under confocal microscopy (LSM 780, Zeiss) with excitation at 488 nm and emission at 598-650 nm range. Cell death was assayed by staining seedlings with 2% (w/v) Evans blue solution for 5 min and subsequent extensive washing. Superoxide staining was performed by dipping seedlings in 0.2% (w/v) solution of nitroblue tetrazolium (NBT) in 50 mM sodium phosphate buffer (pH 7.5) overnight at room temperature and protected from light followed by bleaching with hot ethanol. Staining of starch granules was performed by bleaching 7d-old seedlings with 96% ethanol for 5 h and then by incubating bleached seedlings in Lugol's staining solution (5.7 mM iodine, 43.4 mM KI, 0.2 N HCI) for 30 min and further extensive overnight washing with water.

3.2.1.6. Change of nitrogen source for plant growth

To test the effect of changing the nitrogen source, plants were grown for 11 days in ammonium-supplied MS media without nitrogen and then transferred to media containing either nitrate or ammonium as the only nitrogen source for two additional days.

3.2.1.7. Inhibition of protein synthesis or degradation

To test the effect of proteasome and protein synthesis inhibition, 100  $\mu$ M MG132 and/or 40  $\mu$ M cicloheximide (CHX) were added respectively to liquid media, where seedlings were incubated for 16 h.

# 3.2.2. Bacterial manipulation

# 3.2.2.1. Genetic transformation by heat shock

Chemically competent *E. coli* cells were transformed by heat shock. For doing so, cells were unfrozen on ice for 15 min, 1-5  $\mu$ l of variable concentration of the plasmid were added and after another 30 min of incubation on ice, the mixture was kept at 42 °C for 30 s. After cooling down on ice, 250  $\mu$ l of LB were added and the mixture was incubated at 37 °C and 200 rpm shaking for 1 h. Transformed bacteria were selected by spreading the mixture on solid LB plates with the corresponding antibiotic and were incubated at 37 °C for 24 h.

For further plant transformation, chemically competent *A. tumefaciens* cells were transformed by heat shock with the desired plasmid. Cells were unfrozen on ice, 5  $\mu$ l of variable concentration of the plasmid were added and the mixture was kept at 37 °C and 200 rpm shaking for 5 min. Then the mixture was frozen on liquid nitrogen and unfrozen on ice. 250  $\mu$ l of LB were added and the mixture was incubated at 28 °C and 200 rpm shaking for 2-3 h. Transformed Agrobacteria were selected by spreading the mixture on solid LB plates with the corresponding antibiotic and were incubated at 28 °C for 2-3 days.

# 3.3. Nucleic acid methods

# 3.3.1. Extraction and purification of nucleic acids

# 3.3.1.1. Extraction of genomic DNA from leaves or seedlings

Extraction of genomic DNA from plant material (usually leaves or full seedlings) for genotyping or other purposes was performed as described by Edwards *et al.* (1991) with minor modifications. Liquid nitrogen frozen samples were homogenized in extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) with a polytron. After a 2 min centrifugation at 4 °C, supernatant was incubated on ice with 1 volume of ice cold isopropanol for 30 min. The mixture was centrifuged for 5 min at room temperature and the dried pellet was resuspended in TE buffer. Alternatively, genomic DNA was isolated with hexadecyltrimethylammonium bromide (CTAB)-containing buffer. DNA samples were stored at -20 °C.

# 3.3.1.2. Cleaning of PCR products

Amplified DNA of PCR products was cleaned for further sequencing or manipulation, removing excess primers and unincorporated nucleotides. To achieve this, 5  $\mu$ I of PCR product was incubated with 1  $\mu$ I of ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix). The mix was incubated for 15 min at 37 °C and the reagent was inactivated by incubating for additional 15 min at 80 °C. Alternatively, PCR products were cleaned with the ChargeSwitch PCR Clean-Up Kit (Invitrogen).

#### 3.3.1.3. Extraction of DNA from agarose gel

DNA fragments separated by size through agarose gel electrophoresis were recovered from gel slices of specific bands by using the PureLink Quick Gel Extraction Kit (Invitrogen).

3.3.1.4. Extraction and isolation of plasmidic DNA from bacteria

For the extraction and purification of plasmids from transformed bacteria, a liquid culture was prepared in 4-8 ml of LB medium from a single colony. Culture was incubated for at least 10 h at 37 °C and then was centrifuged at 4500 rpm for 10 min. The pellet was used for the extraction by using the PureLink Plasmid

Miniprep Kit of Invitrogen. Plasmids were collected in MilliQ water. Afterwards, plasmid and transgene were checked by sequencing with specific primes (Table A3). Plasmid samples were stored at -20 °C.

3.3.1.5. Extraction and isolation of total RNA from seedlings

Total RNA from full seedlings was extracted and purified with Nucleospin RNA Plant kit (Macherey-Nagel). RNA was recovered in diethyl pyrocarbonate (DEPC)-treated water and its quality was checked by running a fraction on an agarose gel electrophoresis. Alternatively, for microarray analyses, plants were frozen in liquid nitrogen and the total RNAs were extracted with Trizol and purified with the RNeasy kit (QIAGEN). RNA samples were stored at -80 °C.

# 3.3.2. Manipulation of nucleic acids

# 3.3.2.1. Methods for quantification and concentration

Nucleic acids in extracts were quantified by measuring with an ND1000 spectrophotometer (NanoDrop Technologies), which allows direct measurements using 1 µl of non-diluted samples. Concentration of DNA samples in water was increased by using a centrifugal evaporator SpeedVac Savant at room temperature. Concentration of RNA samples in water was increased by precipitation after adding 0.05 volumes of 1M acetic acid in DEPC-treated water and 0.7 volumes of absolute ethanol. After incubating at -80 °C for 30 min, samples were centrifuged at maximum speed and 4° C for 10 min and pellets were resolubilized in DEPC-treated water.

# 3.3.2.2. Methods for DNA amplification

For genotyping or other purposes, short DNA sequences were amplified by PCR. For each sample, 1  $\mu$ I of purified DNA was added to a reaction mix composed by 2.5  $\mu$ I of 10X reaction buffer with MgCl<sub>2</sub>, 0.75  $\mu$ I of 10mM dNTP mix, 0.75  $\mu$ I of 10  $\mu$ M forward and reverse primers, 0.5  $\mu$ I of Biotools DNA polymerase (1U/  $\mu$ I) and MilliQ water to a total volume of 25  $\mu$ I. Table A3 gathers all oligonucleotides used in this work. Amplification was carried out with an Applied Byosystems 96-well thermocycler. Standard program featured 2 min at 94 °C initialization, 35 cycles of denaturation (30 s at 94 °C), annealing (30 s at 54°C) and extension (2 min at 72 °C), and 7 min at 72 °C of final elongation. Number of cycles, annealing temperature and extension time were modified to suit a few cases. Products of PCR were used immediately for electrophoresis or stored at -20 °C. For cloning purposes, cDNA was used as template for High Fidelity (HF)-PCR, in which polymerase and reaction buffer were substituted by those provided by Fermentas for HF-PCR.

# 3.3.2.3. Reverse transcription (RT) of mRNA

Reverse transcription of the polyadenylated fraction of RNA was performed to obtain cDNA. For each sample, 1-2  $\mu I$  of purified RNA were added to 0.5  $\mu g$  of

oligo(dT)18 in 12.5  $\mu$ l of DEPC-treated water and incubated at 65 °C for 5 min. After cooling down on ice, the following was added to the mix: 4  $\mu$ l of Fermentas 5X reaction buffer, 0.5  $\mu$ l of RiboLock RNase inhibitor of Thermo Scientific (40 U/  $\mu$ l), 2  $\mu$ l of 10mM dNTP mix and 1  $\mu$ l of Fermentas RevertAid H Minus Reverse Transcriptase (200 U/ $\mu$ l). The mix was incubated at 42 °C for 90 min and the reaction was stopped by incubation for 10 min at 70 °C. The resulting cDNA was stored at -20 °C.

3.3.2.4. Quantification of transcripts by quantitative PCR (qPCR)

Real-time or quantitative PCR (qPCR) was used to quantify the expression level of genes. Reaction mix was composed by 10  $\mu$ l of SYBR Green PCR Master Mix (Applied Biosystems), 7.8  $\mu$ l of MilliQ water, 0.6  $\mu$ l of each specific primer (10  $\mu$ M) (Table A3) and 1  $\mu$ l of RT product (cDNA) as template. Reactions were performed in MicroAMPTM Optical 96-well reaction plates (Applied Biosystems) in an ABI 7500 Fast Real-Time Thermocycler, following the recommended program (2 min at 50° C, 10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C). For each reaction a Ct value and a melting curve were obtained. For each quantification assay, 3 biological replicates were used and each was used for 3 technical replicates. Relative expression of genes respecting control or mock samples was calculated with Applied Biosystems 7500 Software v.2.0.4 as indicated in equation 3.1, using *ACT2/8* as endogenous gene.

#### Equation 3.1. Normalized relative expression

 $\begin{aligned} Relative \ expression &= 2^{-\Delta\Delta Ct} \\ \Delta\Delta Ct &= (Ct_{target \ gene} - Ct_{endogenous \ gene}) - (Ct_{calibrator \ gene} - Ct_{endogenous \ gene}) \end{aligned}$ 

# 3.3.2.5. Transcriptomic analysis by microarrays

Microarrays were used for a genome-wide analysis of gene expression. Prior to the transcriptomic analysis, RNAs were checked for their integrity and purity by nanocapillary electrophoresis in Bioanalyzer Agilent 2100. Then, RNAs were ligated to an RNA oligonucleotide adaptor (Invitrogen) using T4 RNA ligase (Ambion). The RNAs were extracted once with phenol- chloroform and nonligated adapter was removed by chromatography with MicroSpin S-300 HR columns (GE Healthcare). Purified ligation products were precipitated in ethanol and used as templates for reverse transcription with Superscript III (Invitrogen) for 3 h at 46 °C, using oligonucleotide oligo(dT) as primer. Template RNA was removed by alkaline hydrolysis and first-strand cDNA purified with S.N.A.P. columns (Invitrogen). Second-strand synthesis was performed with Tag DNA polymerase (Roche) for 5 min at 94 °C, 5 min; 58 °C, 1 min; 72 °C, 10 min. A forward oligonucleotide T7-Adap primer, which was complementary to the RNA adapter and contained the sequence of bacteriophage T7 promoter, was used. Double-stranded cDNA was then purified with MinElute columns (Qiagen) and in vitro transcribed with T7 RNA polymerase, using a MessageAmp aRNA kit (Ambion). Amplified RNA was treated with DNase I (Roche) to remove cDNA templates, purified with an aRNA Purification Module (Ambion) and then used as template for single-stranded cDNA synthesis, according to Affymetrix instructions as follows: aRNA was reverse transcribed with SuperScript II (Invitrogen) for 1 h at 42 °C with oligo(dT) as primer. After alkaline hydrolysis of aRNA and purification (MinElute columns, Qiagen), cDNA was fragmented with 1.5 units of DNase I (GE Healthcare) into fragments in the 50–200 bp range. Finally, 3' ends of fragmented cDNA were biotin- ddUTP labeled with terminal deoxynucleotidyl transferase (Promega) and GeneChip DNA labeling reagent (Affymetrix).

Three biological replicates and their corresponding negative controls were independently hybridized to ATH1 microarrays (Affymetrix), containing 22 500 transcript variants from 24 000 well-characterized Arabidopsis genes. Each sample was added to a hybridization solution containing 100 mM 2-(N-morpholino) ethanesulfonic acid, 1 mM Na<sup>+</sup>, and 20 mM of EDTA in the presence of 0.01% Tween-20. Hybridization was performed for 16 h at 45 °C. Each microarray was washed and stained with streptavidin–phycoerythrin in a Fluidics station 450 (Affymetrix) and scanned at 2.5-µm resolution in a GeneChip Scanner 3000 7G system (Affymetrix). Data analyses were performed using genechip operating software (GCOS), to generate the corresponding CEL files.

Alternatively, sample RNA was amplified and labeled with the Agilent Low Input Quick Amp Labeling Kit and an Agilent Spike-In Kit was used to assess the labeling and hybridization efficiencies. Hybridization and slide washing were performed with the Gene Expression Hybridization Kit and Gene Expression Wash Buffers, respectively. Three biological replicates were hybridized to Arabidposis (V4) Gene Expression Microarray 4x44K, which contained 43,803 probes (60-mer oligonucleotides) and was used in a one-color experimental design. After washing and drying, slides were scanned in an Agilent G2565AA microarray scanner, at 5  $\mu$ m resolution and using the double scanning, as recommended. Image files were analyzed with the Feature Extraction software 9.5.1. Interarray analyses were performed with the GeneSpring 11.5 software.

# 3.3.2.6. DNA sequencing

Each bacterial purified plasmid (200 ng/ $\mu$ l) was checked by DNA sequencing with an ABi 3130XL (LifeTechnologies) using the cyclic sequencing kit BigDye Terminator v3.1 (LifeTechnologies). One oligonucleotide was used for the sequencing of every 700 bp of DNA. Cleaned PCR products (2.5 ng/ $\mu$ l) were similarly sequenced.

# 3.3.2.7. Electrophoretic techniques

DNAs were size-separated by agarose gel electrophoresis. Gel was composed by 1-2% (w/v) agarose and 10 mg/ml ethidium bromide in TAE buffer. Electrophoresis was run at 100 V and gel was visualized in UV transilluminator with

digital camera G:BOX (Syngene). Agarose gel electrophoresis was also used for RNA analysis, but it was performed at 40 V.

Genomic DNA isolated from plants was run in duplicate in 2% (w/v) agarose gels and either ethidium bromide-stained (15  $\mu$ g) or blotted (5  $\mu$ g) onto positively charged nylon membranes. DNA digested with *Msp*I was digoxigenin (DIG)-labelled and used as probe for Southern blot with anti-DIG antibody coupled to alkaline phosphatase to amplify the signal.

# 3.3.2.8. Enzymatic digestion of DNA with restriction enzymes

For different purposes, DNA was cut with restriction endonucleases. Reaction mix was composed by the DNA, 2  $\mu$ I of specific buffer (Thermo Fisher Scientific), 0.5  $\mu$ I of each restriction enzyme (Thermo Fisher Scientific) and MilliQ water to a volume of 20  $\mu$ I. The mix was incubated for 1-18 h at the corresponding temperature for the enzyme. The enzyme was deactivated by incubation at 70 °C for 10 min. Restriction product was analyzed by electrophoresis or stored at -20 °C.

# 3.3.2.9. Southern blot

15  $\mu$ g of CTAB-isolated genomic DNA were separated through 2% (w/v) agarose gel, blotted onto positively charged nylon membranes, and Southern blot performed with a digoxigenin-labelled probe (DIG High Prime DNA Labeling and Detection Starter Kit II from Roche).

# 3.3.2.10. Cloning techniques in bacteria

For the generation of transgenic plants overexpressing Arabidopsis NRs and NIR1 genes, Invitrogen's pCR8/GW/TOPO (Fig. A1) was used as entry vector while pAlligator2 (Bensmihen *et al.*, 2004) (Fig. A2) was the destination vector. 3' A-overhangs were added to HF-PCR products from amplifying the cDNA corresponding to the transcripts of the target genes by incubating with Taq polymerase for 10 min at 72 °C. For the cloning reaction of the desired gene into the entry vector, 4.2  $\mu$ I of HF-PCR product were added to 1  $\mu$ I of salt solution and 0.8  $\mu$ I of vector and incubated for 2 h at 23 °C. This mix was then used for direct bacterial transformation. The gene of interest was cloned to the destination vector by recombination through the Gateway LR reaction. 100-150 ng of purified entry vector, 200 ng of destination vector and 2  $\mu$ I of Clonase LR II were added to a total volume of 10  $\mu$ I of TE buffer. The mixture was incubated at 25 °C for 1-18 h and finished upon addition of 1  $\mu$ I of Proteinase K and an incubation of 10 min at 37 °C. This dilution was then used for bacterial transformation.

# 3.4. Protein methods

# 3.4.1. Extraction and purification of proteins

# 3.4.1.1. Extraction of total proteins from plant tissues

Total crude protein extracts were prepared by grinding liquid nitrogen frozen seedlings with a polytron and further extraction in 50 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl, 5% (v/v) glycerol, 5 mM EDTA, 0.05% (v/v) Triton X-100, 10 mM dithiothreitol (DTT), 1% (v/v) protease inhibitor cocktail (Sigma) and 1 mM phenylmethylsulfonyl fluoride (PMSF). Alternatively, in order to achieve non-reducing conditions, extractions were performed in the absence of DTT. Clean soluble protein-containing supernatant after 20 min centrifugation at 16000 g and 4 °C was stored at -80 °C.

3.4.1.2. Immunopurification of tagged proteins with magnetic beads

HA-tagged proteins overexpressed in transgenic plants were immunopurified with anti-HA-magnetic beads (Miltenyi Biotec, Germany). Purification was performed with the  $\mu$ MAC Epitope Tag Protein Isolation kit (Miltenyi Biotec, Germany). For further proteomic analysis, proteins were eluted under non-denaturing non-reducing conditions by using 0.1 mM triethylamine (pH 11.8) (Sigma), and proteins were collected in 1 M MES buffer (pH 3.0) for neutralization of the eluted immunopurified proteins. Purified proteins were stored at -80 °C.

# 3.4.1.3. Precipitation of proteins

In order to change the buffer of protein extracts or to achieve an increase in their concentration, four volumes of cold acetone were added and incubated overnight at -20 °C. The solution was centrifuged for 10 min at 4 °C and the dried pellet was resuspended in the desired volume of buffer.

# 3.4.2. Manipulation of proteins

#### 3.4.2.1. Quantification of total proteins

Total protein content in extracts was measured by adding Bradford reagent as described by Bradford (1976), measuring absorbance at 595 nm with the 96well Multiskan GO spectrophotometer (Thermo Scientific), and quantifying with a bovine serum albumin (BSA) calibration curve.

# 3.4.2.2. Protein separation by SDS-PAGE

Proteins in extracts were separated by their molecular weight by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were loaded with Laemmli 6X (without DTT if non-reducing conditions were required) in 10% acrylamide gels, after denatured by 95 °C incubation for 10 min. Electrophoresis was performed at 100 V with a protein molecular weight ladder.

Gels were either stained for total protein with Imperial reagent (Thermo Scientific) or semi-dry transferred to nitrocellulose membranes.

# 3.4.2.3. Protein detection by Western blot analyses

Levels of specific endogenous, tagged- or post-translationally-modified proteins were analyzed in total protein extracts by Western blot. Separated proteins in polyacrylamide gels were semi-dry transferred to nitrocellulose membranes with a Semi-Dry Blotter (Thermo Scientific), running at 140 mA for 45 min per gel. After blocking membranes with TTBS buffer supplemented with either 5% (w/v) non-fat dried milk, 3% (w/v) Top-Block (Fluka) or 3% (w/v) BSA, Western blots were performed by incubating membranes overnight at 4°C in the following primary antibodies at the indicated dilution factor: polyclonal anti-NR (Agrisera, 1:1000), anti-NiR1 (Davenport et al., 2015, 1:1000), monoclonal anti-3nitroY (Cayman Chemicals, 1:1000) or monoclonal anti-TMT (Thermo Scientific, 1:1000). Polyclonal anti DE-ETIOLATED 3 (DET3) (donated by David Alabadí, IBMCP Valencia, Spain, 1:10000) was used as loading control (Duek et al., 2004). Alternatively, loading control was assessed by staining nitrocellulose membranes after blotting with Ponceau S. Secondary anti-rabbit or anti-mouse antibodies coupled to horseradish peroxidase (HRP) (GE, 1:5000) and Supersignal WestPico Chemiluniscence reagents (Thermo Scientific) were used to visualize bands. HAtagged proteins were directly detected with monoclonal anti-HA-HRP antibody (Thermo Scientific, 1:1000).

# 3.4.2.4. LC-MS/MS-based proteomic analyses

Protein extracts obtained from plants expressing HA-tagged versions of NRs and NiR1 were used for proteomic analyses for the identification of *in vivo* PTMs. Protein samples immunopurified under non-reducing conditions were precipitated with six volumes of cold acetone and digested overnight, under non-reducing conditions, with trypsin (mass spectrometry grade, Promega Corp.; 1:10 enzyme/substrate ratio) at 37°C in 6 M urea, 200 mM ABC buffer. The resulting peptides, desalted with UltraMicroSpin columns, were fragmented by collision-induced dissociation in a Thermo LTQ Orbitrap Velos Pro mass spectrometer. Fragmented peptides were separated in a packed nanocapillary column (NTCC-360/75-1.9-25L Nikkyo Technos). Raw data were processed and analyzed by using the Mascot Server v2.4 (Matrix Science) database [false discovery rate (FDR) < 5%, A. thaliana UniProt database including 13,140 proteins, with a mass tolerance of 7 ppm for the precursors and 0.5 Dalton for fragments].

# 3.4.2.5. NR and NIR activity assays

Nitrate reductase activity assays were performed as reported (Park *et al.*, 2011) with slight modifications. Proteins were extracted by homogenizing liquid nitrogen frozen seedlings in a buffer composed by 250 mM Tris–HCI (pH 8.0), 1 mM EDTA, 1  $\mu$ M Na<sub>2</sub>MoO<sub>4</sub>, 5  $\mu$ M flavin adenine dinucleotide (FAD), 3 mM dithiothreitol (DTT), 12 mM  $\beta$ -mercaptoethanol and 250  $\mu$ M PMSF. The enzymatic

reaction was carried out by incubating 20  $\mu$ g of protein extract in a final volume of 250  $\mu$ l of NR activity reaction buffer (40 mM NaNO<sub>3</sub>, 80 mM K<sub>2</sub>HPO<sub>4</sub>, 20 mM KH<sub>2</sub>PO<sub>4</sub>, 0.2 mM NADH, pH 7.5) at 25 °C for 30 min. Reaction was initiated by the addition of the NaNO<sub>3</sub> and was stopped upon addition of 100  $\mu$ l of sulfanilamide reagent (1% (w/v) sulfanilamide and 2.4 M HCl) and 100  $\mu$ l of N-(1-napthyl) ethylenediamine hydrochloride (N-NEDA) reagent (0.02% (w/v)) to 100  $\mu$ l of the reaction solution. Formation of nitrite was determined by measuring at 540 nm the absorbance of the resultant mixture with the 96-well Multiskan GO spectrophotometer (Thermo Scientific). Quantification of nitrite formation was performed by a nitrite calibration curve and the comparison between samples incubated for 30 min and samples in which the reaction was stopped at the beginning by the addition of the sulfanilamide reagent. For each genotype and condition, 3 biological replicates were used and each was used for 3 technical replicates.

Nitrite reductase activity was assayed as previously reported (Takahashi et al., 2001) with slight modifications. Proteins were extracted by homogenizing liquid nitrogen frozen seedlings in 400 µl of a buffer composed by 50 mM potassium phosphate buffer (pH 7.5), 1 mM EDTA, 10 mM β-mercaptoethanol, 100 μM PMSF and 5 mg PVP. The enzymatic reaction was carried out by incubating 50 µg of protein extract in a final volume of 250 µl of NIR activity reaction buffer (33 mM potassium phosphate buffer, pH 7.5, 1 mM NaNO<sub>2</sub>, 1 mM methyl viologen, 11.11 mM sodium dithionite) at 30 °C for 10 min. Reaction was initiated upon addition of the sodium dithionite and stopped by oxidizing the samples with a vortex mixer. Nitrite conversion was determined by measuring with the 96-well Multiskan GO spectrophotometer (Thermo Scientific) at 540 nm the absorbance of a mixture composed by 4 µl of the reaction solution, 96 µl water, 100 µl of sulfanilamide reagent and 100 µl of N-NEDA reagent. Quantification of nitrite conversion was performed by a nitrite calibration curve and the comparison between samples incubated for 10 min and samples in which the reaction was stopped at the beginning by vortexing. For each genotype and condition, 3 biological replicates were used and each was used for 3 technical replicates.

# 3.5. Metabolic methods

# 3.5.1. LC/MS- and GC/MS-based analyses of the metabolome

The sample preparation process was carried out using the automated MicroLab STAR system from Hamilton Company. Recovery standards were added prior to the first step in the extraction process for quality Control (QC) purposes. Sample preparation was conducted by series of organic and aqueous extractions to remove the protein fraction while allowing maximum recovery of small molecules. The resulting extract was divided into two fractions; one for analysis by Liquid Chromatography (LC) and one for analysis by Gas Chromatography (GC). Samples were placed briefly on a TurboVap

(Zymark) to remove the organic solvent. Each sample was then frozen, dried under vacuum and prepared for either LC/MS or GC/MS.

The LC/MS portion of the platform was based on a Waters ACQUITY UPLC and a Thermo-Finnigan LTQ mass spectrometer, which consisted of an electrospray ionization (ESI) source and linear ion-trap (LIT) mass analyzer. The sample extract was split into two aliquots, dried, then reconstituted in acidic or basic LC-compatible solvents, each of which contained 11 or more injection standards at fixed concentrations. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns. Extracts reconstituted in acidic conditions were gradient eluted using water and methanol both containing 0.1% Formic acid, while the basic extracts, which also used water/methanol, contained 6.5 mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS2 scans using dynamic exclusion. The Thermo-Finnigan LTQ-FT mass spectrometer had a linear ion-trap (LIT) front end and a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer back end. For ions with counts greater than 2 million, an accurate mass measurement could be performed. Accurate mass measurements could be made on the parent ion as well as fragments. The typical mass error was less than 5 ppm. lons with less than two million counts require fragmentation spectra (MS/MS) typically generated in data dependent manner or targeted MS/MS in the case of lower level signals.

The samples destined for GC/MS analysis were re-dried under vacuum desiccation for a minimum of 24 hours prior to being derivatized under dried nitrogen using bistrimethyl-silyl-triflouroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp is from 40° to 300° C in a 16 minute period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization.

The data extraction of the raw mass spec data files yielded information that was loaded into a relational database and manipulated without resorting to BLOB manipulation. Peaks were identified using peak integration software, and component parts were stored in a separate and specifically designed complex data structure. Compounds were identified by comparison to library entries of more than 1000 commercially available purified standards. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific compound or an isobaric entity. Additional entities could be identified by virtue of their recurrent nature (both chromatographic and mass spectral). A variety of curation procedures were carried out to ensure accurate and consistent identification of true chemical entities, and to remove those representing system artifacts, misassignments, and background noise.

# 3.5.2. Quantification of anthocyanins

Anthocyanins were spectrophotometrically determined in methanolic extracts by reading their absorbance at 530 nm as described (Solfanelli *et al.*, 2006).

# 3.5.3. Phytohormone quantification

Four independent biological replicate samples of around 150-200 mg fresh weight of either non-acclimated or cold-acclimated Col-0 and nia1,2noa1-2 seedlings were suspended in 80% methanol-1% acetic acid containing internal standards and mixed by shaking during one hour at 4°C. The extract was kept at 20 °C overnight, centrifuged, the supernatant dried in a vacuum evaporator, and the dry residue was dissolved in 1% acetic acid and passed through an Oasis HLB (reverse phase) column as described in Seo et al. (2011). The dried eluate was dissolved in 5% acetonitrile-1% acetic acid, and the hormones were separated using an autosampler and reverse phase UHPLC chromatography (2.6 µm Accucore RP-MS column, 50 mm length x 2.1 mm i.d., ThermoFisher Scientific) with a 5 to 50% acetonitrile gradient containing 0.05% acetic acid, at 400 µL/min over 14 min. The phytohormones were analyzed with a Q-Exactive mass spectrometer (Orbitrap detector, ThermoFisher Scientific) by targeted Selected Ion Monitoring (SIM). The concentrations of hormones in the extracts were determined using embedded calibration curves and the Xcalibur 2.2 SP1 build 48 and TraceFinder programs. The internal standard for quantification of ABA was the deuterium-labelled hormone. For JA, dihydrojasmonate (dhJA) was used as internal standard.

# 3.6. In silico and statistical methods

# 3.6.1. *In silico* analyses

DNA and amino acid sequences were analyzed by using the tools available in the Sequence Manipulation Suite (http://www.bioinformatics.org/sms2/) and the performed alignments were with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Gene Ontology enrichment of functional categories in gene lists was performed by the Gene Ontology Consortium tools (http://www.geneontology.org/). Comparison of transcriptome profiles with publicly available datasets was performed with AtCAST3.1 tool (http://atpbsmd.yokohama-cu.ac.jp/cgi/atcast/search input.cgi) by selecting data from different ATH1 experiments with P value of Student's t-test < 0.01. Spearman's rank-order correlation coefficients (SCCs) were used to estimate the functional overlapping/co-expression between experiments (Kakei & Shimada, 2015). Search for the RAP2.3 binding motif MGCCGYM in promoter sequences of Arabidopsis genome was performed with Patmatch tool in the TAIR10 Loci Upstream Sequences -- 1000 bp (DNA) database. Multiple protein sequence alignments were performed by using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Protein Database Bank (<u>https://www.rcsb.org/</u>) files were processed with Yasara software (<u>www.yasara.org/</u>) for 3D structure modelling.

# 3.6.2. Prediction of PTMs

S-nitrosylation and nitration sites on potential NO target proteins were predicted by GPS-SNO (Xue *et al.*, 2010; <u>http://sno.biocuckoo.org/</u>) and iSNOPseAAC (Xu *et al.*, 2013; <u>http://app.aporc.org/iSNO-PseAAC/</u>); and GPS-YNO2 (Liu *et al.*, 2011; <u>http://yno2.biocuckoo.org/</u>) and iNi-tro-Tyr (Xu *et al.*, 2014; <u>http://app.aporc.org/iNitro-Tyr/</u>) tools.

# 3.6.3. Statistical analyses

Differential gene transcript levels or hypocotyl lengths were statistically analyzed by Student's t-test and considered significant with p-value  $\leq 0.05$ . The Linear Model Methods (LiMMA) were used for determining differentially expressed genes in microarray-based analyses. To control the false-discovery rate, P-values were corrected using the method of Benjamini and Hochberg (1995). Criteria for selection of genes were fold value >1.5 and false-discovery rate  $\leq 0.05$ . Statistical analysis and graphical visualization of data were performed with the interactive tool FIESTA (http://bioinfogp.cnb.csic.es/tools/FIESTA/).

For metabolomic analyses, following log transformation and imputation with minimum observed values for each compound, Welch's two-sample t-test and ANOVA contrast were used to identify biochemicals that differed significantly between experimental groups. A Two-way ANOVA was also used to identify biochemicals exhibiting a significant time and treatment main effect and the interaction effect between these two variables. An estimate of the false discovery rate (q-value) was calculated to take into account the multiple comparisons. Statistical analyses are performed with the program "R" (http://cran.r-project.org/). Statistically significant differences in hormone quantification and transcript analyses were computed based on Student's t-tests.

Materials and methods

# Results

Results

#### 4. <u>RESULTS</u>

#### 4.1. <u>Regulation of NR-dependent NO biosynthesis</u>. <u>Post-translational</u> <u>modifications of the biosynthetic enzymes</u>

This section 4.1 is an excerpt from the research article: "Costa-Broseta, Á., Castillo, M. C., & León, J. (2018). Protein Stabilization and Post-translational Modifications Control NO Homeostasis in Arabidopsis". This research article was submitted to Plant Physiology and was under revision when PhD Thesis writing was finished. All the results and figures that appear here are derived from the work of the PhD student in collaboration with the other authors.

#### 4.1.1. Abstract

Nitric oxide (NO) is mainly produced from nitrite in plants through a reductive mechanism involving nitrate reductases (NRs) in balance with nitrite reductases (NiRs). Arabidopsis has two NADH-dependent NRs, NR1/NIA1 and NR2/NIA2, and one Ferredoxin-dependent NiR called NiR1. We found that NRs and NiR1 enzymes were oppositely regulated by two main factors: nitrate-induced signaling controlled by the function of the NIN-like protein 7 (NLP7) transcription factor, and proteasome-mediated degradation. NLP7 positively regulated NRs but negatively regulated NiR1. Moreover, NRs but not NiR1 were controlled by proteasome-mediated degradation. High rates of NO production were sustained under positive regulation of NRs and negative regulation of NiR1 as demonstrated in a *nir1* loss-of-function mutant generated by CRISPR-Cas9. NR stabilization and NO accumulation under treatment with proteasome inhibitors were potentiated in a prt6-1 mutant background, thus connecting NO production with the Arg/N-end rule proteolytic pathway. Moreover, PRT6 protected NRs from NO-triggered degradation in a putative auto-regulatory loop controlling NO homeostasis. NO-triggered changes in NRs and NiR1 protein and activity were likely exerted by post-translational modifications (PTMs). We identified nitration of tyrosine residues as well as S-nitrosylation of cysteine residues in NRs and NiR1, which may account for inactivation of the enzymes. Moreover, NOtriggered PTMs were accompanied by lysine ubiquitylation that is likely required for further proteasome-mediated degradation of the enzymes.

# 4.1.2. Nitrate Reductase is Regulated by Proteasome-mediated Degradation and Nitrate Signaling

Nitrate assimilation, involving the nitrate reductase (NR)-catalyzed conversion of nitrate to nitrite and further reduction to ammonium by nitrite reductase (NiR), may be a source of nitric oxide (NO) when the second redox reaction is replaced by a conversion of nitrite to NO catalyzed by NR or other molybdenum-dependent enzymes (Bender & Schwarz, 2018) (Fig. 4.1A). This alternative function for NR

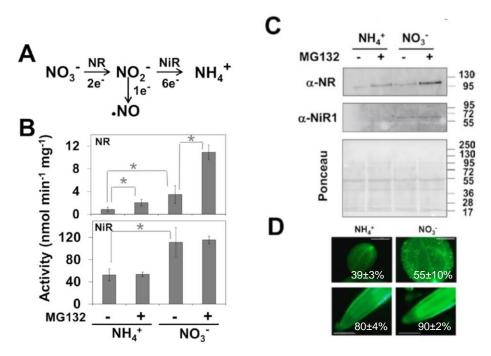


Figure 4.1. Effect of nitrogen source and proteasome-mediated degradation on NR and NiR. A, Scheme of nitrate assimilation and NO production. B, NR and NiR activities; and C, protein levels in plants grown for 11 days in ammonium-supplied media and then transferred to media containing either 5 mM nitrate or 2.5 mM ammonium as indicated for two additional days. By 16 h before collecting samples plants were either untreated (-) or treated with 100  $\mu$ M MG132 proteasome inhibitor (+). D, NO levels detected in DAF-FM DA stained cotyledons and roots of plants grown with 5 mM nitrate or 2.5 mM ammonium. Quantification of fluorescence is shown as the mean of at least 3 replicate experiments ± standard error. \* represents statistically significant with p<0.05 in paired Student's t-test. Size bars represent 100  $\mu$ m.

place this enzyme as a relevant node in regulating developmental and stressrelated responses where NO is involved. However, the conditions favoring this alternative function are not well known. The levels of NR activity and protein are centrally regulated by the availability of nitrate. Nitrate induces gene transcripts, protein synthesis and the activity of nitrate assimilatory enzymes in plants (Aslam & Huffaker, 1989; Cheng et al., 1991). Accordingly, we confirmed that in plants grown in ammonium-supplied media and then transferred to nitrate-supplied media or kept in ammonium as control, the levels of NR and NiR1 proteins and their activities were higher in nitrate-supplied than in ammonium-supplied plants (Fig. 4.1B, C). It is noteworthy mentioning that treatment with the proteasome inhibitor MG132 stabilized NR protein (Fig. 4.1C) and enhanced its activity (Fig. 4.1B), whereas no alterations were detected for NiR1 in the absence or presence of the proteasome inhibitor. The increased NR protein and activity levels correlated well with the increased NO content detected in shoots and roots of nitrate-grown plants when compared to ammonium-grown plants (Fig. 4.1D). All these data together suggest that NR, besides being positively regulated by nitrate, is negatively regulated by a proteasome-mediated proteolytic process that may be relevant in controlling NO production.

Some previous evidences suggested that the above proposed proteasomemediated proteolytic process modulating NR function and the subsequent NO production might be related to the N-end rule proteolytic pathway. We previously reported that the degradation of transcription factors of the group VII of Ethylene Responsive Factors (ERFVII) through the arginylation branch of the N-end rule pathway is on the basis of a NO sensor mechanism (Gibbs et al., 2014a). Degradation of ERFVIIs requires their polyubiquitylation by the E3 ubiquitin ligase PRT6 in such a way that *prt6-1* mutant plants were insensitive to NO and, remarkably, contained increased levels of NO in leaf stomata (Gibbs et al., 2014a). On the other hand, it has been reported that the nitrate-triggered induction of genes coding for nitrate assimilation enzymes NR and NiR1 are due to the function of the NLP7 transcription factor that upon nitrate treatment accumulates in the nuclei (Marchive et al., 2013), and binds to specific sites of the promoters of NR and NiR genes (Guan et al., 2017; Zhao et al., 2018). We have tested whether the levels of NR and NiR1 protein and enzyme activities were altered in the N-end rule pathway prt6-1 mutant as well as in the nitrate signaling nlp7-1 mutant plants in nitratecontaining media. Figure 4.2A shows that the levels of NR and NiR1 proteins did not change significantly in *prt6-1* when compared to wild type plants. By contrast, the levels of NR and NiR1 proteins decreased and increased, respectively in *nlp7*-1 plants (Fig. 4.2A). These changes in the protein levels matched with decreased and enhanced enzyme NR and NiR activities, respectively (Fig. 4.2B). The effect of *nlp7-1* mutation seems to be epistatic on the *prt6-1* mutation as the double *prt6-*1nlp7-1 mutant also showed decreased and increased NR and NiR protein and activities, respectively (Fig. 4.2A, B). Regarding this, *nlp7-1* plants have pale-green normal-size leaves; prt6-1 plants are smaller with green leaves, and nlp7-1prt6-1 plants are small pale-green plants combining the parental phenotypes (Fig. 4.3A). Remarkably, the expression of N terminus-tagged NLP7 under the control of the endogenous promoter or the constitutive 35S promoter in the double nlp7-1prt6-1 background rescued both the small size and the pale green phenotypes (Fig. 4.3A), thus suggesting PRT6 and NLP7 may be somehow functionally linked. It is noteworthy mentioning that expression of NLP7 in the nlp7-1 and nlp7-1prt6-1 mutant backgrounds led to a significant increase in the endogenous NO content even over those detected in *nlp7-1prt6-1* plants (Fig. 4.3B). These data suggest a central role for NLP7 in regulating nitrate assimilation acting oppositely on NRs and NiR1, which might be relevant for the production of NO.

If NLP7 regulates NR positively and NiR negatively it would be expected that the levels of NO decrease in NLP7-defective plants. Figure 4.2C shows that indeed the NO content was slightly lower in *nlp7-1* than in wild type plants. Interestingly, the slightly increased NO levels detected in *prt6-1* plants when compared to wild type plants were substantially larger in *prt6-1nlp7-1* plants (Fig. 4.2C), despite

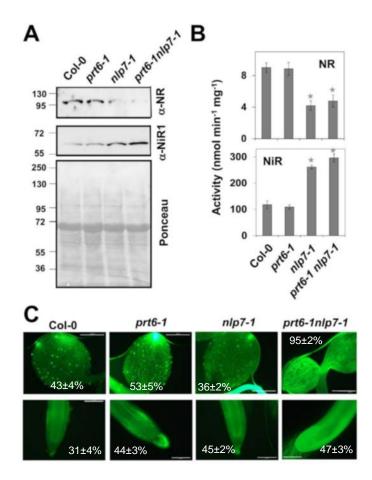


Figure 4.2. Regulation of NR and NiR by NLP7 nitrate signaling factor. A, Protein levels and B, enzyme activities in the indicated mutant plants. Activity values are the mean  $\pm$  SD of three independent biological replicates and shown Western blots with specific anti-NR and anti-NiR1 proteins are representative. Ponceau S staining was used as loading control and molecular size marker (kDa) locations are shown at the left side of A panels. \* Represents statistically significant with p<0.05 in paired Student's t-test comparing to Col-0 levels. C, NO levels detected in DAF-FM DA stained cotyledons and roots of plants of the indicated genotypes. Quantification of fluorescence is shown as the mean of at least 3 replicate experiments  $\pm$  standard error. Size bars represent 100 µm.

having reduced levels of NR protein and activity (Fig. 4.2A, B). In turn, *prt6-1nlp7-1* plants contained significantly enhanced NiR protein and activity (Fig. 4.2A, B). Therefore, the enhanced NO levels and NiR1 function in *prt6-1nlp7-1* plants could be indicative of NiR1 playing a positive role in NO production. To test this hypothesis, we have generated transgenic 35S:*NiR1* plants (Fig. 4.4) and a *nir1* loss-of-function mutant, by using CRISPR-Cas9 technology (Fig. 3.1). After the analysis of the NO content, we found a large accumulation of NO in *nir1* mutant plants whereas no significant alteration was observed in *35S:NiR1* plants (Fig. 4.5) despite these transgenic plants were in a *prt6-1* background that favors NO production (Fig. 4.2C) (Gibbs *et al.*, 2014a). If the enhanced NO content detected in *prt6-1nlp7-1* plants were not due to increased NiR1 function, it might be alternatively explained by a NLP7-independent PRT6-regulated process. As

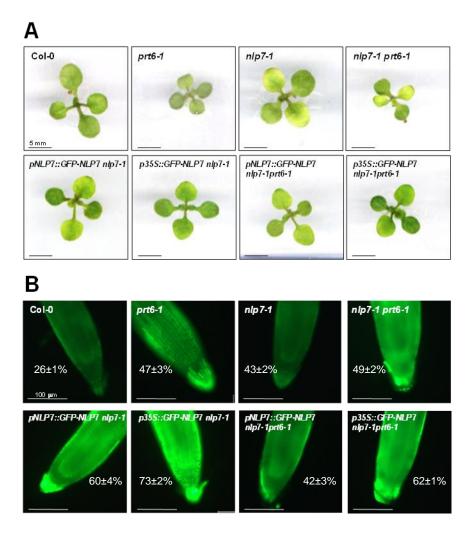


Figure 4.3. Complementation of *nlp7-1* and *prt6-1* related phenotypes and NO production by *NLP7* over-expression. A, Appearance of 11-day old Arabidopsis plants of the indicated genotypes grown under standard conditions. Size bars represent 5 mm. B, NO levels detected by staining with DAF-FM DA in roots of the indicated genotypes. Quantification of fluorescence is shown as the mean of at least 3 replicate experiments ± standard error. Size bars correspond to 100  $\mu$ m.

shown in Figure 4.6, the levels of NR protein increased upon MG132 treatment and decreased after treatment with the protein synthesis inhibitor cicloheximide (CHX) in wild type plants, thus indicating the levels of NR depend on the *de novo* biosynthesis and further stabilization of the protein. However, the NR levels were lower in *nlp7-1* plants and further decreased upon treatment with MG132 or CHX (Fig. 4.6), thus suggesting the NR stabilization induced by the proteasome inhibitor would require NLP7. Interestingly, the MG132-induced accumulation of NR was strongly potentiated either in *prt6-1* or the quintuple ERFVII (*qerfvii*) mutant plants (Fig. 4.6). These data would be compatible with the accumulation of a potential 26S proteasome substrate, different than ERFVIIs and presumably regulated negatively by them. Upon accumulation, this proteasome substrate would regulate positively NR through NLP7.

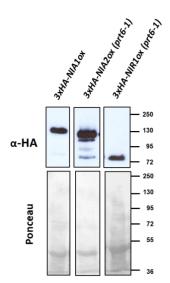
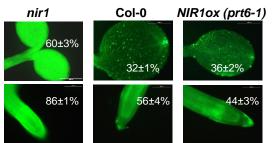


Figure 4.4. Generation of transgenic plants overexpressing HA-tagged versions of NR1, NR2 and NiR1. Representative western blots with specific anti-HA from 27 µg total protein extracted from transgenic lines of Arabidopsis plants overexpressing HA-tagged versions of the NR (NIA1 and NIA2) and NIR1 proteins. Ponceau S staining was used as loading control and molecular size marker (kDa) locations are shown at the right side of panels.



**Figure 4.5. NO accumulation in plants altered in nitrite reduction to ammonium.** NO levels in CRISPR-Cas9 *nir1* mutant or *NIR1* overexpressing *prt6-1* plants were detected by DAF-FM DA staining of cotyledons and roots. Quantification of fluorescence is shown as the mean of at least 3 replicate experiments ± standard error. Size bars represent 100 µm.

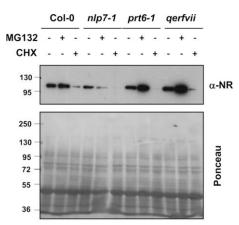
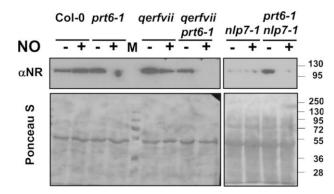


Figure 4.6. NR protein in nitrate signaling and Arg/N-end rule pathway mutants. Effect of proteasome and protein synthesis inhibitors MG132 and cicloheximide (CHX), respectively, on NR protein levels was shown by Western blot using specific anti-NR from 27  $\mu$ g total protein extracted from plants pretreated with 100  $\mu$ M MG132 or 40  $\mu$ M CHX as indicated. Ponceau S staining was used as loading control and molecular size marker (kDa) locations are shown at the left side of panels.

#### 4.1.3. NR and NiR1 proteins are post-translationally regulated by NO

The fact NR function and NO production seems to be tightly controlled by nitrate derived signals points to nitrate signaling as a relevant process in regulating endogenous NO levels. We analyzed the effect of treatment with a pulse of exogenous NO on the levels of NR protein. As shown in Figure 4.7, NR protein accumulated by 1h after exposure of plants to a NO pulse, whereas it was fully degraded in *prt6-1* plants. Because Arabidopsis NR1 and NR2 proteins are not MC-proteins their relationship with PRT6 function must be mediated by an intermediary protein. NR degradation in response to NO exposure occurred also in double *qerviiprt6-1* and *nlp7-1prt6-1* mutant plants (Fig. 4.7), thus suggesting a still unknown negative regulator of NR that is substrate of PRT6 is a target of the negative regulation exerted by NO. All together these data point to the existence of a NO-triggered NR protein stabilization process requiring PRT6 function



**Figure 4.7. Effect of exogenous NO on NR protein stability.** Plants of the indicated genotypes were exposed to a 5 min 300 ppm NO pulse (+) or untreated (-) as control and 1 h later samples collected for total protein extraction. Samples of 50 µg protein were loaded per lane and NR levels analyzed by Western blot with anti-NR antibodies. Ponceau S- staining of membranes was performed as loading control. The position of protein size markers (M) in kDa is located at the right side of panels.

independently of ERFVIIs or NLP7. This NO-related protein stabilization processes suggest that NO might act by self-controlling its own biosynthesis by acting on NRs.

Regulation of NR by NO through a PRT6-mediated mechanism, strongly points to post-translational modifications (PTMs) of NR by polyubiquitylation, but also likely by NO-related PTMs. By using proteomic methodologies based on LC-MS/MS techniques on protein extracts from plants expressing HA-tagged protein versions (Fig. 4.4), we have identified that NR1 and NR2 undergo multiple PTMs including ubiquitylation of lysine residues, S-nitrosylation of cysteine residues and nitration or amination of tyrosine residues. As shown in Figure 4.8A, these PTMs were detected in both NR1 and NR2. It is noteworthy mentioning that both proteins were ubiquitylated in lysine residues spanning the whole protein sequences with the only exception of NR2 not being modified in lysine or tyrosine residues inside the cytochrome b5 heme-binding and FAD-binding FR-type domains nor in the lysine residue involved in the sumoylation process of NRs (Fig. 4.9). Moreover,

А

K203       NO       Yes         K204       NO       Yes         K204       NO       Yes         K206       NO       Yes         K206       NO       Yes         K207       K269       NO       Yes         Y271       NO       NO       Yes         Y289       Y289       NO       Yes         Y289       NO       Yes       Yes         Y330       Yes       Yes       Yes         Y331       Yas       Yes       Yes         Y332       Yes       No       Yes         Y333       Yes       Yes       Yes         Y334       Yes       Yes       Yes         Y333       Yes       Yes       Yes         Y334       Yes       Yes       Yes         Y334       Yes       Yes       Yes         Y333       Yes       Yes       Yes         Ya34       Yes       Yes       Yes         Ya34       Yes       Yes       Yes         K402       K399       Yes       Yes         K433       K400       Yes       Yes         K433<	NIA1	NIA2	CONSERVED	Y-NO2	Y-NH2	C-NO	K-GG			
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		Moco binding							Heme binding	FAD binding

Figure 4.8. Post-translational modifications identified in planta for NR1/NIA1 and NIR2/NIA2. A, Tyrosine (Y), cysteine (C), and lysine (K) residues in red, magenta, violet, blue and green were identified as nitrated, aminated, nitrated and aminated, S-nitrosylated and ubiquitylated, respectively. Homologue residues in gray were not found to be modified. B, Highly conserved cysteine and tyrosine and lysine residues in the motif Y-Y----KK in the N-terminal an central domains of assimilatory NRs, respectively, from rice (Os), Populus (POPTR), Arabidopsis (AT) and maize (GRMZM) plants that were identified as S-nitrosilated (blue vertical arrow), nitrated (red vertical arrow) and ubiquitylated (green vertical arrow) in both NR1 and NR2 proteins. C, Scheme showing the NR protein sequence with the position of the above mentioned conserved residues that were identified as modified and the relative position of Molibdenum Cofactor (MoCO), Heme and Flavin (FAD) binding sites together with the dimerization interface, and cytochrome b5, FAD and NAD binding pockets.

some of the identified PTMs were detected in residues largely conserved not only in Arabidopsis NRs but also in NRs from other plants. Remarkably, we identified S-nitrosylation of the C<sup>197</sup> and C<sup>191</sup> from NR1 and NR2, respectively (Fig. 4.8B), which are the amino acids involved in Molybdenum Cofactor (MoCo) binding (Fig. 4.8C). Also nitration and amination of two tyrosine residues in tandem (Y<sup>395</sup> and Y<sup>397</sup> from NR1 and Y<sup>392</sup> and Y<sup>394</sup> from NR2), and ubiquitylation of two consecutive lysine residues (K<sup>402</sup> and K<sup>403</sup> from NR1 and K<sup>399</sup> and K<sup>400</sup> from NR2) closed to the tyrosine cluster were identified in NR1 and NR2 (Fig. 4.8A). All these residues were fully conserved in NRs (Fig. 4.8B). This PTM cluster is located in the central part of NR proteins that separate the dimerization domain from the cyt b5-, FAD- and NAD-binding domains in the C-terminal part of the proteins (Fig. 4.8C and 4.9).

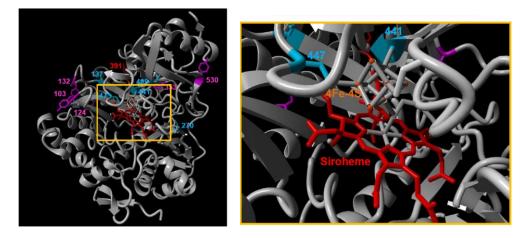
As mentioned above, an increased NO production could be the result of enhanced NR function or eventually of reduced NiR1 function (Fig. 4.1 and Fig. 4.5). Therefore, we also checked whether NiR1 may be also target of NO-related PTMs and polyubiquitylation. Similar proteomic analyses than those performed for NRs were also implemented for HA-tagged versions of NiR1. As shown in Figure 4.10, NiR1 was heavily polyubiquitylated in both N- and C-terminal domains in largely conserved lysine residues in nitrite reductases from different plants. NiR1 was also ubiquitylated in non-conserved lysine residues (Fig. 4.10B). Besides, both conserved and non-conserved tyrosine residues were nitrated and/or aminated (Fig. 4.10A, B). Remarkably, conserved C<sup>464</sup> and C<sup>470</sup>, involved in binding 4Fe-4S cluster as well as C522 involved in siroheme binding were identified as Snitrosylated (Fig. 4.10B, C). Taking advantage of the homology between the spinach and Arabidopsis NiRs and the reported 3D structure of spinach NiR (Protein Database Code 2akj) we have located the equivalent spinach residues to that identified as modified in Arabidopsis NiR1 (Fig. 4.10). Figure 4.11 shows that whereas nitrated tyrosine residues were located in the uppermost part of the structure relatively far from the 4Fe-4S cluster and siroheme binding pocket, the Snitrosylation of the two cysteines involved in binding the 4Fe-4S cluster in the catalytic center is very likely to affect the activity of the modified enzyme, presumably by blocking the proper electron transference and thus inhibiting the activity of the enzyme. In comparison, on the other hand, figure 4.12 shows how taking advantage of the availability of the 3D structural model of the corn NR cytochrome FAD- binding domain (PDB code 2cnd) and by amino acid sequence homology with Arabidopsis NRs, we have located a nitrated tyrosine residue in NR1 (Y<sup>733</sup>) which corresponded to Y<sup>83</sup> very close to the FAD in the 3D model.

	P11832 NIA1_ARATH P11035 NIA2_ARATH	MATSVDNRHYPTMNGVAHAFKPPLVPSPRSFDRHRHQNQTLDVILTETKIVKETEV MAASVDNRQYARLEPGLNGVVRS <mark>X</mark> KPPVPGRSDSPKAHQNQTTNQTVFLKPAKVHDDDE- **:*****:* * :***:****: * .*.*::*. *:*. *:*					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	ITTVVDSYDDSSSDDEDESHNRNVPYYKELVKKSNSDLEPSILDPRDESTADSWIQRNSS DVSSEDENETHNSNAVYYKEMIRKSNAELEPSVLDPRDEYTADSWIERNPS * **:**:** *. ****:::***::****:*********					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	MLRLTGKHPFNAEAPLPRLMHHGFITPVPLHYVRNHGAVPKANWSDWSIEITGLVKRPAK MVRLTGKHPFNSEAPLNRLMHHGFITPVPLH <mark>W</mark> VRNHGHVPKAQWAEWTVEVTGFVKRPMK *:*********					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	FTMEELISEFPSREFPVTLV <mark>C</mark> AGNRRKEQNMVKQTIGFNWGSAGVSTSLWKGIPLSEILR FTMDQLVSEFA <mark>M</mark> REFAATLV <b>A</b> GNRRKEQNMV <mark>KK</mark> SMGFNWGSAGVSTSVWRGVPLCDVLR ***::*:***	236 230				
	P11832 NIA1_ARATH P11035 NIA2_ARATH	RCGIYSRRGGALNVCFEGAEDLPGGGGS <mark>X</mark> GTSI <mark>K</mark> KEMAMDPARDIILAYMQNGELL RCGIFSRKGGALNVCFEGSEDLPGGAGTAGSKYGTSIK <mark>KEM</mark> AMDPSRDIILA <mark>Y</mark> MQNGE <mark>Y</mark> L ****:****************					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	TPDHGFPVRVIVPGFIGGRMVKWLKRIIVTPQESDS <mark>YM</mark> HYK <mark>D</mark> NRVLPSLVDAELANSEAW TPDHGFPVRIIIFGFIGGRMVKWLKRIIVTTKESDNFYHFKDNRVLPSLVDAELADEEGW **********					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	WY <mark>RPE</mark> YIINELNINSVITTPGHAEILPINAFTTQKPYTLKG <mark>WA</mark> SGGG <mark>KW</mark> VTRVEVTLDG W <mark>YRPE</mark> YIINELNINSVITTPCHEEILPINAFTTQRPYTLKGWASGG <mark>WW</mark> VTRVEVTVDG ******************					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	GDTWSVCELDHQEKFNK <mark>B</mark> C <mark>R</mark> FWCWCFWSLDVEVLDLLSAKDVAVRAWDESFNTQPDKLIW GETWNVCALDHQEKPNKYCKFW <mark>C</mark> WBFWSLEVEVLDLLSAKEIAVRAWDETLNTQPEKMIW *.**.**					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	NLMGMMNNCWFRIRTNVCKPHRGEIGIVFEHPTRPGNQSGGWMAKERQLEISSESNNTLK NLMGMMNNCWFRVKTNVCKPHKGEIGIVFEHPTLPGNESGGWMAKERHLEKSADAPPSLK ************************************					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	KSVSSPFMNTAS MYSISEVR <mark>H</mark> HNTADSAWIIVHGHI <mark>WDO</mark> TRFL <mark>H</mark> HPGGTDSILINAGT SVSTPFMNTTAMYSSSEVKKHNSADSCWIIVHGHIYDCTRFLMHPGGSDSILINAGT					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	DCTEEFEA H SDKAKKLLEDERIGELITTGYDSSPNVSVHGASNFGPLLAPIKELTP D TEEFEA H SDKAKKMLEDYRIGELITTGYSSDSSSPNNSVHGSSAVFSLLAPIGEATP					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	QKNIALVNPREKIPVRLIEKTSISHDVR <mark>M</mark> FRFALPSEDQQLGLPVGKHVFVCANINDKLC VRNLALVNPRAKVPVQLVEKTSISHDVRKFRFALPVEDMVLGLPVGKHIFLCATINDKLC					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	LRAYTPTSAIDAVGHIDLVVKV FKDVHPRFPNGGLMSQHLDSLPIGSMIDIKGPLGHIE LRAYTPSSTVDVVGYFELVVKIYFGGVHPRFPNGGLMSQYLDSLPIGSTLEIKGPLGHVE					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	KCKGNFLVSGKFKFAKKLAMLAGGTGITFIYQIIQSILSDPEDETEMYVVYANRTEDDI YLGKGSFTVHGKFKFADKLAMLAGGTGITFVYQIIQAILKDPEDETEMYVIYANRTEEDI					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	UVREELEGWASKHKERLKIW <mark>W</mark> VVEIAKEGWSYSTGFITEAVLREHIPEGLEGESLALACG LLREELDGWAEQYPDRLKVWYVVESAKEGWAYSTGFISEAIMREHIPDGLDGSALAMACG *:****:***: :***.**** *****					
	P11832 NIA1_ARATH P11035 NIA2_ARATH	PPPMIQFALQPNLEKMGYNVKEDLLIF 917 PPPMIQFAVQPNLEKMQYNIKEDFLIF 917 ********:******* **:***					
Cytochrome b5 heme-binding domain 💶 FAD-binding FR-type domain 💷							
	MoCo binding	Fe Hemo binding binding Sumoylation site YKPE					

Figure 4.9. Post-translationally modified residues in aligned NR1/NIA1 and NR2/NIA2 proteins. Residues with previously reported regulatory functions and domains with key roles in prosthetic group binding are located as indicated in the sequences. Ubiquitylated lysines, S-nitrosylated cysteines and nitrated or aminated tyrosines are highlighted in green, blue, red and pink, respectively.



**Figure 4.10.** Post-translational modifications identified in planta for NiR1. A, Multiple aligned amino acid sequences of different plant NiRs showing conserved residues highlighted in yellow. The Arabidopsis NiR1 tyrosine (Y), cysteine (C), and lysine (K) residues that were identified as nitrated, aminated, nitrated and aminated, S-nitrosylated and ubiquitylated are shown in white characters on red, magenta, violet, blue and green backgrounds, respectively. B, Amino acid residues that were identified as modified (same colour code than in A) in NiR1. Those highly conserved are highlighted in gray background. C, Scheme showing the NiR1 protein sequence with the position (marked with vertical arrows) of the above mentioned conserved residues that were identified as modified, and the relative position of 4Fe-4S cluster and Siroheme binding sites.



**Figure 4.11. Location of S-nitrosylated cysteines and nitrated/aminated tyrosines in the 3D model of NiR1.** The NiR1 3D structure was modelled in the basis of the 3D structure obtained from spinach feredoxin-nitrate reductase crystals (PDB code 2akj) using the Yasara application (www.yasara.org). The position of the siroheme (red) and 4Fe-4S cluster (orange) cofactors together with the number residue of each modified amino acid are shown in left panel. Right panel shows a close-up image of the cofactor binding pocket.

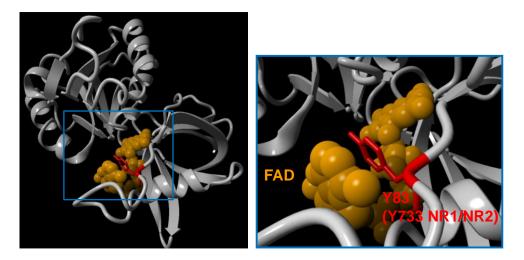


Figure 4.12. 3D modelling of Arabidopsis NRs showing the position of nitrated conserved tyrosine residues close to the FAD binding site. The right panel represents a zoom of the area enclosed by the blue rectangle in the left side image.

### 4.2. <u>NO-triggered transcriptomic and metabolomic responses. Role of</u> <u>hormones in NO sensing</u>

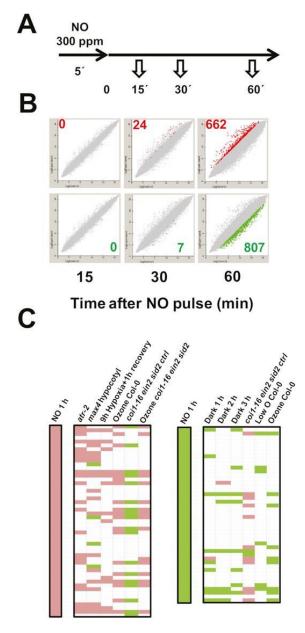
This section 4.2 is composed by excerpts from the research articles: "Castillo, M. C., Coego, A., Costa-Broseta, Á., & León, J. (2018). Nitric oxide responses in Arabidopsis hypocotyls are mediated by diverse phytohormone pathways. Journal of experimental botany, 69(21), 5265-5278", and "León, J., Costa, Á., & Castillo, M. C. (2016). Nitric oxide triggers a transient metabolic reprogramming in Arabidopsis. Scientific Reports, 6, 37945". All the results and figures that appear here are derived from the work of the PhD student in collaboration with the other authors.

#### 4.2.1. Abstract (1)

Plants are often exposed to high levels of nitric oxide (NO) that affects development and stress-triggered responses. However, the way in which plants sense NO is still largely unknown. Here we combine the analysis of early changes in the transcriptome of plants exposed to a short acute pulse of exogenous NO with the identification of transcription factors (TFs) involved in NO sensing. The NO-responsive transcriptome was enriched in hormone homeostasis- and signaling-related genes. To assess events involved in NO sensing in hypocotyls, we used a functional sensing assay based on the NO-induced inhibition of hypocotyl elongation in etiolated seedlings. Hormone-related mutants and the TRANSPLANTA collection of transgenic lines conditionally expressing Arabidopsis TFs were screened for NO-triggered hypocotyl shortening. These approaches allowed the identification of hormone-related TFs, ethylene perception and signaling, strigolactone biosynthesis and signaling, and salicylate production and accumulation that are essential for or modulate hypocotyl NO sensing. Moreover, NO inhibits hypocotyl elongation through the positive and negative regulation of some abscisic acid (ABA) receptors and transcripts encoding brassinosteroid signaling components thereby also implicating these hormones in NO sensing.

### 4.2.2. Overrepresentation of hormone- and oxygen-related regulatory components in the early NO-responsive transcriptome

To unravel the sensing mechanism underlying the early plant responses to NO, we have designed an experimental system based on *A. thaliana* seedlings exposed to a short pulse of pure NO gas. We previously reported an increase in NO-related post-translational modification of proteins and an extensive metabolic rearrangement by 1 h and 6 h after treatment, respectively (León *et al.*, 2016; see next sections 4.2.8-14). These data suggested changes should occur in the time frame between a few minutes and 1 h after NO exposure. Thus, we analyzed



**Figure 4.13. Identification and characterization of the NO-responsive transcriptome.** A, Experimental scheme of sampling. B, Plots representing the up-regulated (red dots) and down-regulated (green dots) genes at the indicated times after exposure to NO. C, AtCAST3.1-based comparison of the up- and down-regulated transcriptomes at 30 min and 1 h after exposure to NO, respectively, with publicly available hormone- and redox-related transcriptome datasets.

changes in the Arabidopsis transcriptome shortly after exposure to an NO pulse for 5 min. Samples were harvested at 15, 30, and 60 min for subsequent transcriptome analyses (Fig. 4.13A). Overall analyses indicated that by 15 min after exposure to NO, no significant change was observed in any gene transcript when compared to those of untreated seedlings (Fig. 4.13B). By 30 min, 24 and 7 genes were significantly up- or down-regulated, respectively (Fig. 4.13B). After that, more extensive changes in transcript levels were detected by 60 min, with ~1500 genes differentially expressed, representing ~5% of the Arabidopsis genome. A total of

662 and 807 genes were up- and down-regulated, respectively (Fig. 4.13B). The complete list of differentially expressed genes including annotation, fold changes, and P-values corrected for FDRs, as well as a full MIAME-description of the transcriptome analyses is shown in Supplementary Table S2 (in Castillo et al., 2018). A PANTHER over-representation test of Gene Ontology analysis, using the Ontology Α. thaliana database of the Gene Consortium (http://www.geneontology.org/), with genes differentially expressed by 1 h after NO exposure allowing identification that the response to chitin, the responses to hormones, particularly to ethylene and jasmonates, as well as the responses to hypoxia functional categories were significantly over-represented (Supplementary Table S3 in Castillo et al., 2018). On the other hand, a comparison of the 50 top up-regulated NO-responsive genes identified here at 1 h after NO with publicly available transcriptome data by using AtCAST3.1 (Kakei & Shimada, 2015) (http://atpbsmd.yokohama-cu.ac.jp/cgi/atcast/search\_input.cgi) showed significant co-regulation profiles with those also up-regulated in *atr-2* (GEO code GSE63355) and max4 (GEO code GSE6151) mutants, as well as with the re-oxygenated plants after hypoxia (Branco-Price et al., 2008) (GEO code GSE9719), or the ozonetreated wild-type and coi1-16ein2sid2ctrl (GEO code GSE65740) plants (Fig. 4.13 C). Also a significant anti-regulation was observed for the NO-responsive transcriptome at 1 h with the transcriptome of the untreated coi1-16ein2sid2ctrl (GEO code GSE65740) mutant (Fig. 4.13C). Genes that were down-regulated by NO showed only a significant co-regulation with the transcriptome of plants under darkness (Fig. 4.13C).

# 4.2.3. A sensing test based on inhibition of hypocotyl elongation allowed identification of hormone-related transcription factors modulating NO sensitivity

Several of the transcriptomes that display partial overlapping with the transcriptome of NO-exposed plants (Fig. 4.13) corresponded to experiments performed with hypocotyl samples, thus suggesting NO-exerted regulation could be relevant in hypocotyls. Etiolated plants exposed to exogenous NO are characterized by root growth arrest and hypocotyl shortening (Fig. 4.14A). The inhibition of hypocotyl elongation was proportional to NO concentrations, with a 50% inhibition after exposure to 300 ppm NO (Fig. 4.14A). We have used this simple and quantitative NO sensing test to screen 968 Arabidopsis TRANSPLANTA transgenic lines (Supplementary Table S4 in Castillo et al., 2018), conditionally expressing 263 TFs under a β-estradiol-inducible promoter (Coego et al., 2014). Different levels of induced expression ranging from 5- to 250-fold were detected upon  $\beta$ -estradiol treatment of transgenic lines, as shown for a randomly selected group (Fig. 4.15). Several independent transgenic lines for each TF were analyzed for hypocotyl length in etiolated untreated (MS) or β-estradiol-induced (MSE) seedlings, or those conditions plus NO treatment, MS+NO and MSE+NO, respectively. Our screen searched for TFs causing either attenuated inhibition

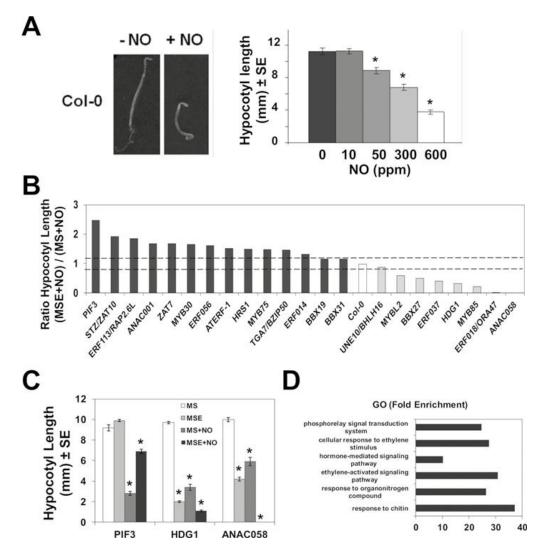


Figure 4.14. Screening of TPT transgenic lines conditionally expressing transcription factor encoding genes through a NO-triggered hypocotyl shortening assay in etiolated seedlings. A, NO triggers a dose-dependent hypocotyl shortening. B, Ratio of the hypocotyl lengths in TPT lines exposed to NO in the absence (MS) or presence of the inducer  $\beta$ -estradiol (MSE). Dashed lines represent the upper and lower variability limits of the ratios of wild type Col-0 hypocotyl lengths. C, Hypocotyl lengths of TPT lines expressing PIF3, HDG1 and ANAC058 transcription factors are altered by  $\beta$ -estradiol treatment even in the absence of NO. D, Gene Ontology (GO) analysis point to a significant enrichment of the hormone and organonitrogen compounds categories among genes triggering significant hypo- or hyper-sensitivity to NO in the screening of TPT lines.

(hyposensitivity) or enhanced inhibition (hypersensitivity) to NO upon conditional  $\beta$ -estradiol-induced expression. The  $\beta$ -estradiol-induced expression of some TFs such as ZAT10 and MYB85 attenuated and potentiated the NO-triggered inhibition of hypocotyl elongation, thus inducing NO hyposensitivity and hypersensitivity, respectively. The ratios between hypocotyl length of  $\beta$ -estradiol-treated and untreated NO-exposed seedlings determined whether the expression of a given TF brings about hyposensitivity or hypersensitivity to NO relative to wild-type Col-0 plants with ratios of ~1 and variability <12% (Fig. 4.14B). Table 4.1 shows the 56

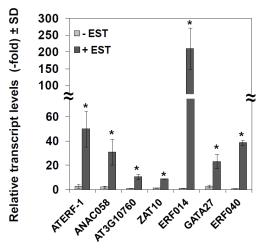


Figure 4.15.  $\beta$ -estradiol induced transcript accumulation in randomly selected TRANSPLANTA transgenic lines. The relative transcript levels were analyzed by RT-qPCR from RNAs obtained from seedlings treated (+EST) or not (-EST) with  $\beta$ -estradiol. Values are normalized to the levels in wild type plant and represent the mean  $\pm$  SD of three independent replicates. \* representing p-values <0.005 in Student's t-test.

lines corresponding to 22 TFs that showed  $\beta$ -estradiol-dependent hyposensitivity or hypersensitivity to NO. As proofs of concept in our screening, the TF inducing the strongest NO hyposensitivity was PIF3, which has already been reported to promote hypocotyl elongation (Feng et al., 2008). Another TF identified, MYB30, has been reported to be functionally related to NO-triggered responses (Tavares et al., 2014). Moreover, this sort of screening also allows identification of TFs that themselves regulate the elongation of the hypocotyl in the absence of NO treatment, such as HDG1 or ANAC058 that induced shortening, or the abovementioned PIF3, which promoted elongation (Fig. 4.14C). Paralleling the effects in the absence of NO, those factors also caused hyposensitivity and hypersensitivity to NO, respectively (Fig. 4.14C). Gene Ontology analysis points to a significant over-representation of the functional categories of hormone signaling pathways, particularly ethylene, among the TFs listed in Table 4.1 (Fig. 4.14D). Among the identified TFs, we found the AP2-related and integrase-type ORA47 and RAP2.6L (Krishnaswamy et al., 2011; Chen et al., 2016), as well as four additional integrasetype ERF TFs, ERF014, ERF037, ERF056, and ERF113/RAP2.6L, and the ethylene-responsive element-binding factor 1 (ATERF-1), all of them related to ethylene signaling. Also TFs that are functionally related to ABA homeostasis or signaling such as the MYB-type HRS1 and MYB30 (Wu et al., 2012; Lee & Seo, 2016) and the NAC058 TF (Coego et al., 2014) were identified in the screening. The functional interaction between NO sensitivity and hormone signaling was not restricted to ABA and ethylene. Among TFs identified in the screening, ORA47 and ZAT10 have also been characterized as positive and negative regulators, respectively, of jasmonic acid (JA) signaling (Pauwels et al., 2008), and MYB30 regulates BR signaling (Li et al., 2009). Finally, PAP1/MYB75 and MYBL2 regulate the biosynthesis of anthocyanins and flavonoids, the latter functioning as a node

between JA and gibberellin (GA) signaling (Xie *et al.*, 2016); and MYB75/PAP1 and MYB85 seems to be also involved in the secondary cell wall formation or thickening, and in the lignification of stems (Zhong *et al.*, 2008; Bhargava *et al.*, 2013), a process that tightly controls the growth of hypocotyls and other plant organs (Hamant & Traas, 2010). These data, together with the Gene Ontology analyses (Supplementary Table S3 in Castillo *et al.*, 2018) and the co-regulation of transcriptomes (Fig. 4.13) shown above, strongly suggest a determinant involvement of hormone biosynthesis and signaling in NO sensing.

AGI code	Annotation	TPT lines	NO sensitivity
AT1G09530	PIF3_ phytochrome interacting factor 3	1.09530.1F4	Hyposensitive
AT4G17500	ATERF-1_ ethylene responsive element binding factor 1	4.17500.1B3, E2, I7	Hyposensitive
AT1G01010	ANAC001_NAC domain containing protein 1	1.01010.1E5, F9, G3	Hyposensitive
AT1G13300	HRS1myb-like transcription factor family protein	1.13300.1A9, D3, E5	Hyposensitive
AT1G56650	MYB75_ PAP1_ production of anthocyanin pigment 1	1.56650.1C5, H3	Hyposensitive
AT3G28910	MYB30_ myb domain protein 30	3.28910.1C1, E5	Hyposensitive
AT5G13330	Rap2.6Lrelated to AP2 6I	5.13330.1D9, G9, I4	Hyposensitive
AT2G22200	ERF056_Integrase-type DNA-binding superfamily protein	2.22200.1B1, E7, G8	Hyposensitive
AT1G44830	ERF014_Integrase-type DNA-binding superfamily protein	1.44830.1A99, F99, G99	Hyposensitive
AT3G46090	ZAT7C2H2 and C2HC zinc fingers superfamily protein	3.46090.1B99,E99,H99,I99	Hyposensitive
AT1G27730	STZ_ZAT10salt tolerance zinc finger	1.27730.1E5, I1	Hyposensitive
AT3G21890	BBX31_B-box type zinc finger family protein	3.21890.1A8, B5, H8	Hyposensitive
AT4G38960	BBX19_B-box type zinc finger family protein	4.38960.1A3, G3	Hyposensitive
AT1G77920	TGA7_bZIP transcription factor family protein	1.77920.1B3, E1, G3, H2	Hyposensitive
AT3G18400	ANAC058_NAC domain containing protein 58	3.18400.1D3, G9	Hypersensitive
AT1G71030	MYBL2_ MYB-like 2	1.71030.1C5, H9	Hypersensitive
AT4G22680	MYB85_ myb domain protein 85	4.22680.1F1	Hypersensitive
AT1G77200	ERF037_Integrase-type DNA-binding superfamily protein	1.77200.1C99, H99	Hypersensitive
AT1G74930	ERF018/ORA47_Integrase-type DNA-binding protein	1.74930.1E2, F8, H4	Hypersensitive
AT2G30250	WRKY25_WRKY DNA-binding protein 25	2.30250.1C5, D2, F2	Hypersensitive
AT1G68190	BBX27_B-box zinc finger family protein	1.68190.1D1	Hypersensitive

Table 4.1. TPT lines showing hypo- or hyper-sensitivity to NO on hypocotyl elongation of etiolated seedlings upon conditional expression of TF encoding genes

# 4.2.4. Ethylene perception and signaling as well as salicylate and strigolactone biosynthesis are required for NO sensing

The transcriptome analyses of NO-exposed seedlings and the screening of transgenic plants conditionally expressing TFs suggested the involvement of ethylene signaling in NO-triggered responses. To check whether ethylene perception and signaling are involved in sensing NO, we tested the sensitivity to NO in hypocotyl shortening assays with the ethylene-insensitive *etr1-3* and *ein2-5* mutants (Roman *et al.*, 1995). Figure 4.16A shows that *etr1-3* and *ein2-5* seedlings

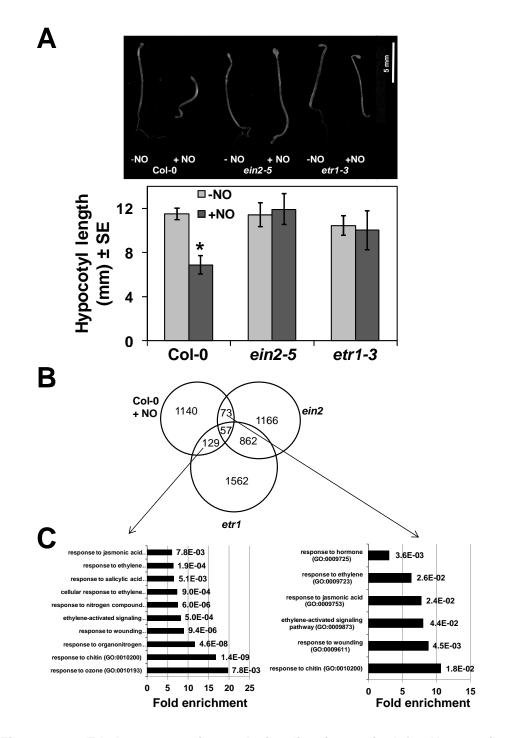


Figure 4.16. Ethylene perception and signaling is required for NO sensing. A, Hypocotyl length of untreated (-NO) and NO-treated (+NO) wild type and ethylene signalingrelated mutant seedlings. Values are the mean (n=25)  $\pm$  standard error with \* representing pvalues <0.05 in Student's t-test. B, Venn diagrams representing the intersection between the NO-responsive transcriptome and those differentially regulated in *etr1* and *ein2* mutants. C, Gene Ontology analysis of the genes found in the intersection between the NO-responsive and *etr1*regulated (left panel) or *ein2*-regulated (right panel) transcriptomes. Fold enrichment (black bars) and the FDR-corrected p-values (at the right side of bars) for the functional categories are shown. were fully insensitive to NO in inhibiting hypocotyl elongation, thus suggesting that ethylene perception and signaling were essential for NO sensing. The comparison of the NO-responsive transcriptome with the differential transcriptomes previously reported for the ein2-1 and etr1-1 mutants (Cheng et al., 2009) (GEO Accession GSE12715) points to a significant overlap (Fig. 4.16B). There were 57 genes that were common for the three transcriptomes and an additional 73 and 129 genes in the intersections of the NO-responsive genes and ein2-1, and the NO-responsive genes and etr1-1, respectively (Fig. 4.16B), thus supporting a potential relevant involvement of ETR1 and EIN2 in NO sensing. We can rule out that the involvement of ETR1 and EIN2 in NO sensing was due to transcriptional regulation of the corresponding genes by NO, as only slight increases below 1.6-fold in the corresponding transcripts were detected in NO-treated plants (Fig. 4.17). A Gene Ontology analysis with these groups of commonly regulated genes yielded a significant over-representation of the expected functional categories related to ethylene responses but, of note, also of responses to JA- and salicylic acid (SA)related functional categories (Fig. 4.16C).

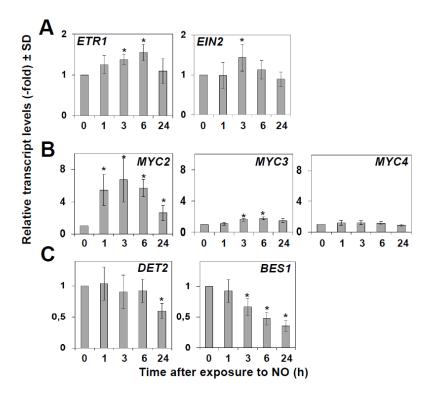


Figure 4.17. Effect of NO treatment on the transcript levels of A, ethylene, B, jasmonate and C, brassinosteroid biosynthetic/or signaling encoding genes. The relative transcript levels were analyzed by RT-qPCR from RNAs obtained at the indicated times after NO exposure of seedlings. Values are the mean  $\pm$  SD of three independent replicates. \* representing p-values <0.05 in Student's t-test.

Among genes that were similarly regulated by NO treatment in wild-type plants or in untreated ethylene-insensitive mutants (Fig. 4.16B), we found some that participate in SA-triggered responses. SA is synthesized in Arabidopsis mostly through the isochorismate pathway involving the function of the chloroplast transporter EDS5/SID1 and the isochrorismate synthase 1 (ICS1)/SID2 and ICS2 proteins (Serino *et al.*, 1995; Wildermuth *et al.*, 2001; Garcion *et al.*, 2008). We have tested the sensitivity to NO of SA-deficient *sid2-1eds5-3nahG* plants, which overexpress the *nahG* bacterial gene coding for a SA hydroxylase converting SA to catechol (Gaffney *et al.*, 1993; Delaney *et al.*, 1994) in a genetic background carrying mutations in EDS5/SID1 and ICS1/SID2 genes. Figure 4.18A shows that, in contrast to wild-type plants, the hypocotyls of etiolated *sid2-1eds5-3nahG* plants were not shortened upon exposure to NO, thus suggesting that the biosynthesis and accumulation of SA is essential for NO sensing in hypocotyls.

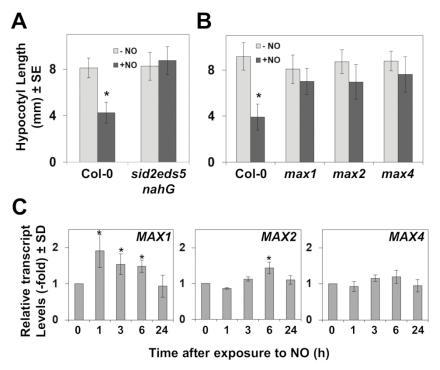


Figure 4.18. Involvement of salicylates and strigolactones in NO sensing. A, Hypocotyl length of untreated (–NO) and NO-treated (+NO) wild-type and salicylate-deficient mutant seedlings; and B, wild-type and strigolactone biosynthesis and signaling mutants. Values are the mean  $\pm$ SE (*n*=25). C, Effect of NO treatment on the transcript levels of strigolactone biosynthetic/or signaling-encoding genes. The relative transcript levels were analyzed by RT-qPCR from RNAs obtained at the indicated times after NO exposure of seedlings. Values are the mean  $\pm$ SD of three independent replicates. \**P*-values <0.05 in Student's *t*-test.

On the other hand, we found a significant co-regulation between up-regulated genes in NO-treated wild-type plants and in the SL biosynthesis *max4* mutant (Fig. 4.13C). SLs are synthesized from the diterpene all-*trans*- $\beta$ -carotene by the sequential catalysis of D27, MAX3, MAX4, and MAX1, and then SLs are perceived by the D14 receptor, which interacts with the E3 ubiquitin ligase MAX2 that polyubiquitylates negative regulators of the SMXL family and sends them for proteasome-mediated degradation (Morffy *et al.*, 2016). We have analyzed whether SLs may be relevant for sensing NO by testing NO-triggered hypocotyl

shortening with the biosynthetic and signaling *max1*, *max2*, and *max4* mutants (Sorefan *et al.*, 2003; Booker *et al.*, 2005; Stirnberg *et al.*, 2007). Figure 4.18B shows that all three SL mutants were insensitive to NO, thus indicating that the NO-triggered inhibition of hypocotyl elongation required the biosynthesis and signaling of SLs, and thus the involvement of these hormones in NO sensing. Only the *MAX1* gene was significantly, although moderately, up-regulated upon exposure to NO (Fig. 4.18C), thus suggesting that the transcriptional activation of SL biosynthetic and signaling genes was not the limiting step in the SL-mediated NO response mechanism.

### 4.2.5. NO sensing and ABA signalling

We have previously reported that NO antagonizes ABA in regulating multiple processes from seed germination and seedling establishment to abiotic stress responses (Lozano-Juste & León, 2010; León et al., 2014). More precisely, the negative effect of NO on ABA perception is mediated, at least in part, by the posttranslational Y-nitration and the subsequent inactivation and degradation of PYR/PYL receptors (Castillo et al., 2015). Other positive regulators of the ABA core signaling pathway, such as the kinase OST1/SnRK2.6, were reported to be inactivated through post-translational S-nitrosylation of key C residues (Wang et al., 2015b; Wang et al., 2015c). Despite NO exerting regulation on ABA signaling at the post-translational level, our data suggest that NO also regulates ABA signaling at the transcriptional level. The ABA hypersensitivity detected in the NOdeficient nia1,2noa1-2 mutant plants (Lozano-Juste & León, 2010) correlated well with a significant over-representation of ABA-related genes among up-regulated genes in nia1,2noa1-2 plants (Gibbs et al., 2014a). Finally, further support for the involvement of ABA signaling in NO-triggered responses comes from the identification of several TFs related to ABA signaling in our screening of NOtriggered shortening of hypocotyls in transgenic conditional overexpressing lines (Fig. 4.14B; Table 4.1). Those include BBX31, which is one of the ABA-specific marker genes, as well as the ABA-up-regulated STZ/ZAT10, PIF3, ERF056, and RAP2.6L genes and the down-regulated MYB30 and HRS1 genes (Nemhauser et al., 2006). Because ABA perception and signaling involve multicomponent families of receptors and regulators, we decided to check first whether a specific NOregulated ABA signaling pathway might exist in Arabidopsis. To test this hypothesis, the transcript levels of genes coding for core components of the ABA signaling pathway were analyzed by RT-qPCR at different times after exposure to NO. Figure 4.19A shows a transient up-regulation of several ABA receptorencoding genes by 1-6 h after exposure to NO. PYL3, PYL6, and PYL7 were strongly up-regulated, whereas others, such as PYL4, PYL5, and PYL8, were moderately up-regulated (Fig. 4.19A). Only PYR1 was significantly down-regulated (Fig. 4.19A). In the next step of the signaling pathway, members of the clade A of type 2C phosphatases (PP2Cs) act as negative regulators of ABA signaling. Although most of the genes coding for ABA-related phosphatases, with the

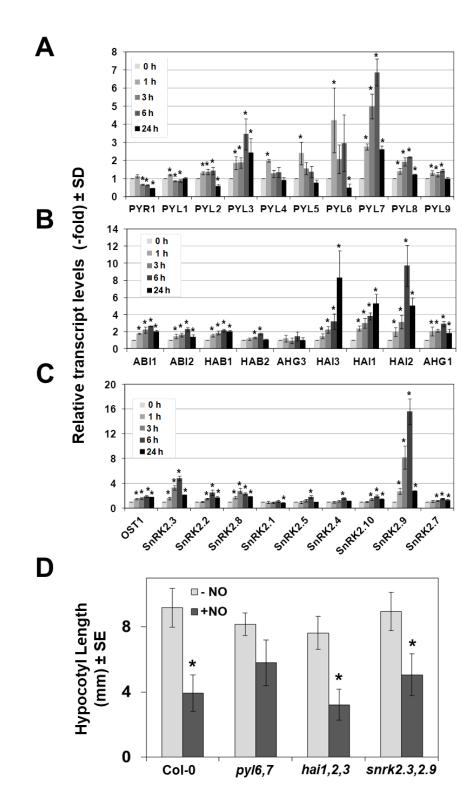


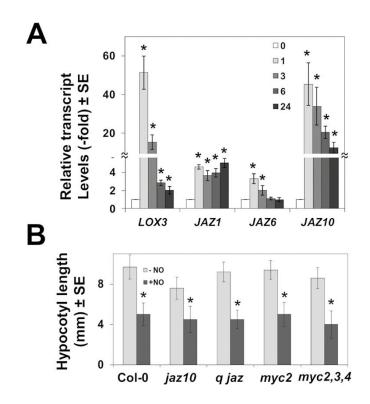
Figure 4.19. Involvement of ABA signaling in NO sensing. The relative transcript levels of A, ABA receptor; B, clade A of protein 2C phosphatase; and C, SnRK2 family of protein kinase encoding genes, were analyzed by RT-qPCR from RNAs obtained at the indicated times (hours) after NO exposure of seedlings. Values are the mean  $\pm$  SD of three independent replicates. D, Hypocotyl length of untreated control (-NO) and NO-treated (+NO) wild type and ABA signaling mutant seedlings. Values are the mean (n=25)  $\pm$  standard error with \* representing p-values <0.05 in Student's t-test.

exception of HAB2 and AHG3/PP2CA, were slightly up-regulated in NO-treated plants, only those from the highly ABA-induced subfamily (HAI1, HAI2/AIP1, and HAI3) were strongly up-regulated (>4-fold) by 6-24 h after NO treatment (Fig. 4.19B). Finally, the next step in ABA signaling is carried out by the positive regulation exerted by protein kinases of the Sucrose non-fermenting 1-Related protein Kinase 2 (SnRK2) family. Among SnRK2-encoding genes, only SnRK2.9 and to a lesser extent SnRK2.3 were up-regulated upon NO treatment (Fig. 4.19C). From these data, we identified a subset of NO-responsive ABA signaling components including the receptors PYL6, PYL7, and PYL3, the phosphatases HAI1, HAI2/AIP1, and HAI3, and the SnRK2.9 and SnRK2.3 kinases. To check whether these NO-responsive ABA signaling-encoding genes were involved in regulating the plant sensitivity to NO, we tested the effect of exogenously supplied NO on the elongation of hypocotyls from etiolated seedlings of ABA signaling mutant combinations in the NO-responsive genes identified. We generated double pyl6,7 and snrk2.3,2.9 mutants, and, together with the available hai1,2,3 mutant (Bhaskara et al., 2012), these were analyzed in hypocotyl shortening assays. Figure 4.19D shows that hai1,2,3 and snrk2.3,2.9 mutants showed a hypocotyl shortening not significantly different from that detected in wild-type plants, and only the hypocotyls of *pyl6*,7 plants were significantly insensitive to NO.

### 4.2.6. NO sensing and jasmonate signaling

Three independent transcriptome analysis-based lines of evidence suggest that JA may be important for NO sensing mechanisms. We found a significant overrepresentation of the JA signaling categories among NO-responsive genes (Supplementary Table S3 in Castillo et al., 2018) but also among the particular set of genes that were also differentially expressed in ethylene signaling-deficient mutants (Fig. 4.16C). Moreover, we found a significant anti-regulation of NOresponsive genes in plants affected in JA perception and signaling (Fig. 4.13C, D). Among NO-responsive genes listed in Supplementary Table S2 (in Castillo et al., 2018), we found that the lipoxygenase-encoding genes LOX3 and LOX4, the 12oxophytodienoate reductase-encoding gene OPR1, and the allene oxide cyclaseencoding gene AOC3 were all >2-fold up-regulated. Similarly, JAZ1, JAZ5, JAZ8, and JAZ10 genes coding for different components of the JAZ family of negative regulators of JA signaling were also up-regulated (Supplementary Table S2 in Castillo et al., 2018). Interestingly enough, the gene JMT coding for the JA carboxyl methyltransferase, which metabolizes JA to methyl-JA, was strongly downregulated (Supplementary Table S2 in Castillo et al., 2018). Together, these data suggest the existence of NO-sensitive branches of the JA biosynthetic and signaling pathways. We have confirmed that some JA biosynthetic and signaling genes were up-regulated by NO, thus supporting that the NO-responsive transcriptome identified in the microarray analyses is truly representative. Figure 4.20A shows that LOX3 and JAZ10 were strongly up-regulated (>40-fold), whereas JAZ1 and JAZ6 were also up-regulated though more slightly (3- to 4-fold) by 1 h

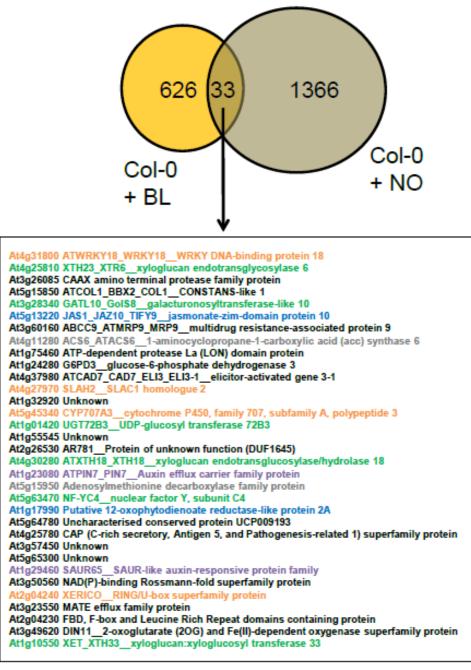
after NO treatment. To explore whether NO-regulated components of the JA signaling pathway modulate NO sensitivity, the response to exogenously supplied NO of JA signaling (*jaz10*, quintuple *jaz1,3,4,9,10*, the single *myc2*, and the triple *myc2,3,4*) mutants was assayed in hypocotyl shortening assays. Figure 4.20B shows that none of them was significantly different in sensitivity to NO-triggered hypocotyl shortening when compared with wild-type plants. We also checked whether the levels of *MYC* transcripts were regulated in NO-treated plants as shown above for some of the *JAZ* genes (Fig. 4.20A). *MYC2* was strongly upregulated upon exposure to NO, whereas *MYC3* was only slightly up-regulated and *MYC4* was not significantly altered (Fig. 4.17B). These findings together suggest that despite the fact that many of the JA signaling component-encoding genes are regulated by NO, this hormone is not involved in NO sensing.



**Figure. 4.20. Involvement of jasmonate signaling in NO sensing.** A, Relative transcript levels of indicated genes were analyzed by RT-qPCR from RNAs obtained at the indicated times (hours) after NO exposure of seedlings. Values are the mean  $\pm$  SD of three independent replicates. B, Hypocotyl length of untreated (-NO) and NO-treated (+NO) wild type and jasmonate signaling-related mutant seedlings. *q jaz* stands for the quintuple *jaz1,3,5,9,10* mutant. Values are the mean (n=25)  $\pm$  standard error with \* representing p-values <0.05 in Student's t-test

#### 4.2.7. Involvement of brassinostereoids in NO sensing

Among genes that were up-regulated by NO at 1 h (Supplementary Table S2 in Castillo *et al.*, 2018), we found a significant overlap (Fig. 4.21) with those that were also up-regulated in responses to BRs (Nemhauser *et al.*, 2006). We also



Involved in ABA signaling, ethylene biosynthesis, auxin signaling, jasmonate signaling, cell wall loosening and cell elongation besides NO- and BR-triggered responses

Figure 4.21. Genes up-regulated by either brassinolide (BL) or NO treatments. The color codes corresponded to the functional categories outlined at the end of the table.

found several TF-encoding genes involved in BR-regulated processes. BRs are synthesized from campesterol through a complex pathway involving 11 steps (Fig. 4.22A). The second step catalyzed by DET2 seems to be determinant for BR biosynthesis, in such a way that *det2* mutant plants are severely impaired in BR production (Fujioka *et al.*, 1997; Noguchi *et al.*, 1999). Once the active BR

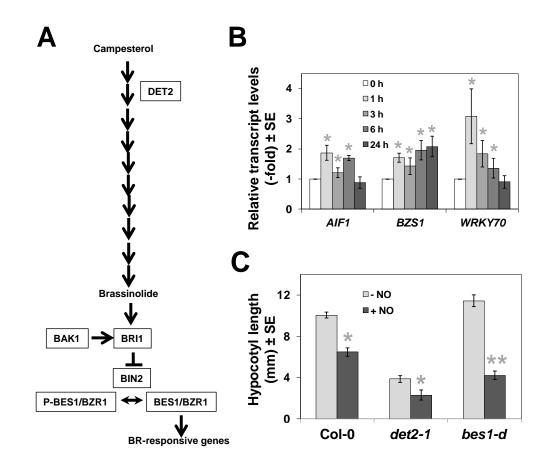


Figure 4.22. Involvement of brassinosteroid signaling in NO sensing. A, Scheme of brassinosteroid biosynthesis and signaling pathways. B, The relative transcript levels of BR-related transcription factor encoding genes were analyzed by RT-qPCR from RNAs obtained at the indicated times (hours) after NO exposure of seedlings. Values are the mean  $\pm$  SD of three independent replicates. \* represents p-values <0.05 in Student's t-test. C, Hypocotyl length of untreated (-NO) and NO-treated (+NO) wild type and brassinosteroid biosynthesis and signaling mutant seedlings. Values are the mean (n=25)  $\pm$  standard error. \* and \*\* represent p-values <0.05 and <0.01, respectively, in Student's t-test.

brassinolide is synthesized, it is further perceived by the BRI1 receptor (Jiang *et al.*, 2013), which is controlled by reversible phosphorylation by BAK1 (He *et al.*, 2013). Then, BRI1 negatively regulates BIN2, which in turn modulates the phosphorylation state of the transcriptional activators BES1 and BZR1 that accumulate in the nucleus (Yin *et al.*, 2002), triggering BR-responsive gene expression (Fig. 4.22A). We have confirmed that *AIF1*, *BZS1*, and *WRKY70*, which are involved in BR-regulated control of plant growth and responses to stress (He *et al.*, 2005; Wang *et al.*, 2009; Chen *et al.*, 2017), were up-regulated by NO to different extents (Fig. 4.22B), thus pointing to BR signaling as a target of NO action. To check whether BR biosynthesis was necessary for NO sensing in hypocotyls, we analyzed the loss-of-function *det2-1* mutant in DE-ETIOLATED2 that is severely BR deficient and dwarf (Chory, 1991). The hypocotyls of etiolated *det2-1* seedlings, despite being short, were still fully sensitive to NO in inhibiting hypocotyl

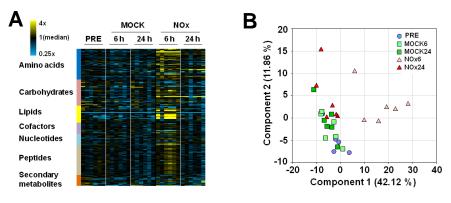
elongation, thus indicating that biosynthesis of BRs is not required for NO sensing. Moreover, the dominant gain-of-function *bes1-D* mutant in BRI1-EMS-SUPPRESSOR 1 (BES1)/BRASSINAZOLE-RESISTANT 2 (BZR2) (Shin *et al.*, 2016), despite displaying longer hypocotyls in non-treated seedlings, was hypersensitive to NO in shortening their hypocotyls (Fig. 4.22C), thus suggesting that BES1-mediated BR signaling potentiates NO sensing. It is worth mentioning that in accordance with BES1 being an NO target, the *BES1* gene was strongly down-regulated by NO (Fig. 4.17C), thus representing a potential node for selfcontrolled sensing.

### 4.2.8. Abstract (2)

Nitric oxide (NO) regulates plant growth and development as well as responses to stress that enhanced its endogenous production. Arabidopsis plants exposed to a pulse of exogenous NO gas were used for untargeted global metabolomic analyses thus allowing the identification of metabolic processes affected by NO. At early time points after treatment, NO scavenged superoxide anion and induced the nitration and the S-nitrosylation of proteins. These events preceded an extensive though transient metabolic reprogramming at 6 h after NO treatment, which included enhanced levels of lipid catabolism and accumulation of polyamines, phospholipids, chlorophyll breakdown, protein and nucleic acid turnover and increased content of sugars. Accordingly, lipid-related structures such as root cell membranes and leaf cuticle altered their permeability upon NO treatment. Besides, NO-treated plants displayed degradation of starch granules, which is consistent with the increased sugar content observed in the metabolomic survey. The metabolic profile was restored to baseline levels at 24h posttreatment, thus pointing up the extraordinary plasticity of plant metabolism in response to nitroxidative stress conditions.

# 4.2.9. Metabolomic analyses reflect a transient reprogramming response to exogenous NO

Arabidopsis thaliana seedling exposed to a pulse of NO gas were used as model for mimicking an acute exposure of plants to a peak in environmental levels of NO or to intracellular accumulation of large amounts of NO in the production foci in plants under stressful conditions. Seedlings were exposed to a single dose of 300 ppm NO for 5 min and then, samples were harvested for untargeted metabolomic analyses comprising a total of 232 named biochemicals. Similar analyses were also performed in mock treated plants. Samples were collected at the time of the treatment (PRE), and then at 6 h and 24 h after exposure to NO or mock treatment (Fig. 4.23A). Plants exposed to NO underwent a transient though remarkable metabolic alteration by 6 h after treatment. A statistically significant increase in the amount of lipid, carbohydrate, amino acid and nucleotide categories of analyzed metabolites was detected in plants by 6 h after exposure to NO (Fig. 4.23A). By 24 h, the metabolomic status of treated plants was restored to that of untreated plants (Fig. 4.23A). Supplementary Table S1 (can be found in León et al., 2016) includes the complete dataset with pathway heat maps and box and line plots for every analyzed metabolite showing the comparative fold- levels for every metabolite in the different samples as well as the statistical significance for the different comparisons. Only a few metabolites showed a significant difference when PRE and mock 6 h samples were compared, thus indicating only a small proportion of the metabolites analyzed were potentially regulated by circadianrelated events in the experiment (Fig. 4.23A and Supplementary Table S1 in León et al., 2016). By contrast, around 70% of the metabolites showed significant alteration in their endogenous levels by 6 h after exposure to NO (Fig. 4.23A and Supplementary Table S1 in León et al., 2016). Significantly lower number of changes were detected in samples collected at 24 h after exposure (Fig. 4.23A and Supplementary Table S1 in León et al., 2016), thus suggesting NO treatment has an extensive though transient metabolic effect on Arabidopsis plants. The Principal Component Analysis (PCA) confirms this general pattern. The PRE and MOCK 6 h or 24 h samples did not separate well, the NOx 24 h samples had only partial separation, and indeed the NOx 6 h samples were all clearly separated from this grouping (Fig. 4.23B). Moreover, to assess the compounds which meet the statistical criteria for significant differentiation between the analyzed samples, we applied two types of tests to compare the five experimental groups. The first approach was to compare each treated or mock-treated group to the PRE samples, using Welch's Two Sample t-Test. This approach yields information on the effect of time parameter on the experiment. A summary of the numbers of biochemicals that achieved statistical significance ( $p \le 0.05$ ), as well as those approaching significance (0.05<p<0.1), is shown in Table 4.2. One would expect approximately 10-15 compounds to reach significance by chance alone with 232 compounds tested. In these comparisons the mock samples showed only 12 or 24 compounds



**Figure 4.23. Metabolomic Changes after Applying an NO Pulse to Arabidopsis Plants.** A, Heat map showing the increased (yellow) or decreased (blue) concentration of metabolites at the indicated times after treatment of plants (NOx) or untreated (MOCK) control. B, Principle Component Analysis (PCA) plot. Blue circles represent the samples before treatment, green squares the mock-treated samples and red triangles to NO treated samples at the indicated times after pulse.

at 6 h and 24 h, respectively, which were significant (Table 4.2), thus suggesting the mock treatment had little effect on the metabolome. By contrast, the NOx treatment resulted in 133 compounds being significant at the 6 h time point, and 55 at 24 h (Table 4.2), consistent with a robust experimental effect. To eliminate any diurnal effects in the data, we used an ANOVA Contrast test to compare the NOx to MOCK plants at each time point individually, and to score the time-related differences within each treatment pair. Again, the results were quite consistent with the observations taken from the heat map (Fig. 4.23A), PCA (Fig. 4.23B), and t-test results (Table 4.2). There were 154 compounds meeting the cut-off when comparing NOx 6 h to MOCK 6 h, but only 29 when compared at the 24 h time point (Table 4.2 and Supplementary Table S1 in León *et al.*, 2016), consistent with the idea that plants experienced a transient and acute response to NO exposure, and then, most metabolites returned to baseline levels after 24 h.

Total	Metabolites	Metabolites	Total	Metabolites	Metabolites
number of	up	down	number of	up	down
metabolites	p≤0.05	p≤0.05	metabolites	0.05 <p<0.1< td=""><td>0.05<p<0.1< td=""></p<0.1<></td></p<0.1<>	0.05 <p<0.1< td=""></p<0.1<>
p≤0.05			0.05 <p<0.1< td=""><td></td><td></td></p<0.1<>		
40	4	44	47	4	40
	-			-	16
24	2	22	22	4	18
133	114	19	15	10	5
55	17	38	29	7	22
154	138	16	14	9	5
29	18	11	17	7	10
27	16	11	17	6	11
155	12	143	9	2	7
	p≤0.05 12 24 133 55 154 29 27	metabolites         p≤0.05           12         1           24         2           133         114           55         17           154         138           29         18           27         16	metabolites $p \le 0.05$ $p \le 0.05$ 12       1       11         24       2       22         133       114       19         55       17       38         154       138         154       138       16         29       18       11         27       16       11	metabolites $p \le 0.05$ $p \le 0.05$ metabolites $0.05 121111724222221331141915551738291541541381614191727161117$	metabolites $p \le 0.05$ $p \le 0.05$ metabolites $0.05 0.05 121111712422222413311419151055173829715413816149291811177271611176$

Table 4.2. Statistica	l comparisons in the	e metabolomic analyses
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PRE, Pre-treatment; MOCK6, Mock-treated; harvested after 6 h; MOCK24, Mock-treated harvested after 24 h; NOx6, Nitric oxide treatment harvested after 6 h; NOx24, Nitric oxide treatment harvested after 24 h. n=6 biological independent replicates.

# 4.2.10. Altered levels of amino acids and dipeptides suggests NO treatment increased protein breakdown

The elevated content of proteinogenic amino acids and dipeptides in the NOx 6 h compared to the MOCK 6 h or PRE samples (Supplementary Table S1 in León

et al., 2016) strongly suggest NO is inducing protein degradation. The exceptions to this pattern were amino acids that serve important metabolic functions in addition to protein synthesis. This includes glutamate, glutamine, and arginine, which are involved in nitrogen trafficking and storage, and glycine and serine, which are utilized in the photorespiration reactions, which endogenous content did not change significantly by NO treatment (Supplementary Table S1 in León et al., 2016). The branched chain amino acids, leucine, isoleucine, and valine are typically at relatively low levels in cells, and function almost exclusively in protein synthesis, so an increase in their levels often indicates net protein turnover. These compounds, as well as the aromatic amino acids were more than twice the levels in NOx 6 h samples relative to MOCK 6 h (Supplementary Table S1 in León et al., 2016). The aromatic amino acids are involved in other important pathways, such as the large phenylpropanoid family pathways (phenylalanine), tocopherol production (tyrosine), and hormone and glucosinolate synthesis (tryptophan), which were relatively unchanged or even dropped by NOx treatment (Supplementary Table S1 in León et al., 2016). Moreover, the content of a common precursor of aromatic amino acid biosynthesis such as shikimate was lower in NOtreated relative to mock-treated plants at both 6 and 24 h time points (Supplementary Table S1 in León et al., 2016), thus suggesting either the increased levels of aromatic amino acids caused a feed-back inhibition on the biosynthetic pathway or, alternatively, their increased levels are the result of active protein turnover.

We have observed that by 30 min after exposure to NO the nitroblue tetrazolium staining of superoxide anion in Arabidopsis seedlings was significantly reduced, thus suggesting NO efficiently scavenged superoxide (Fig. 4.24A). Peroxynitrite, a potent nitrating agent, is formed upon superoxide scavenging by NO, thus suggesting nitration of target proteins may occur in plants exposed to NO. We have analyzed the total and NO-related modified protein profile in plants after the NO pulse. No general changes were observed in the total protein pattern detected after monodimensional electrophoresis (Fig. 4.24B). Exogenous NO enables the modification of proteins through S-nitrosylation or nitration of cysteine and tyrosine residues, respectively (Astier & Lindermayr, 2012). We found that many nitrated and S-nitrosylated proteins started to accumulate by 1 h and reached maximum accumulation between 3 and 6 h after exposure of plants to NO (Fig. 4.24B).

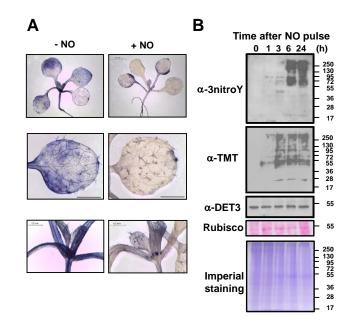
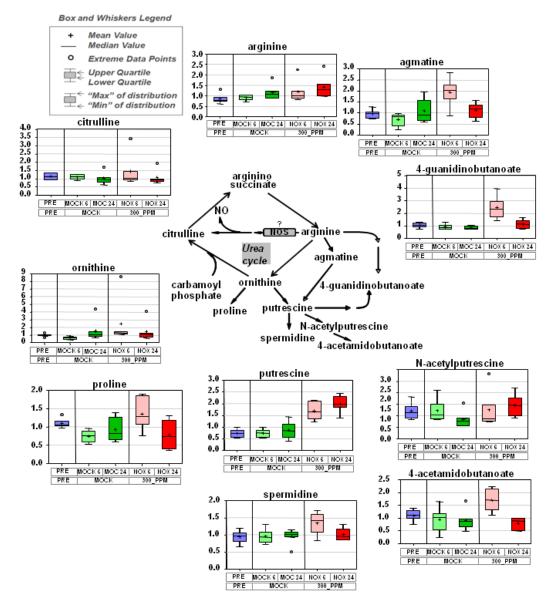


Figure 4.24. Superoxide content reduction and post-translational modifications induced by NO. A, Control untreated seedlings (-NO) and NO-exposed seedlings (+ NO) were stained with Nitroblue tetrazolium from 30 min to 20 h after treatment. B, Nitration and S-nitrosyltion of proteins in NO-treated plants. Each lane was loaded with 10  $\mu$ g of proteins. The marks and numbers at the right side of panels indicate the position of molecular weight markers in kDa. The general pattern of protein is shown by staining of a replicate gel with Imperial (Thermo Scientific). Blot replicates were probed with the antibodies indicated at the left side of panels.  $\alpha$ 3-nitroY, anti-3-nitrated-Y;  $\alpha$ TMT, anti-Tandem Mass Tagged-S-nitrosylated proteins. Equal loading was checked by Coomassie-stained large subunit of Ribulose-1,5-Bisphosphate Carboxylase / Oxygenase (Rubisco) and by using  $\alpha$ DET3, anti-De-Etiolated 3. Data shown are representative of three independent experiments with similar results.

### 4.2.11. Acute exposure to NO triggers the accumulation of polyamines and responses to oxidative stress

Plants exposed to NO also showed specific effects on  $\alpha$ -ketoglutarate-derived amino acids of the glutamate family. The levels of  $\gamma$ -aminobutyrate (GABA) and 2aminobutyrate were slightly increased, and the increase was higher (4.3-fold) for  $\gamma$ -hydroxybutyrate (GBH) by 6 h after exposure to NO (Supplementary Table S1 in León *et al.*, 2016). It has been reported that the metabolism of GABA leads to accumulation of GBH when plants are exposed to low oxygen or high light conditions, and also that under those stress conditions accumulation of GABA and alanine was also detected (Allan *et al.*, 2008), which is in agreement with our data (Supplementary Table S1 in León *et al.*, 2016). Also in the glutamate family of amino acids, the levels of proline and ornithine were 1.8- and 4-fold increased, respectively, and also their corresponding N-acetyl derivatives were 1,7- and 5.8fold augmented, respectively (Supplementary Table S1 in León *et al.*, 2016). The increased levels of proline and ornithine were also accompanied by significant increases in the content of polyamines such as putrescine (2.3-fold) and spermidine (1.4-fold) as well as the precursor agmatine (2.8-fold) in NO-exposed plants after 6 h (Fig. 4.25 and Supplementary Table S1 in León *et al.*, 2016).

Cysteine is an unique example where active synthesis of an amino acid was evident in response to NO treatment. It is formed from serine in two steps, through the O-acetylation of serine by acetyl-CoA to form O-acetylserine, followed by the incorporation of hydrogen sulfide, displacing the acetyl group (Romero *et al.*, 2014). Cysteine synthesis is often the rate limiting step for the formation of glutathione, a critical compound in cellular redox responses (Noctor *et al.*, 2012). We found an increase above 2-fold in all these compounds in NOx 6 h plants. O-

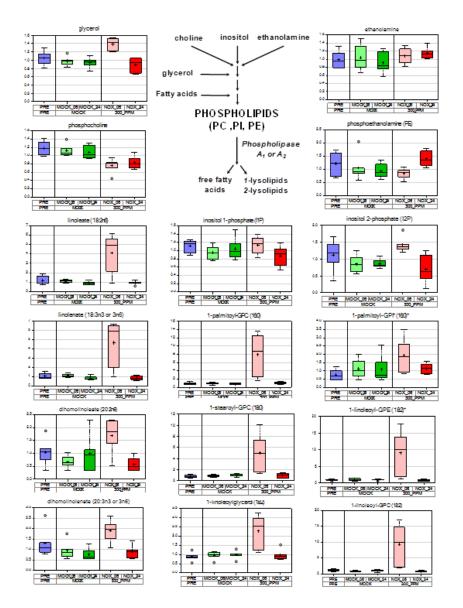


**Figure 4.25. Enhanced polyamine content in plants exposed to NO.** Scheme showing the biosynthesis of polyamines derived from the urea cycle and the box plots for every metabolite before treatment (blue) and at indicated times after mock- (green) or NO-treatment (red). Light and dark tones correspond to 6 h and 24 h, respectively, after treatment.

acetylserine and the non-enzymatically rearranged N-acetylserine were 17- and 11-fold increased, respectively (Supplementary Table S1 in León et al., 2016), thus suggesting the exposure to NO triggered a strong demand for cysteine production as a mean to remediate oxidative stress. In agreement with that, perturbations of several other oxidative markers, namely increased of oxylipins 13-HODE, 9-HODE and 9,10-hydroxyoctadec-12(Z)-enoic acid produced through the LOX pathway (Feussner & Wasternack, 2001), were detected. Also consistent with NO-treated plants undergoing oxidative stress, the endogenous content of threonate, which is a downstream product of ascorbate metabolism (Green & Frv. 2005), were more than 4-fold higher in NO-treated plants by 6 h, and also a moderate increase in both oxidized and reduced glutathione content was detected (Supplementary Table S1 in León et al., 2016). By contrast, the content of  $\alpha$ -tocopherol, which together with glutathione and ascorbate constitute the triad of main antioxidants in plants undergoing oxidative stress (Szarka et al., 2012), were around 50% lower by 6 h after NO treatment (Supplementary Table S1 in León et al., 2016). These data together suggest that NO-exposed plants respond to oxidative stress through a complex process.

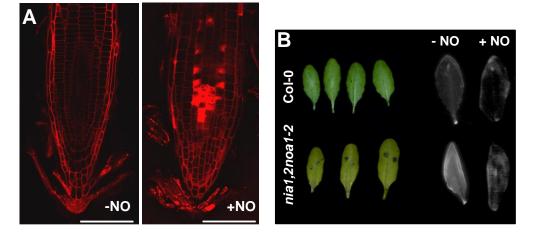
## 4.2.12. Altered lipidome reflects changes in lipidic structures such as membranes and cuticle in NO-exposed plants

The content of compounds belonging to the lipid category underwent large changes in NO-treated plants. Alterations were strictly limited to the six hour time point (Fig. 4.26). A significant increase in the free polyunsaturated fatty acids (PUFAs) content of around 4-fold for linoleate and linolenate, and around 2-fold increase for the C20 PUFAs dihomolinoleate and dihomolinolenate was detected (Supplementary Table S1 in León et al., 2016 and Fig. 4.26). We also found a strong increase in several lyso-lipids such as 1-palmitoyl-GPC (8.9-fold), 1palmitoyl-GPE (13.2-fold), 1-stearoyl-GPC (5.7-fold), 1-linoleoyl-GPC (10.8-fold), 1-linoleoyl-GPE (8.9-fold), 2-linoleoyl-GPC (4.8-fold) and 2-linoleoyl-GPE (5.4-fold) (Supplementary Table S1 in León et al., 2016 and Fig. 4.26). Altered content of free fatty acids and phospholipids are likely connected to processes of lipid trafficking in either membrane remodeling or alteration of lipidic leaf structures such as cuticles (Hurlock et al., 2014). We tested whether cell membranes or cuticles were altered in Arabidopsis plants upon exposure to NO. Nitrooxidative stress produced by the acute pulse of NO can severely damage membranes by lipid peroxidation (Blokhina et al., 2003), thus altering their permeability. The integrity of membranes was monitored in vivo by staining roots with propidium iodide that only stains nuclei if membranes become permeable, but it remains staining membranes and walls if cell membranes are intact. Roots of untreated plants display non altered plasma membranes as demonstrated by propidium iodide decorating the cell membrane with no permeation into the cells (Fig. 4.27A). By contrast, by 30 min after exposure to NO the roots of NO-treated plants displayed numerous cells



**Figure 4.26.** Altered phospholipid catabolism in plants after exposure to NO. Scheme showing the metabolism of phospholipids and the box plots for fatty acids and lipids before treatment (blue) and at indicated times after mock- (green) or NO-treatment (red). Light and dark tones correspond to 6 h and 24 h after treatment, respectively.

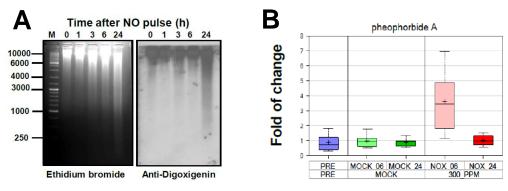
with propidium iodide-stained nuclei, thus indicating the permeability of their membranes was altered (Fig. 4.27A). Also the formation, integrity and permeability of the leaf cuticle are highly connected to lipid metabolism in Arabidopsis (Yeats & Rose, 2013). We checked whether the permeability of the leaf cuticle was influenced by the exposure to NO. Wild type Arabidopsis plants treated with exogenous NO as well as the Arabidopsis *nia1nia2noa1-2* triple mutant, depleted of endogenous NO (Lozano-Juste & León, 2010), were tested for cuticle permeability. Assays of cuticle permeability after application of toluidine blue or calcofluor white demonstrated an enhanced permeability of the cuticle in NO-deficient leaves that was reversed by treatment with exogenous NO (Fig. 4.27B).



**Figure 4.27.** Alterations in the permeability of lipidic structures. A, Permeability of plasma membrane in roots of NO-treated plants. Untreated (-NO) or NO-treated (+NO) roots from wild type Col-0 plants were stained with propidium iodide at 30 min after exposure to NO. Images were obtained with a confocal microscope Leica TCS SL with excitation at 488 nm and emission at 598-650 nm range. Scale bars: 50 µm. B, Effect of NO on leaf cuticle permeability. In the left panel, two drops of 10 µl of toluidine blue solution were applied on the upper side of undetached leaves from wild type Col-0 and NO-deficient *nia1,2noa1-2* mutant plants. After 2 h, leaves were extensively washed with water, excised and photographed to show penetration or not of toluidine blue through cuticle. In the right panel, leaves from untreated (-NO) or NO-treated (+NO) plants were detached at 6 h after NO pulse, bleached with ethanol, stained for 1 min with calcolfluor white and extensively rinsed with water before photographed under UV light. Data shown are representative of five and three independent toluidine blue and calcofluor white experiments, respectively, with similar results.

# 4.2.13. Altered purine, pyrimidine and chlorophyll metabolism suggest NO enhanced nucleic acid turnover, chlorophyll degradation and non-programmed cell death

The metabolomic data also reflected a significant increase in metabolites involved in purine and pyrimidine metabolism. The levels of allantoin, guanine, urate, cytidine, cytosine-2',3'-cyclic monophosphate, pseudouridine, uridine and uracil were all increased (between 1.5- and 7.6-fold) by 6 h after NO treatment (Supplementary Table S1 in León *et al.*, 2016). However, all these metabolites returned to the baseline levels by 24 h, with special emphasis on uracil, which moved from a 7.6-fold increase by 6 h to non-significantly changed levels by 24 h (Supplementary Table S1 in León *et al.*, 2016). It is noteworthy mentioning that, in turn, the levels of nucleosides such as adenosine, adenosine-2´-monophosphate and inosine are between 20 and 45% reduced by 6 h after plants were exposed to NO (Supplementary Table S1 in León *et al.*, 2016). In agreement with the enhanced metabolism of purines and pyrimidines, we found a pattern of progressive genomic DNA degradation upon exposure of plants to the NO pulse (Fig. 4.28A).



**Figure 4.28. NO triggers DNA and chlorophyll degradation.** A, Genomic DNA isolated from NO-exposed plants at the indicated times after application of NO pulse. Genomic DNA was run in duplicate in 2% agarose gels and either ethidium bromide-stained (15 μg) or blotted (5 μg) onto positively charged nylon membranes. DNA digested with *Msp*I was digoxigenin (DIG)-labelled and use as probe for Southern blot with anti-DIG antibody coupled to alkaline phosphatase to amplify the signal. DNA ladder marker (M) in base pairs is shown in the left side. B, y-axis shows Fold of Change as Group Means Ratios in Scaled Imputed Data for pheophorbide a. + represents mean value; \_\_\_\_\_ represents median value; boxes indicate the limits of upper and lower quartile; and error bars represent the maximum and minimum of distribution.

On the other hand, the metabolomic data also suggest NO induces chlorophyll degradation. Pheophorbide a, which has been identified as a genuine intermediate of chlorophyll breakdown (Hörtensteiner, 2013), was 3.7-fold increased by 6 h after NO treatment (Fig. 4.28B), thus suggesting Pheophorbide a oxidase (PAO) activity should be reduced in NO-exposed plants. The absence of PAO activity in different plants has been correlated with increased cell death, and pheophorbide a has been reported to be involved in both light-dependent and light-independent cell death mechanisms (Pruzinská et al., 2005; Hirashima et al., 2009). We checked whether treatment with exogenous NO alters the endogenous levels and leads to cell death in exposed leaves. Figure 4.29A shows the endogenous levels of NO after staining roots with DAF-FM diacetate. The endogenous levels slightly decreased by 2 and 4 h after exposure to exogenous NO and modest increases were observed by 6 and 24 h (Fig. 4.29A) but increases were far below those detected in plants treated with a known inducer of NO such as salicylic acid (Zottini et al., 2007) (Fig. 4.29B). Evans blue staining of plants at different times after NO treatment showed blue stained spots of dead cells at 6 h and 24 h after exposure to NO (Fig. 4.29C) and although seedlings were slightly chlorotic by 24 h plants they were fully viable showing normal growth by seven days after treatment (Fig. 4.29C).

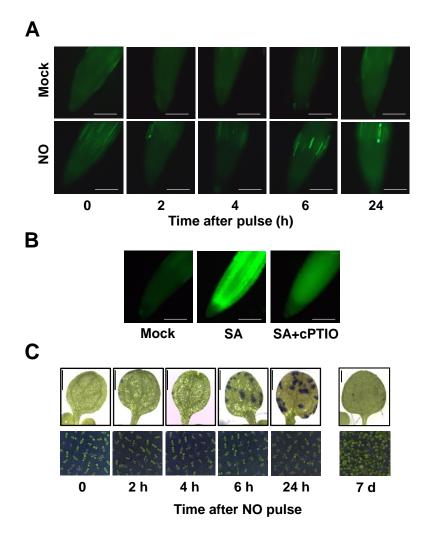
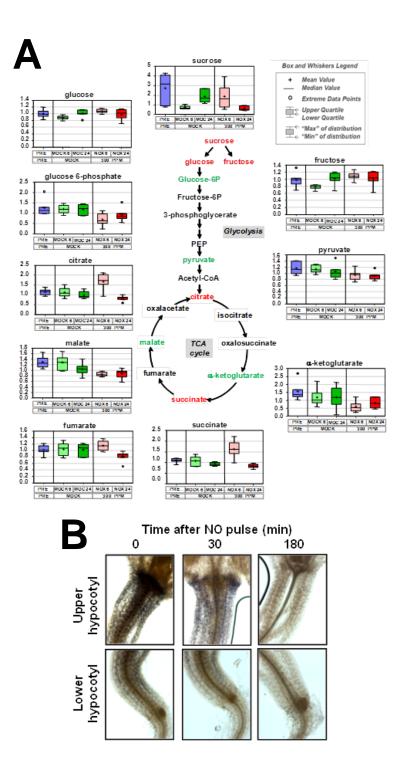


Figure 4.29. Endogenous NO content and cell death upon exposure to exogenous NO. Seedlings (8 day-old) were treated with A, exogenous NO or treated with air as control (Mock) and B, mock treated or treated with 0.5 mM salicylic acid (SA) or with SA plus 0.5 mM 2- (4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (SA+cPTIO) for 1 h, and at the indicated times after exposure, the endogenous NO content was assayed by root staining with DAF-FM DA. Scale bars: 100  $\mu$ m. C, Cell death was assayed by Evans blue staining of plants at the indicated times after exposure to NO. Scale bars: 1 mm. The appearance of the plants for every time point is shown in the bottom panels. The experiments were repeated three times with similar results and representative images are shown.

### 4.2.14. Exposure to NO affected photorespiration and central carbon metabolism

By 6 h after exposure to NO, the content of the glycolisis intermediates glucose-6-phosphate, glycerate and pyruvate decreased (0.57-, 0.59- and 0.83-fold, respectively) if compared to mock-treated plants at the same time point (Fig. 4.30A and Supplementary Table S1 in León *et al.*, 2016). It is noteworthy mentioning that glycerate, which is an intermediate in photorespiration, kept levels significantly reduced by 24 h after exposure to NO (Supplementary Table S1 in León *et al.*, 2016). Also the levels of two metabolites of the TCA cycle such as  $\alpha$ -



**Figure 4.30.** Metabolites of glycolysis and TCA cycle in plants exposed to NO. A, Scheme showing the intermediate metabolites (in red increased content; in green reduced content) of the glycolysis and the TCA cycle and the box plots for every metabolite before treatment (blue) and at indicated times after mock- (green) or NO-treatment (red). Light and dark tones correspond to 6 h and 24 h, respectively, after treatment. B, Starch granules were stained with Lugol's reagent in 7-day old seedlings exposed to a 300 ppm NO pulse for the indicated times. Images for hypocotyls are representative of at least six independent plants per time point. ketoglutarate and malate were significantly reduced by 6 h in NO-treated plants (Fig. 4.30A and Supplementary Table S1 in León et al., 2016). Finally, sedoheptulose-7-phosphate and ribose-5-phosphate, both intermediates of the Calvin Cycle were not different at the six hour time point, but were significantly lower (0.32-, 0.58-fold, respectively) at 24 hours (Supplementary Table S1 in León et al., 2016). The content of sucrose, the downstream product of photosynthesis, although increased (2.5-fold) by 6 h was significantly reduced (0.33-fold) by 24 h (Fig. 4.30A and Supplementary Table S1). Also different sugars including amino and nucleotide sugars as well as disaccharides were increased (between 1.4- and 3-fold) in the 6 h time point in NO-treated plants (Supplementary Table S1 in León et al., 2016). Remarkably, three of those compounds arabitol, ribulose and ribitol were strongly reduced (0.04-, 0.54- and 0.74-fold, respectively) by 24 h after NO treatment (Supplementary Table S1 in León et al., 2016). In agreement with exogenous NO altering photosynthetic metabolism and triggering differential accumulation of sugars, we have detected a significant reduction in the starch granules in the hypocotyls of NO-treated seedlings by 3 h after exposure to NO (Fig. 4.30B).

### 4.3. <u>Function of the ERFVII RAP2.3 on the regulation of NO homeostasis</u> and signaling

This section 4.3 is an excerpt from the research article: "León, J., Costa-Broseta, Á., & Castillo, M. C. (2018). RAP2.3 negatively regulates nitric oxide biosynthesis and sensing through a rheostat-like mechanism in Arabidopsis". This research article was submitted to The Plant Journal and was under revision when PhD Thesis writing was finished. All the results and figures that appear here are derived from the work of the PhD student in collaboration with the other authors.

#### 4.3.1. Abstract

Nitric oxide (NO), which regulates development and defense responses in plants, is sensed through a mechanism involving the N-end rule pathway mediated degradation of the group VII of ERF transcription factors (ERFVIIs). A member of the ERFVIIs, RAP2.3, negatively regulates both the NO biosynthesis and their triggered responses. RAP2.3 seems to work as a molecular rheostat maintaining a brake on NO synthesis and responses when accumulated, either by avoiding its degradation or by enhancing its expression. Its degradation would thus release those brakes allowing NO production and the subsequent activated responses. The use of conditionally expressing transgenic lines for RAP2.3 together with NO treatment and genome-wide transcriptome analyses allowed finding that the enhanced expression of RAP2.3 largely attenuated the changes in the NOtranscriptome both qualitatively and quantitatively. responsive Transcriptome data uncover the existence of RAP2.3-dependent and independent NO-responsive branches of jasmonate, and ABA signaling pathways, which seems to be functionally relevant for NO sensing.

### 4.3.2. A rheostat-like mechanism based on RAP2.3 degradation controls endogenous NO content

RAP2.3 is one of the members of the group VII ERF transcription factors that we previously reported to be involved in NO sensing (Gibbs *et al.*, 2014a). As a substrate of the arginylation branch of the N-end rule pathway of proteolytic degradation (Gibbs *et al.*, 2011; Licausi *et al.*, 2011), the stability of the RAP2.3 protein depends on its N-terminal sequence, in such a way that a wild type version containing the MC- N-terminal motif is degraded upon polyubiquitylation by the proteasome, whereas a mutated MC2A version is resistant to proteasome-mediated degradation. By using plants overexpressing either MC-RAP2.3 or MA-RAP2.3 (Gibbs *et al.*, 2014a), we analyzed the effect of RAP2.3 on the endogenous NO content. After staining with the NO specific fluorophore DAF-FM, plant cotyledons overexpressing the degradable MC-RAP2.3 version contained a 2.3-fold increase in NO-associated fluorescence compared to wild type plants (Fig. 4.31). However, the levels in MC-RAP2.3 overexpressing roots were not

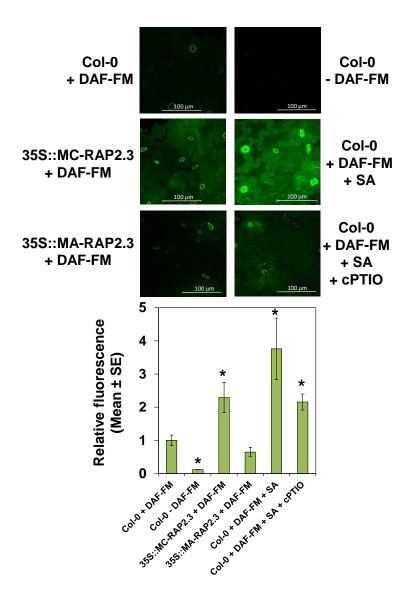


Figure 4.31. NO in cotyledons of plants overexpressing MC- and MA-RAP2.3 versions. Cotyledons from plants of the indicated genotypes were treated (+) or untreated (-) with 10  $\mu$ M DAF-FM DA, 500  $\mu$ M of the NO scavenger cPTIO, and 500  $\mu$ M of the NO inducer salicylic acid (SA) as indicated. Fluorescence was detected by confocal microscopy with Z-stacks equivalents for all the genotypes and conditions tested. Images are representative of 3 to 5 replicate experiments and relative fluorescence is shown as the mean of replicate experiments ± standard error.\* means significant with p-value <0.05 in Student's t-test.

significantly different than those of wild type roots (Fig. 4.33A). The increased fluorescence levels were nevertheless not as high as those detected in plants treated with SA (3.8-fold; Fig. 4.31), which has been reported to be a strong inducer of NO production both in roots and shoots (Zottini *et al.*, 2007). The detected fluorescence was confirmed to be associated to the endogenous content of NO, as samples treated with SA and the NO specific scavenger cPTIO showed lower fluorescence levels than plants treated with SA (Fig. 4.31). Remarkably, the enhanced NO content in *35S::MC-RAP2.3* plants was not directly associated to the protein expression levels, as the over-expression of the non-degradable MA-

RAP2.3 version led to cotyledon fluorescence not significantly different than wild type plants (Fig. 4.31). These data point to a requirement for RAP2.3 degradation to release a brake exerted by RAP2.3 on NO biosynthesis. Since RAP2.3 degradation by the proteasome requires the previous polyubiquitylation mediated by the E3 ubiquitin ligase PRT6, we tested whether NO content in cotyledons was altered in prt6-1 mutant plants. The overall NO-associated fluorescence in prt6-1 plants was not significantly different than those from wild type plants either in cotyledons (Fig. 4.32) or in roots (Fig. 4.33B). However, increases in NO levels were detected in the guard cells of stomata (Fig. 4.32), in agreement with previous report (Gibbs et al. 2014a). Interestingly, the guintuple mutant with loss of function of all ERFVII transcription factors (gerfvii), which contained NO levels similar to wild type plants both in shoots (Fig. 4.32) and roots (Fig. 4.33B), displayed almost no fluorescence in the stomata (Fig. 4.32). A similar pattern of endogenous NO content was also detected in the sextuple gerfvii prt6-1 mutant (Fig. 4.32 and 4.33B), thus indicating the enhanced NO levels caused by the *prt6* mutation was not dependent on the actual ERFVII levels but on their capacity of being degraded through a PRT6-mediated process. Regarding this, plants expressing a non-

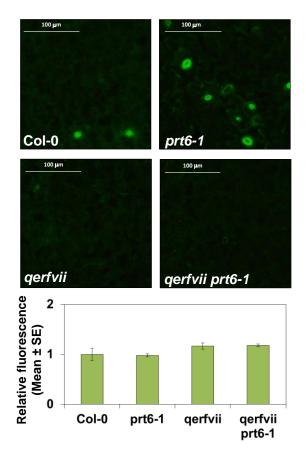


Figure 4.32. NO in cotyledons of N-end rule pathway mutant and wild type plants. Cotyledons from plants of the indicated genotypes were treated with 10  $\mu$ M DAF-FM DA. Fluorescence was detected by confocal microscopy with Z-stacks equivalents for all the genotypes and conditions tested. Images are representative of 3 to 5 replicate experiments and relative fluorescence is shown as the mean of replicate experiments ± standard error.

degradable version (35S::MA-RAP2.3) of the protein or not expressing the protein at all (*qerfvii* mutant) would behave similarly with low levels of NO. These findings point to a potential role for RAP2.3 working as a molecular rheostat in such a way that the NO synthesis would be limited when RAP2.3 is not degraded but activated upon degradation.

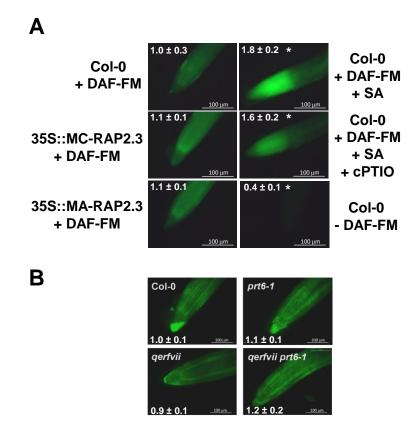


Figure 4.33. NO in roots of mutant and transgenic plants. Roots of the indicated genotypes of A, plants overexpressing MC- and MA-RAP2.3 versions and B, N-end rule pathway mutant plants genotypes were treated (+) or untreated (-) with 10  $\mu$ M DAF-FM DA, 500  $\mu$ M of the NO scavenger cPTIO, and 500  $\mu$ M of the NO inducer salicylic acid (SA) as indicated were treated with 10  $\mu$ M DAF-FM DA. Plants shown in B were only treated with DAF-FM DA. Fluorescence was detected by fluorescence microscopy. Images are representative of 3 to 5 replicate experiments and quantification of fluorescence is shown as the mean of replicate experiments ± standard error .\* means significant with p-value <0.05 in Student's t-test.

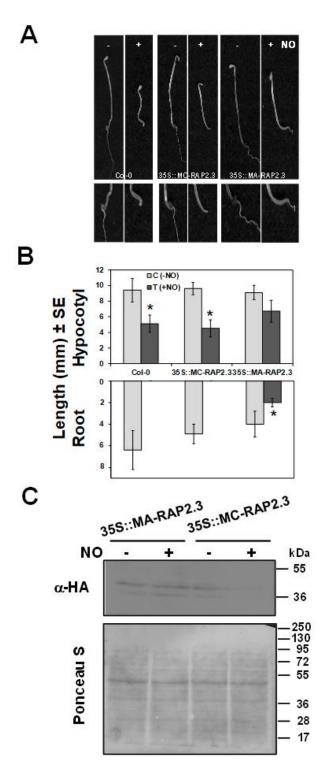
#### 4.3.3. RAP2.3 modulates NO sensing in shoots and roots

Despite the potential role of RAP2.3 in regulating the endogenous NO levels as described above, RAP2.3, together with the rest of ERF VII transcription factors, has been proposed to participate in NO sensing through the arginylation branch of the N-End Rule pathway (Gibbs *et al.*, 2014b; Gibbs *et al.*, 2014a). According to the central role exerted by the E3 ubiquitin ligase PRT6 in that process, we reported that the NO-triggered inhibition of the hypocotyl elongation was impaired in the *prt6* 

mutant plants (Gibbs, et al., 2014a). Thus, the stabilization of ERFVIIs seems to be a key factor for the sensitivity of plants to NO. By using plants over-expressing either the wild type MC-version or the mutated MA-version of RAP2.3, we tested whether enhanced levels of RAP2.3 altered the sensitivity to NO in etiolated hypocotyl shortening assays. Figures 4.34A and 4.34B show that plants overexpressing an MC-RAP2.3 version were as sensitive to NO-triggered inhibition of hypocotyl elongation as Col-0 plants. In turn, the over-expression of the nondegradable MA-RAP2.3 version almost fully released the NO-triggered inhibition of hypocotyl elongation. To check whether these differential phenotypes were related to the stability of the RAP2.3 version expressed, we took advantage of the Cterminal HA-tags of both proteins in our constructs. Whereas MC-RAP2.3 was efficiently degraded by NO, the levels of MA-RAP2.3 protein remained high under NO treatment, thus correlating with the NO-sensitive and -resistant phenotypes observed, respectively (Fig. 4.34C). Besides hypocotyl shortening, the response of plants to NO was also characterized by a drastic inhibition of primary root growth in wild type plants (Fig. 4.34A,B). As for hypocotyl shortening, 35S::MC-RAP2.3 plants were also fully inhibited in root growth but, in turn, 35S::MA-RAP2.3 displayed a significant yet defective root growth under the NO treatment applied in this experiment (Fig. 4.34A,B). These data thus suggest the stabilization of RAP2.3 protein made plants less sensitive to NO, so that RAP2.3 likely acts also as a negative regulator of NO sensing and signaling.

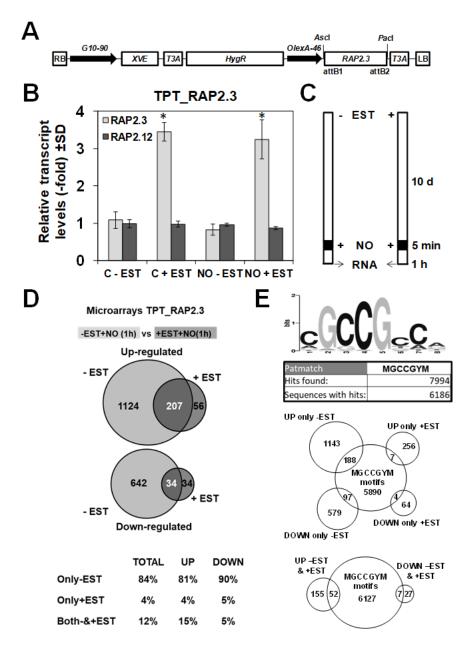
## 4.3.4. Genome-wide transcriptome analyses revealed RAP2.3 as a general negative regulator of NO-triggered responses

Inhibition of root and hypocotyl growth is only part of the NO-triggered responses in plants. We have previously reported that a pulse of NO triggers a transient but extensive metabolic reprogramming that includes enhanced levels of polyamines, lipid catabolism and accumulation of phospholipids, chlorophyll breakdown, protein and nucleic acid turnover and decreased and increased content of starch and sugars, respectively (León et al., 2016). To assess, at the molecular level, how NO triggers multiple responses and what is the regulatory role exerted by RAP2.3, we conducted comparative transcriptome analyses of NOtreated versus untreated plants in TPT (TRANSPLANTA) Arabidopsis transgenic lines (Coego et al., 2014) conditionally expressing RAP2.3 under a  $\beta$ -estradiolinducible promoter (Fig. 4.35A). Upon treatment with  $\beta$ -estradiol, those plants specifically expressed RAP2.3, as demonstrated by the large accumulation of the RAP2.3 transcript and the absence of enhanced expression for the highly related RAP2.12 gene (Fig. 4.35B). Following the experimental scheme shown in Figure 4.35C, β-estradiol treated or control untreated plants were grown for 10 d under standard growing conditions, exposed to a 300 ppm NO pulse for 5 min, and 1 h later samples were collected for RNA isolation and further transcriptome analyses using Arabidopsis whole genome Agilent microarrays. Normalized and filtered data (p-value corrected for FDR < 0.05 and fold change in absolute values > 1.5) from



**Figure 4.34. Overexpression of non-degradable MA-RAP2.3 confers hyposensitivity to NO.** A, Hypocotyls (upper panels) and roots (detail in lower panels) of etiolated seedlings either treated (+) or not (-) with 300 pm NO. B, The length of hypocotyls and primary roots was measured, after scanning at least 20 individuals for every genotype and condition, with Image J. Values are the mean ± SD. \* means significant with p-value <0.05 in Student's t-test. C, The levels of RAP2-3-HA protein were analyzed by Western blot with anti-HA antibodies in protein extracts from NO treated (+) or not (-) etiolated seedlings of the indicated genotypes. Total protein staining with Ponceau S is shown as loading control. The position of molecular weight markers is shown at the right side of panels.

all transcriptome analyses are summarized in Table A9. The NO pulse triggered the rapid and differential regulation of 2097 genes representing roughly 10% of the Arabidopsis genome (Fig. 4.35D), thus pointing to relevant transcriptional regulation of the NO-induced responses. Remarkably, the NO-triggered responses at the transcriptome level were largely attenuated when NO-treated plants overexpressed RAP2.3 upon activation by  $\beta$ -estradiol treatment (Fig. 4.35D). Around 81% of the up-regulated and 90% of the down-regulated genes by NO in TPT RAP2.3 plants were differentially expressed only in the absence of β-estradiol treatment (Fig. 4.35D and Table A9). The 1124 and 642 genes that were up- and down-regulated, respectively, by NO in plants not treated with β-estradiol but not in those over-expressing RAP2.3 represent potential NO targets negatively regulated by RAP2.3. By contrast, only 263 and 68 genes were up- and downregulated, respectively, by NO regardless of the levels of RAP2.3 expression (Fig. 4.35D). From those, 56 and 34 genes were up- and down-regulated, respectively, by NO only when RAP2.3 was over-expressed (Fig. 4.35D), thus suggesting RAP2.3 might act also as a positive regulator of NO-triggered changes in this gene subset. These data support that RAP2.3, in addition to play a role in controlling NO biosynthesis and sensing, exerted an important quantitative and qualitative modulation of the NO-regulated transcriptome. Remarkably, RAP2.3 seems to act mainly as a repressor of NO-responsive genes, although also potential targets of its positive regulation were pinpointed in these analyses. An in silico screening of GCC-like boxes, defined previously as putative RAP2.3 binding motifs (Franco-Zorrilla et al., 2014), was performed. Searching the 1000bp promoter sequence upstream of the initiation codon of genes in the whole Arabidopsis genome for the (C/A)GCCG(C/T)(C/A) motif sequence allowed us to find 7994 hits corresponding to 6186 sequences (Fig. 4.35E and Table A7). Among these putative RAP2.3binding targets, 296 corresponded to genes that were differentially regulated in NO-treated plants of the TPT\_RAP2.3 transgenic lines (Fig. 4.35E and Table A7). We found 188 and 97 genes containing the consensus RAP2.3 binding motif that were up- or down-regulated, respectively, by NO only in TPT plants that were not treated with  $\beta$ -estradiol (Fig. 4.35E and Table A7). By contrast, only 7 and 4 genes containing the consensus RAP2.3 binding motif were up- or down-regulated, respectively, by NO only in TPT plants that were treated with  $\beta$ -estradiol (Fig. 4.35E and Table A7), thus suggesting the number of targets negatively regulated by RAP2.3 in NO-triggered responses are by far larger than those positively regulated. We also found a relatively large amount of genes carrying putative RAP2.3-binding motifs, 52 and 7 that were up- or down-regulated by NO in TPT\_RAP2.3 plants independently of being treated or not with  $\beta$ -estradiol (Fig. 4.35E and Table A7), thus suggesting they were not truly targets of RAP2.3 regulation.



**Figure 4.35. Transcriptome analysis of β-estradiol inducible RAP2.3 transgenic lines.** A, Construct used to conditionally express *RAP2.3* under the control of a β-estradiol-inducible *XVE* factor that activates the *OlexA-46* promoter. B, *RAP.3* and *RAP2.12* transcript levels were quantified in plants, either not treated (C) or treated (NO) in the presence (+) or absence (-) of β-estradiol (EST), by RT-qPCR with specific primers. Values are the mean ± SD of three independent biological replicates. \* mean statistically significant with p-value < 0.05 in Student's t-test. C, Scheme displaying the times for growing, treatments and sampling designed for the transcriptome analyses. D, Venn diagrams showing differentially up- and down-regulated genes in conditionally expressing TPT-RAP2.3 transgenic lines treated (+) or not (-) with the transgene inducer β-estradiol (EST). E, Putative RAP2.3 binding element and in silico analysis of the presence in promoter genes on the whole Arabidopsis genome. Venn diagrams represent the intersection between genes that were identified as differentially expressed in NO-treated plants and those carrying the binding motif in their promoters.

## 4.3.5. The NO-responsive RAP2.3-independent transcriptome includes jasmonic acid and ethylene core signaling gene sets

The group of 241 genes that were up- or down-regulated by NO in TPT RAP2.3 plants independently of being treated or not with  $\beta$ -estradiol (Fig. potential represents the NO-Responsive RAP2.3-independent 4.35D) transcriptome. A Gene Ontology analysis on these genes revealed that the functional categories related to jasmonic acid (JA) biosynthesis, metabolism and signaling as well as the response to ethylene stimulus were the most significantly over-represented. The set of JA-related genes included some coding for biosynthetic enzymes such as LOX3, LOX4, AOC1, AOC3, OPR3 and OPCL1 as well as some coding for different components of JA signaling including the transcription factor MYC2 and the negative regulators JAZ2, 5, 6, 7, 9, 10, CYP94b1 and CYP94c1, which were all strongly up-regulated from 6- to more than 50-fold (Table A9). Similarly, several ethylene response-related genes such as those coding for the ethylene response (ERF) transcription factors RRTF1, ERF13, ERF-2, ERF6, ERF-1, CEJ1/DEAR1 and RAP2.9/DEAR5 were also included in this group of genes. Since these JA- and ethylene-related genes resulted to be upregulated, it seems that JA and ethylene signaling were both enhanced in response to NO through a process that was not modulated by RAP2.3. Besides JA- and ethylene-regulated processes, also ABA-related processes such as dehydration were also over-represented among up-regulated genes of this group. This subset included genes coding for several dehydrins such as ERD10, ERD12/AOC1, ERD15 or the E3 ubiquitin ligases PUB23 and PUB24, as well as genes coding for water stress-related transcription factors such as MYBR1, MYC2, MBF1C and DREB2b (Table A9). Some of these ABA-responsive genes resulted to be also regulated by other stress-related hormones such as JA or salicylic acid (SA) thus pointing to NO as an enhancer of general RAP2.3-independent stress responses in the plant. Remarkably, some of the above mentioned genes contained (C/A)GCCG(C/T)(C/A) motifs in their promoter sequences (Table A7), thus suggesting not all the in silico identified motifs are truly RAP2.3-related and/or that RAP2.3 binding on that motifs are not modifying the regulation exerted by NO on them.

### 4.3.6. The NO-responsive RAP2.3-dependent transcriptome suggest the existence of NO-sensitive gene-specific hormone signaling pathways

Besides RAP2.3-independent NO-responsive genes, we found two other set of NO-responsive genes showing a RAP2.3-regulated pattern. The smaller set comprised 90 genes that required the over-expression of *RAP2.3* to be up- or down-regulated by NO (Fig. 4.35D). It is noteworthy mentioning that among genes in this set, the cytokinin negative regulator ARR22 encoding gene as well as the auxin responsive proteins SAUR29, 65 and 19, and the auxin metabolic IAA carboxylmethyltransferase 1 (IAMT1) and IAA-amido synthetase GH3.9 encoding genes were strongly down-regulated (Table A9). By contrast, the auxin signaling

enhancer MYB77 transcription factor encoding gene was up-regulated by NO only in RAP2.3 over-expressing plants (Table A9). All together these data suggest NO may regulate the responses to auxins and cytokinins through RAP2.3-mediated processes. However, only 7 and 4 genes among those up- or down-regulated by NO in  $\beta$ -estradiol treated plants contained the (C/A)GCCG(C/T)(C/A) motif in their promoters, and only SAUR65, from the above mentioned as related to auxin and cytokinin signaling (Table A7). Also remarkably, the phosphate transporters PHT1;4 and PHT2;1 were down-regulated by NO in RAP2.3 over-expressing plants (Table A9). Regarding up-regulated genes in this set, we found that the transcription factors RAV1, BBX20/BZS1 and WRKY28 encoding genes, which are involved in regulating ABA-related stress signaling, brassinosteroid and strigolactone signaling, and SA biosynthesis, respectively (van Verk et al., 2011; Feng et al., 2014; Wei et al., 2016), were only induced by NO in plants that overexpress RAP2.3 (Table A9). However, none of them contained RAP2.3-related motifs in their promoters (Table A7), thus suggesting the regulatory effects exerted by RAP2.3 should be not direct on those targets.

The second gene set, by far the greatest group, comprised genes that were up- or down-regulated in NO-treated plants only when RAP2.3 was not overexpressed, thus suggesting RAP2.3 acted as a negative modulator of the NOtriggered regulation for those genes. Gene Ontology analyses of the genes comprised in this set pointed to the over-representation of the functional categories related to responses to chitin, JA, ABA, temperature, light and SA stimuli. Figure 4.36 shows that JA-related genes coding for biosynthetic and metabolic enzymes (PLC7, PLDv1, PLA1/LCAT3, LOX2, AOS/CYP74A, AOC2, OPR1, JAR1, SOT16, CYP94C3, CYP94B1), for repressors and co-repressors of signaling (JAZ1, JAZ3, JAZ12 and the NINJA-like AFP2, AFP3, AFP4) and for JA-responsive markers (VSP1, VSP2, TAT3, JRG21, JR1, CORI3) were all up-regulated by NO only when RAP2.3 was not overexpressed, thus suggesting there is a complete JA biosynthesis and signaling pathway induced by NO and repressed by RAP2.3. Remarkably, only the PLC7, LOX4 and JAR1 jasmonate biosynthetic encoding genes, the latter key for the synthesis of the active form jasmonoyl-isoleucine (Staswick & Tiriyaki, 2004) contained (C/A)GCCG(C/T)(C/A) motifs in their promoters (Table A7). Moreover, as described above there is also a JA pathway, activated by NO independently of RAP2.3, that involve the function of the biosynthetic module comprised by LOX3, AOC1 or AOC3, OPR3, and the signaling module comprising the activator MYC2 and the repressors JAZ5, 6, 7 and 10 (Fig. 4.36 and Table A9). Noteworthy, despite being at principal regulated by NO independently of RAP2.3, the biosynthetic AOC1 gene and the regulatory MYC2 and JAZ6 genes contained (C/A)GCCG(C/T)(C/A) motifs in their promoters (Table A7).

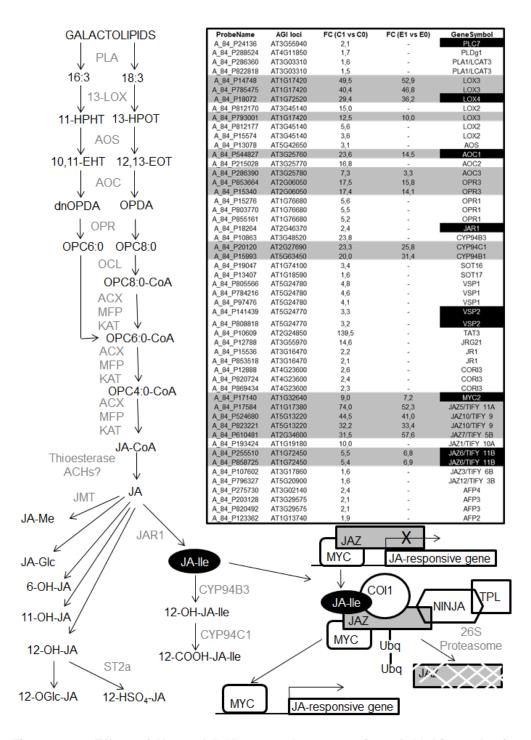


Figure 4.36. Effect of NO and RAP2.3 on the expression of JA biosynthesis and signaling genes. The scheme shows the biosynthesis and signaling JA pathway and the table the fold change (FC) values for the different transcripts in the comparisons C1 vs C0 of plants not treated with  $\beta$ -estradiol at 1 or 0 h after exposure to NO, and E1 vs E0 for the corresponding  $\beta$ -estradiol-treated plants. Highlighted in grey are NO-regulated genes that were not significantly affected by RAP2.3 overexpression. Those highlighted in black contained putative RAP2.3 binding sites in their promoters.

Similarly, ABA-related biosynthetic and signaling genes were also included in this set. The biosynthetic BCH1 and ABA1 genes coding for β-carotene hydroxylase 1 and zeaxanthin epoxidase, respectively, and the metabolic UGT71B6 gene coding for UDP-glucosyl transferase 71b6 were down- and upregulated by NO, respectively, only in TPT RAP2.3 plants that were not treated with  $\beta$ -estradiol (Fig. 4.37). Only the promoter of UGT71B6 gene contained the (C/A)GCCG(C/T)(C/A) motif (Table A7). We also found that only some genes coding for regulatory components of the core ABA signaling were regulated by NO, and some of them were dependent and other independent of RAP2.3 expression (Table A9). The ABA receptors PYL7 and PYL4 encoding genes were up-regulated by NO and this induction was repressed by RAP2.3 (Fig. 4.37 and Table A9). In turn, the gene coding for PYL5 receptor was strictly dependent on RAP2.3 overexpression for the NO-induced expression (Fig. 4.37 and Table A9). Finally, the PYL6 encoding gene was up-regulated by NO independently of RAP2.3 (Fig. 4.37 and Table A9). All together these data suggest that NO may control the ABA perception through gene-specific pathways controlled or not by RAP2.3. This sort of gene-specific regulatory effect can be also applied to the positive protein kinase regulators encoded for the SnRK2 gene family. Only SnRK2.3 and SnRK2.9 were up-regulated by NO, being the induced expression of SnRK2.3 abolished by RAP2.3 over-expression whereas SnRK2.9 expression was not modulated by RAP2.3 (Fig. 4.37 and Table A9). Only the promoters of genes coding for the positive ABA regulators PYL7. SnRK2.3 and SnRK2.9 contained the (C/A)GCCG(C/T)(C/A) motif (Table A7). However, the existence of gene-specific branch pathways inside the ABA signaling process did not match with the expression pattern of ABA target genes coding for either signaling components or transcription factors, which were mostly up-regulated by NO through a RAP2.3repressed mechanism (Fig. 4.37). Only the SLAH2 and AIB genes coding for a nitrate-specific anion channel and an ABA-inducible bHLH-type transcription factor, respectively, were up-regulated by NO independently of RAP2.3 (Fig. 4.37 and Table A9). Moreover, we found (C/A)GCCG(C/T)(C/A) motifs in the promoter of genes such as MYB51 and LTI30 up-regulated by NO only in the absence of RAP2.3 over-expression, but also in the SLAH2 gene, which was up-regulated by NO independently of RAP2.3 expression levels (Fig. 4.37 and Table A7). Thus our findings suggest that RAP2.3 exerted a very efficient repression of the NO-induced expression of ABA targets genes through either the direct regulation of key targets by binding the promoters containing (C/A)GCCG(C/T)(C/A) motifs or, alternatively, by regulating just some master ABA signaling component, likely coding for a transcription factor.

B-carotene						
				FC ([C1] vs	FC ([E1] vs	
BCH2		ProbeName	AGI loci	[C0])	[E0])	GeneSymbol
		A_84_P763996		-1,6	-	BCH1
Zeaxanthin		A_84_P832189		-1,5	-	ABA1
		A_84_P20247		3,0	-	UGT71B6
ABA1/ZEP		A_84_P174151		4,5	5,5	PYL6
ADATIZEE		A_84_P20389		2,4	-	PYL7
VC-1	/ 	A_84_P816095		2,3	-	PYL7
Violax	anthin	A_84_P197484		2,2	-	PYL4
		A_84_P146199		-	3,4	PYL5
ABA2/NSY		A_84_P852047		-	3,3	PYL5
$\checkmark$		A_84_P817892		-	3,3	PYL5
Neoxanthin		A_84_P12575		3,6	2,7	SnRK2.9
	1	A_84_P16007		1,6	-	SnRK2.3
NCED3 Xanthoxin		A_84_P159915		5,9	2,4	SLAH2
		A_84_P22976		1,6	-	ABI5
		A_84_P164953		1,9	-	ABF1
		A_84_P80879		3,1	2,7	AIB
	1	A_84_P151138		-1,7	-	MARD1
ABA2		A_84_P19130		1,7	-	GBF3 ATAF1
\	V	A_84_P784302 A 84 P19986		2,1 2.1	-	ATAF1 ATAF1
ABA-aldehyde		A 84 P14948			-	
				2,0 4,9	-	RVE2 MYB108
AAO3		A_84_P18334 A_84_P19074		3,0	-	MYB51
ABA3/ MoCo		A 84 P17577		1,9	-	MYB13
sulfurase ABA		A_84_P14542		-1.6	-	RVE8
		A 84 P860855		2.4	-	ZF2
		A_84_P21219		2,4	-	ZF2
		A_84_P18822		-2,3	-	MYB28
		A 84 P22292		-2,3	-	FIB
$\checkmark$		A 84 P79549		1.6	-	RGS1
PYR/PYL L PP2C L		A 84 P23251		1.8	-	CPK4
		A_84_P18763		-1.8	-	CIPK20
		A_84_P21126		3,9	_	GCN5-related
		A 84 P853160		3.0	-	SYP121
		A 84 P15485		2.7	-	SYP121
		A 84 P810688		1.7	-	KIN1
		A 84 P258350		1.9	-	KIN2
		A_84_P15372		1.7	-	ANNAT4
		A_84_P10874	AT3G50970	3.5	-	LTI30
SnRK2		A 84 P21026		1.8	-	RD20
		A_84_P22571		1.8	-	LT178
		A 84 P809384		1.6	-	ERD4
		A 84 P809423		1.6	-	ERD4
le e	Transford	A_84_P12888		2.6	-	CORI3
lon	Transcription	A_84_P820724	AT4G23600	2,4	-	CORI3
channels	factors	A_84_P869434	AT4G23600	2,3	-	CORI3
	1					
	V					

Dehydrins

Figure 4.37. Effect of NO and RAP2.3 on the expression of ABA biosynthesis and signaling genes. The scheme shows the biosynthesis and signaling ABA pathway and the table the fold change (FC) values for the different transcripts in the comparisons C1 vs C0 of plants not treated with  $\beta$ -estradiol at 1 or 0 h after exposure to NO, and E1 vs E0 for the corresponding  $\beta$ -estradiol-treated plants. Highlighted in grey are NO-regulated genes that were not significantly affected by RAP2.3 overexpression. Those highlighted in black contained putative RAP2.3 binding sites in their promoters.

#### 4.4. <u>Role of NO in constitutive and cold acclimation induced freezing</u> tolerance

This section 4.4 is composed by excerpts from the research articles: "Costa-Broseta, Á., Perea-Resa, C., Castillo, M. C., Ruíz, M. F., Salinas, J., & León, J. (2018). Nitric Oxide Controls Constitutive Freezing Tolerance in Arabidopsis by Attenuating the Levels of Osmoprotectants, Stress-Related Hormones and Anthocyanins. Scientific Reports, 8(1), 9268" and "Costa-Broseta, Á., Perea-Resa, C., Castillo, M. C., Ruíz, M. F., Salinas, J., & León, J. (2018). Nitric Oxide Deficiency Reduces CBF Induction, ABA Signaling, Anthocyanin Synthesis and Cold Acclimation in Arabidopsis". The second of these research articles was submitted to Journal of Experimental Botany and was under revision when PhD Thesis writing was finished. All the results and figures that appear here are derived from the work of the PhD student in collaboration with the other authors.

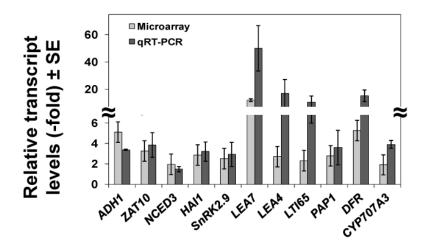
#### 4.4.1. Abstract (1)

Plant tolerance to freezing temperatures is governed by endogenous constitutive components and environmental inducing factors. Nitric oxide (NO) is one of the endogenous components that participate in freezing tolerance regulation. A combined metabolomic and transcriptomic characterization of NO-deficient *nia1,2noa1-2* mutant plants suggests that NO acts attenuating the production and accumulation of osmoprotective and regulatory metabolites, such as sugars and polyamines, stress-related hormones, such as ABA and jasmonates, and antioxidants, such as anthocyanins and flavonoids. Accordingly, NO-deficient plants are constitutively more freezing tolerant than wild type plants.

# 4.4.2. The transcriptome of NO-deficient *nia1,2noa1-2* mutant plants is enriched in cold-related transcripts

We previously reported that *nia1,2noa1-2* mutant plants, carrying mutations in both NIA1 and NIA2 nitrate reductases, as well as in the NO-Associated 1 (NOA1) protein, accumulated very low levels of endogenous NO under control and stressed conditions (Lozano-Juste & León, 2010). The strong NO deficiency of the mutant plants correlated with hypersensitivity to ABA in seed germination, stomata closure and tolerance to dehydration (Lozano-Juste & León, 2010). Intriguingly, our transcriptome analysis of *nia1,2noa1-2* mutants grown at 20°C (GEO identification number GSE41958) (Gibbs *et al.*, 2014a) revealed that around 20% (88/465) of the genes that were up-regulated in the mutant compared to wild-type plants (>2-fold; FDR<0.05) had been related to cold responses (Lee *et al.*, 2005; Kilian *et al.*, 2007). Among those genes (Table A8), some coded for Late Embryogenesis Abundant (LEA) proteins and for transcription factors belonging to the ERF/DREB, Zinc finger and WRKY families. Cold-induced *BCH2* and *NCED3* genes, encoding  $\beta$ -carotene hydroxylase 2 and 9-cis-epoxycarotenoid dioxygenase 3 enzymes involved in ABA biosynthesis, as well as *LOX4* and *OPR1* coding for jasmonate

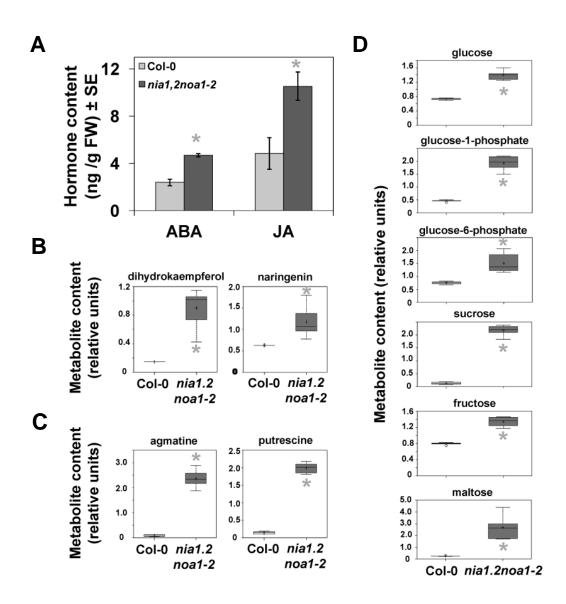
biosynthesis enzymes were also up-regulated in NO-deficient plants (Table A8). ABA and jasmonates have been reported to positively regulate freezing tolerance in Arabidopsis (Hu et al., 2013; Lee & Seo, 2015). Furthermore, a Gene Ontology analysis performed with the Arabidopsis thaliana dataset of the Gene Ontology Consortium (http://www.geneontology.org/) showed that 7 out of 19 and 15 out of 67 genes (20- and 12-fold enrichment with p-values of 2.51E-04 and 1.71E-08) involved in the anthocyanin and flavonoid metabolism functional categories, respectively, were up-regulated in the NO-deficient mutant plants. Accordingly, we found the anthocyanin and flavonoid biosynthesis and metabolism genes CHS, F3'H/TT6, DFR/TT3, PAP1/MYB75 and UF3GT among the cold-induced genes that were up-regulated in *nia1,2noa1-2* plants (Table A8). In addition, genes coding for SUS3, SSP2, and ADC2 enzymes involved in the biosynthesis of sugars and polyamines, respectively, were among the cold-inducible genes up-regulated in NO-deficient plants (Table A8). Sugars and polyamines had been reported to enhance plant-freezing tolerance (Kasukabe et al., 2004; Guy et al., 2008; Korn et al., 2008). To assess the robustness of the over-representation of cold-inducible genes detected in our transcriptomic analysis, the expression levels of 11 coldinduced genes, including ADH1, ZAT10, NCED3, HAI1, SnRK2.9, LEA7, LEA4-5, LTI65, PAP1/MYB75, DFR and CYP707A3, were determined by RT-qPCR in independent RNA samples from the triple *nia1,2noa1-2* mutant and Col-0 plants grown at 21 °C. In all cases, the transcript levels were significantly higher in mutant than in wild-type plants (Fig. 4.38), thus validating the microarray data. These observations indicated that, under control conditions, NO functions as a negative regulator of cold-induced gene expression in Arabidopsis.



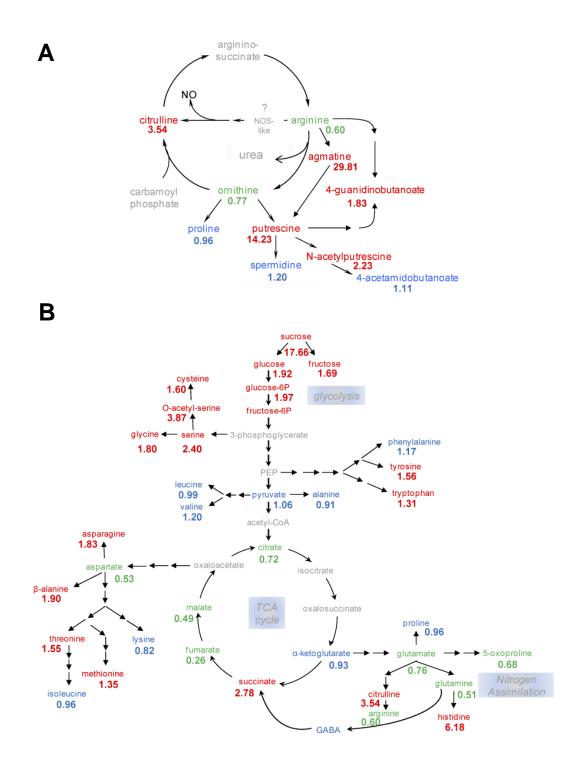
**Figure 4.38.** Levels of cold-inducible transcripts in Col-0 and *nia1,2noa1-2* plants. Comparative transcript analysis based on microarray data and RT-qPCR of wild-type Col-0 and NO-deficient *nia1,2noa1-2* plants. Ratio (*nia1,2noa1,2*/Col-0) values of RT-qPCR analysis are the mean of three independent biological replicates ± standard deviation.

## 4.4.3. Enhanced biosynthesis of ABA, JA and osmoprotective metabolites in NO-deficient plants

Data from microarray analyses strongly suggested that NO-deficient mutants should have increased levels of ABA, JA, anthocyanins, flavonoids, sugars and polyamines. Ultra Performance Liquid Chromatography-Mass Spectrometry analysis confirmed that, in fact, the levels of ABA and JA were around 2-fold higher in nia1,2noa1-2 than in wild-type plants (Fig. 4.39A). On the other hand, a combination of GC-MS and LC-MS techniques allowed quantifying 180 biochemicals including amino acids, carbohydrates, lipids, cofactors and prosthetic groups, nucleotides and secondary metabolites in wild-type and NO-deficient mutant plants (Table S2, which can be found in Costa-Broseta et al., 2018). As expected from the microarray data, the endogenous levels of flavonoids, anthocyanins, polyamines and sugars were significantly higher in mutant than in wild-type plants (Fig. 4.39B-D). The content of the flavonoids/anthocyanins dihydrokaempferol and naringenin in mutant plants were around 6- and 2-fold higher than in wild-type plants, respectively (Fig. 4.39B Table S2 in Costa-Broseta et al., 2018). Similarly, the polyamines agmatine and putrescine were around 30and 14-fold higher, respectively, in mutant than in wild-type plants (Fig. 4.39C; Table S2 in Costa-Broseta et al., 2018). Finally, the levels of glucose, glucose-1phosphate, glucose-6-phosphate, sucrose, fructose and maltose were increased from 2- to 18-fold in *nia1,2noa1-2* when compared to wild-type plants (Fig. 4.39D; Table S2 in Costa-Broseta et al., 2018). As shown in Figure 4.40A, the increased levels of polyamines correlated with reduced content of arginine and ornithine and increased levels of citrulline. On the other hand, the increased levels of sugars in nia1,2noa1-2 plants reflected a general accumulation of glycolisis metabolites and phosphoglycerate-derived amino acids of the serine family (Fig. 4.40B). In turn, metabolites of the tricarboxylic acids (TCA) cycle were significantly less abundant in *nia1,2noa1-2* than in wild-type plants (Fig. 4.40B). Accordingly, the levels of  $\alpha$ ketoglutarate-derived amino acids of the glutaminate synthetase-glutamine oxoglutarate aminotransferase (GS-GOGAT) cycle were also lowered in nia1,2noa1-2 plants (Fig. 4.40B), likely as a reflection of the impaired nitrate assimilation of the mutant plants. In summary, NO seems to exert a metabolic brake in the production of ABA, JA, anthocyanins, flavonoids, sugars and polyamines under standard conditions.



**Figure 4.39.** Levels of hormones and osmoprotective metabolites in Col-0 and *nia1,2noa1-2* plants. A, Quantification of ABA and JA, B, flavonoids / anthocyanins, C, polyamines, and D, sugars, was performed by GC- and LC-mass spectrometry. Hormone content values represent the mean values of four independent biological replicate samples for each genotype ± standard error. \* indicates significantly different with p-value < 0.05 in Student's t-test. For the metabolomic analyses of the other metabolites, Welch's two-sample t-test was used to identify biochemicals that differed significantly between experimental groups. An estimate of the false discovery rate (q-value) was calculated to take into account the multiple comparisons.



**Figure 4.40. Glycolysis and TCA cycle metabolite ratios between** *nia1,2noa1-2* and **Col-0 plants.** Metabolites in red and green were significantly more or less abundant in *nia1,2noa1-2* than in Col-0 plants, respectively. Metabolites in blue were not significantly changed. Values indicated for each metabolite are the mean of six independent replicates performed in the complete metabolomic analyses described in Table S2 in Costa-Broseta *et al.*, 2018.

#### 4.4.4. Increased levels of antioxidant metabolites in *nia1,2noa1-2* plants

As shown in Table S2 (Costa-Broseta et al., 2018), the ascorbate and oxidized glutathione (GSSG) were both elevated in *nia1,2noa1-2* plants. Moreover, other metabolites with antioxidant activity such as the flavonoids dihydrokaempferol and naringenin (Fig. 4.39B) as well as sinapate (Table S2 in Costa-Broseta et al., 2018) accumulated also in NO-deficient plants. We also found around 3-fold accumulation of the oxylipins 9-hydroxyoctadecadienoic acid (9-HODE) and 13hydroxyoctadecadienoic acid (13-HODE) (Table S2 in Costa-Broseta et al., 2018), which can be synthesized enzymatically by lipoxygenases but also nonenzymatically from ROS (Berger et al., 2001), and are considered good markers of oxidative stress (Yoshida et al., 2013). These data strongly suggested that NOdeficient nia1,2noa1-2 mutant plants were subjected to constitutive oxidative stress. Under those conditions, the ascorbate-glutathione cycle is in charge of detoxifying reactive oxygen species. As shown in Figure 4.41, the increased levels of ascorbate and oxidized glutathione were accompanied by significant increases of glutathione precursors, such as methionine, S-adenosylhomocysteine, cysteine and glycine, as well as by a reduced content of nitrogen-related amino acids including glutamate, glutamine and aspartate.

#### 4.4.5. NO negatively regulates constitutive freezing tolerance of Arabidopsis

The results described above strongly suggested that NO should have a negative role in the constitutive freezing tolerance of Arabidopsis. To test this possibility, we analyzed the constitutive freezing tolerance of 2-week-old wild-type and *nia1,2noa1-2* plants. Freezing tolerance was determined as the percentage of surviving plants after exposure to different freezing temperatures for 6 h. Figure 4.42A shows that triple nia1nia2noa1-2 mutant plants displayed significantly greater freezing tolerance than did wild-type plants, the LT<sub>50</sub> (temperature that causes 50% lethality) value being -5.6°C and -4.5 °C, respectively. However, the double *nia1nia2* mutant plants (LT<sub>50</sub> -4.6 °C) were not significantly different than wild type plants and the single *noa1-2* mutant plants were slightly more tolerant (LT<sub>50</sub> -4.8 °C) than wild type plants. Despite *nia1,2noa1-2* plants being slightly delayed in their development compared to Col-0 plants, the increased freezing tolerance manifested by the mutant with respect to the wild-type plants was very apparent (Fig. 4.42B). The endogenous NO levels of wild type and mutant plants were measured by staining with the NO-specific fluorophore staining DAF-FM DA and we found that, as expected, *nia1,2noa1-2* plants contained significantly less NO than Col-0 plants (Fig. 4.42C). These results demonstrated that NO negatively regulates constitutive freezing tolerance in Arabidopsis, in all likelihood, by controlling the levels of osmoprotectant, hormones and redox metabolites.

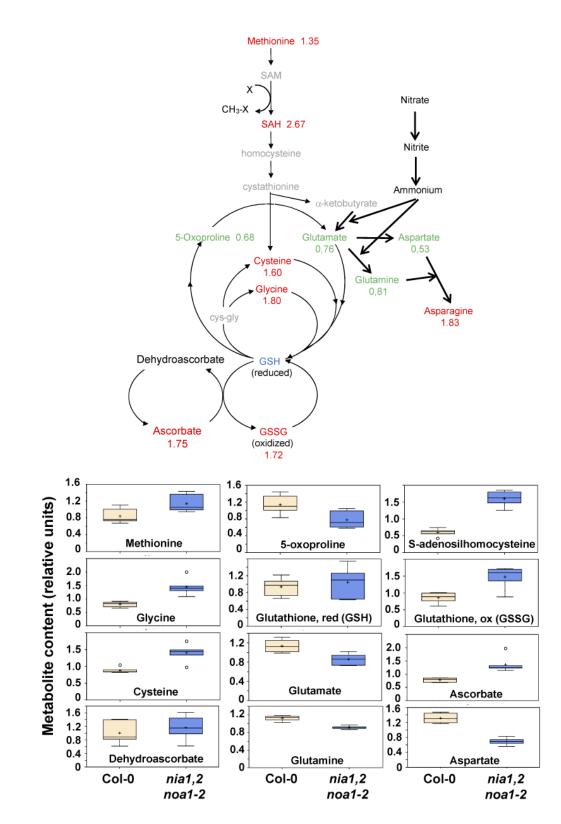


Figure 4.41. Endogenous content of ascorbate-glutathione cycle metabolites in wild type and NO-deficient plants. A diagram of the ascorbate-glutathione cycle is shown at top of the figure. Metabolites in red and green were significantly more or less abundant in *nia1,2noa1-2* than in Col-0 plants, respectively. The box plots corresponding to the metabolites significantly different in both genotypes are shown in the bottom part of the figure. Values indicated for each metabolite are the mean of six independent replicates performed in the complete metabolomic analyses described in Table S2 in Costa-Broseta *et al.*, 2018.

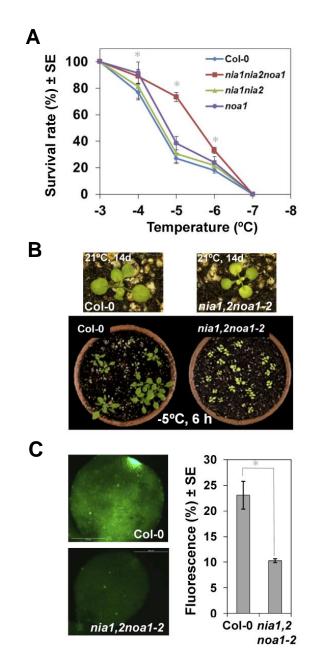


Figure 4.42. Constitutive freezing tolerance of Col-0 and *nia1,2noa1-2* plants. A, Freezing tolerance of 2-week-old plants exposed for 6 h to the indicated freezing temperatures was estimated as the percentage of plants surviving each specific temperature after 7 d of recovery under control conditions at 21°C. Data are expressed as means of three independent experiments with around 50 plants each indicated genotype  $\pm$  standard deviation. Asterisks indicate significant differences between *nia1,2noa1-2* and wild-type plants (p-value < 0.05). b, Upper panels show individual plants of wild type and mutant genotypes before freezing to show the difference in size. The bottom panel shows a representative image of plants from both genotypes after freezing at -5°C and recovery at standard growing temperature for additional 7 days. C, NO levels in Col-0 and *nia1,2noa1-2* plants. Plants were maintained at standard growing conditions for 14 days. The fluorescence of DAF-FM DA-treated plants was detected by confocal microscopy. Shown images are representative of four to six different analyzed plants per genotype and condition, and the quantification values are the mean  $\pm$  standard error. \* indicates significant differences between *nia1,2noa1-2* and wild-type plants (p-value < 0.05).

#### 4.4.6. Abstract (2)

Plant tolerance to freezing temperatures is governed by endogenous components and environmental inducing factors. Exposure to low non-freezing temperatures has been characterized as the main exogenous factor inducing freezing tolerance in a process named cold acclimation. To define the role of nitric oxide (NO) in cold acclimation, we have used triple *nia1,2noa1-2* mutant plants that have impaired not only the nitrate-dependent but also the nitrate-independent NO production, and are thus severe NO-deficient plants. We demonstrate that the cold-induced accumulation of NO is essential to promote full cold acclimation response through the up-regulation of *C-repeat Binding Factor* (*CBF*) gene expression, the perception and signaling of ABA, and the cold-induced production of anthocyanins. Our results reveal ABA perception and signaling as well as anthocyanin production as new relevant factors in the NO-regulated capacity of plants to cold acclimate.

## 4.4.7. NO functions as a positive regulator of cold acclimation in Arabidopsis

The accumulation of large amounts of NO in Arabidopsis during cold acclimation was previously reported (Zhao *et al.*, 2009; Cantrel *et al.*, 2011). We also found that after cold-acclimation for 7 days at 4°C, wild-type leaves accumulated 16-fold more NO than non-acclimated leaves (Fig. 4.43). In turn, the levels of NO in triple *nia1,2noa1-2* mutant leaves subjected to the same conditions accumulated only less than 4-fold compared to that in non-acclimated leaves (Fig.

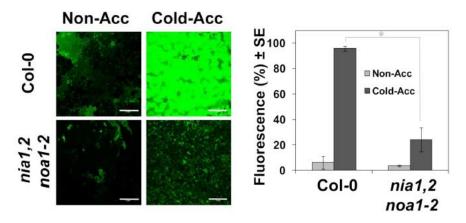
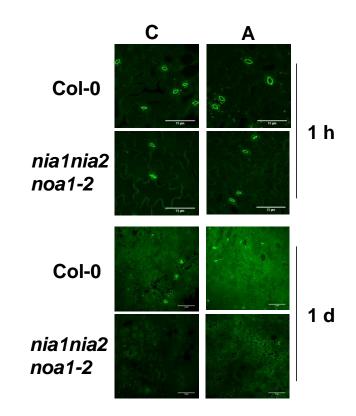


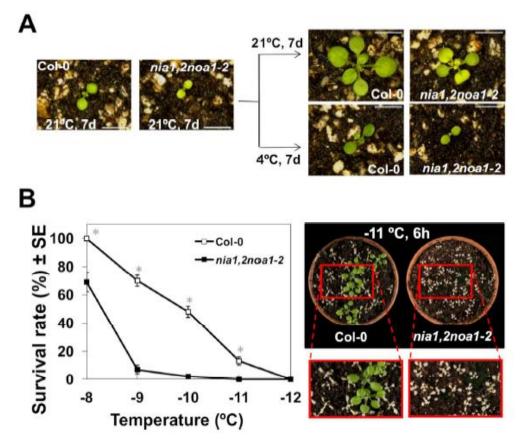
Figure 4.43. NO levels in cotyledons of Col-0 and *nia1,2noa1-2* plants after cold acclimation. Plants were either maintained at standard growing conditions (Non-Acc) or cold acclimated at 4°C (Cold-Acc) for 7 days. The fluorescence of DAF-FM DA-treated plants was detected by confocal microscopy. Shown images are representative of four to six different analyzed plants per genotype and condition, and the quantification values are the mean  $\pm$  standard error. \* indicates significant differences between *nia1,2noa1-2* and wild-type plants (p-value < 0.05). Scale bars correspond to 25 µm.

4.43). We did not find significant changes in the NO content at shorter times of cold treatment, when full cold acclimation has still not been fully accomplished (Fig. 4.44).



**Figure 4.44. NO levels in Col-0 and** *nia1,2noa1-2* **plants at different times after cold treatment.** Plants were grown in soil under standard conditions long day photoperiod light conditions and 21°C for 14 d, and then, treated with the specific NO stain DAF-FM DA for 1h under darkness. After extensive washings, plants were transferred to 4°C (A) or kept at 21°C as control (C) under similar light regime for the indicated times in the right sides of panels. Green fluorescence was visualized with confocal microscopy (40x) of stained cotyledons. Shown images are representative of four to six different analyzed plants per genotype and condition.

Given the remarkable low levels of NO that the triple mutant accumulated in response to 4°C we decided to determine its capacity to cold acclimate. The analysis of the freezing tolerance of cold acclimated (7d, 4°C) Col-0 and NO-deficient *nia1,2noa1-2* plants revealed significant differences. As previously reported (Lozano-Juste & León, 2010), *nia1,2noa1-2* plants display smaller size than Col-0 plants when growing under control conditions but both retarded their growth upon exposure at 4°C (Fig. 4.45A). Freezing tolerance was determined as the percentage of surviving plants after exposure to different freezing temperatures for 6 h. Cold-acclimated mutant plants exhibited significantly lower capacity to tolerate freezing temperatures than cold-acclimated wild-type plants, the LT<sub>50</sub> being -8.3°C and -9.8°C, respectively (Fig. 4.45B). These results indicated that NO plays a positive role in Arabidopsis cold acclimation and also that is required for full development of this adaptive process. However, the defective nitrogen assimilation



**Figure 4.45. Cold acclimation-induced freezing tolerance of Col-0 and** *nia1,2noa1-2* **plants.** A, Panels show representative individual plants of wild type and mutant genotypes grown under standard conditions for 7 d before being shifted to 4°C for additional 7 d (cold-acclimated) or kept for 7 d more at 21°C (non-acclimated as control). B, Freezing tolerance of 2-week-old plants exposed for 6 h to the indicated freezing temperatures after being acclimated for 7 d at 4°C. In all cases, freezing tolerance was estimated as the percentage of plants surviving each specific temperature after 7 d of recovery under control conditions at 21°C. Data are expressed as means of three independent experiments with around 50 plants each ± standard deviation. \* indicates significant differences between *nia1,2noa1-2* and wild-type plants (p-value < 0.05). The right panel in B shows a representative image of plants from both genotypes after cold acclimation, freezing and recovery at standard growing temperature for additional 7 days. Close-up images corresponding to the framed areas are included. Scale bars correspond to 5 mm in panel A.

of *nia1,2noa1-2* plants could also be responsible of their reduced cold acclimationinduced freezing tolerance. Regarding this, we have observed that wild type shoots produced more NO when growing in nitrate-containing media than when growing on nitrite or ammonium as unique N sources, or subjected to N starvation (Fig 4.46). This enhanced NO production in nitrate-grown plants was dependent on nitrate reductase activity as it was not detected in *nia1,2* mutant plants (Fig. 4.46).

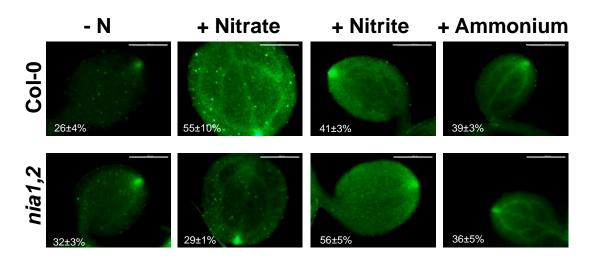


Figure 4.46. Effect of different nitrogen sources on NO production. The NO content was visualized by staining with DAF-FM DA in wild type Col-0 and *nia1,2* seedlings grown in MS media depleted of N (-N), or supplemented with 5 mM of nitrate, 5 mM of nitrite or 2.5 mM of ammonium as indicated as unique nitrogen sources. Quantification of fluorescence is shown as the mean of at least 3 replicate experiments ± standard error. Size bars represent 100  $\mu$ m.

## 4.4.8. NO promotes the cold-induced expression of *CBF* genes in Arabidopsis

As already mentioned, the process of cold acclimation involves many physiological and biochemical changes, most of them being controlled by low temperature through changes in gene expression (Knight & Knight, 2012). In Arabidopsis, different signaling pathways have been identified that regulate these changes. The best characterized and, likely, the most relevant is the pathway mediated by the CBFs, a small family of transcription factors (CBF1-3) whose corresponding transcripts accumulate transiently in response to low temperature (Medina et al., 2011; Knight & Knight, 2012). We, therefore, analyzed if the impaired ability to cold acclimate of *nia1,2noa1-2* plants might be due to a reduced cold induction of the CBF genes. Figure 4.47A shows that, after 6h of exposure to 4°C, the induction of CBF1, 2 and 3 in nia1,2noa1-2 was significantly lower than in wild-type plants. This defective induction was specially accentuated in the case of CBF1 gene. Accordingly, COR15A, LTI65 and LTI78 genes, which are well characterized targets of CBFs (Thomashow, 1999), were significantly less induced upon cold treatment in nia1,2noa1-2 than in Col-0 plants (Fig. 4.47B). NO thus functions as a positive regulator of cold acclimation likely by promoting the coldinduced expression of CBF genes and their corresponding gene targets.

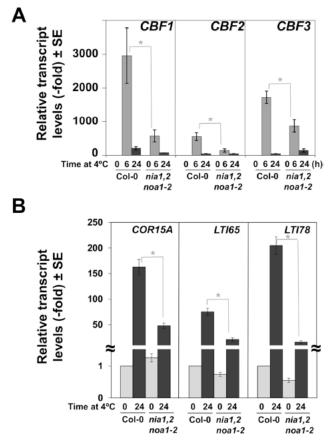


Figure 4.47. Effects of cold acclimation on the *CBFs* and their targets in Col-0 and *nia1,2noa1-2* plants. A, *CBF1*, *CBF2*, *CBF3* and B, *COR15A*, *LTI65* and *LTI78* transcript levels were quantified by RT-qPCR from total RNAs isolated from plants of the genotype and time of  $4^{\circ}$ C incubation indicated. Values are the mean of three independent biological replicate samples for each genotype and condition ± standard error. \* indicates significantly different with p-value  $\leq 0.05$  in Student's t-test.

### 4.4.9. NO regulates the sensitivity of Arabidopsis to ABA in response to low temperature

Phytohormones have also been reported to play important roles in regulating the cold acclimation process in Arabidopsis (Eremina *et al.*, 2016a). In particular, ABA is essential for the adequate development of this adaptive response as evidenced by the fact that low temperature induces increased levels of ABA, and also because ABA-deficient and -insensitive mutants of Arabidopsis are defective in cold acclimation (Nakashima *et al.*, 2014; Ding *et al.*, 2015). To assess whether NO could regulate cold acclimation in Arabidopsis by controlling ABA levels or ABA sensitivity, we first measured the levels of ABA in *nia1,2noa1-2* mutants and wild-type plants after 24 h exposure to 4°C. Both wild type and NO-deficient plants increased their ABA content and reached values that were not significantly different after exposure to low temperature (Fig. 4.48A), suggesting that ABA homeostasis was not significantly altered in NO-deficient plants during the cold acclimation process. Then, we examined the capacity of *nia1,2noa1-2* mutants to correctly sense and signal ABA by analyzing the levels of different ABA-related signaling

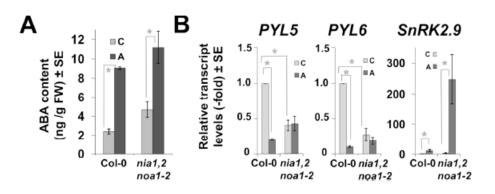


Figure 4.48. ABA homeostasis and signaling in cold acclimated wild-type and NOdeficient plants. A, Quantification of ABA in control non-acclimated C, and cold-acclimated A Col-0 and *nia1,2noa1-2* plants. Values represent the mean values of four independent biological replicate samples for each genotype and condition  $\pm$  standard error. B, The transcript levels of the ABA signaling genes *PYL5, PYL6* and *SnRK2.9* were quantified by RT-qPCR from three independent total RNAs isolated from plants by 24 h of 4°C incubation. Values are the mean of three independent biological replicate samples for each genotype and condition  $\pm$  standard error. \* indicates significantly different with p-value  $\leq$  0.05 in Student's t-test.

transcripts. As shown in Figure 4.48B, the transcripts of two closely related genes coding for the ABA receptors PYL5 and PYL6 were strongly down-regulated in response to low temperature in wild-type plants. In turn, no such repression was observed in the *nia1,2noa1-2* plants, which indeed showed levels in control plants comparable to those detected under cold conditions in Col-0 plants (Fig. 4.48B). Moreover, the levels of *SnRK2.9* transcripts in *nia1,2noa1-2* plants were more than 20-fold higher than in Col-0 plants after cold treatment (Fig. 4.48B). These findings, together with the ABA hypersensitive phenotype we previously reported for *nia1,2noa1-2* plants (Lozano-Juste & León, 2010), strongly suggested that NO acts on ABA perception and signaling more than in ABA homeostasis to regulate cold acclimation.

## 4.4.10. NO activates anthocyanin accumulation in Arabidopsis during cold acclimation

It is well documented that, in response to low temperature, anthocyanins accumulate to protect photosystems from photoinhibition, avoiding the concentration of high levels of reactive oxygen species (Krol *et al.*, 1995; Korn *et al.*, 2008; Schulz *et al.*, 2016). Consistent with these results, it has been described that the expression of genes coding for critical enzymes in the anthocyanin and flavonoid biosynthetic pathway were induced by low temperature, and that the accumulation of these pigments was required to ensure full development of cold acclimation in Arabidopsis (Catala *et al.*, 2011; Schulz *et al.*, 2016). Hence, we analyzed the content of anthocyanins in *nia1,2noa1-2* and Col-0 plants after being subjected to 4°C for 7 days. As shown in Figure 4.49A, wild-type plants increased their anthocyanin content more than 4-fold in response to low temperature. In

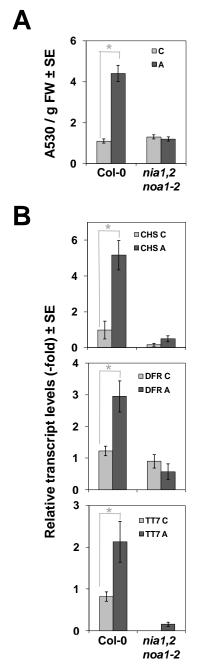


Figure 4.49. Effects of cold acclimation on anthocyanin synthesis in Col-0 and *nia1,2noa1-2* plants. A, Anthocyanin content was spectrophotometrically quantified. Data represent the means of three independent experiments with 25 and 10 plants each. B, Transcript levels of anthocyanin biosynthetic genes chalcone synthase (*CHS*), dihydroflavonol reductase (*DFR*) and flavonol 3-hydroxylase (*TTT/F3H*) genes were quantified by RT-qPCR from total RNAs isolated from plants of the indicated genotype either acclimated A for 24 h at 4°C or non-acclimated as control C. Values are the mean of three independent biological replicate samples for each genotype and condition  $\pm$  standard error. \* indicates significantly different with p-value  $\leq 0.05$  in Student's t-test.

contrast, *nia1,2noa1-2* plants did not accumulate anthocyanins when exposed to cold. As expected from these findings, the cold induction of several genes involved

in anthocyanin biosynthesis, such as *CHS*, *DFR* and *TT7*, was blocked in the NOdeficient plants (Fig. 4.49B). Together, these results indicated that NO positively regulates cold acclimation in Arabidopsis also by activating the cold-induced expression of genes implicated in anthocyanin biosynthesis, hence promoting anthocyanin accumulation. Results

Discussion

Discussion

#### 5. DISCUSSION

The figures 5.2, 5.3, 5.5 and 5.6, and part of the information displayed in this section 5 are excerpts of the research articles: "León, J., Costa, Á., & Castillo, M. C. (2016). Nitric oxide triggers a transient metabolic reprogramming in Arabidopsis. Scientific Reports, 6, 37945", "Costa-Broseta, Á., Perea-Resa, C., Castillo, M. C., Ruíz, M. F., Salinas, J., & León, J. (2018). Nitric Oxide Controls Constitutive Freezing Tolerance in Arabidopsis by Attenuating the Levels of Osmoprotectants, Stress-Related Hormones and Anthocyanins. Scientific Reports, 8(1), 9268", "Castillo, M. C., Coego, A., Costa-Broseta, Á., & León, J. (2018). Nitric oxide responses in Arabidopsis hypocotyls are mediated by diverse phytohormone pathways. Journal of experimental botany, 69(21), 5265-5278", "Costa-Broseta, Á., Castillo, M. C., & León, J. (2018). Protein Stabilization and Post-translational Modifications Control NO Homeostasis in Arabidopsis", "León, J., Costa-Broseta, A., & Castillo, M. C. (2018). RAP2.3 negatively regulates nitric oxide biosynthesis and sensing through a rheostat-like mechanism in Arabidopsis" and "Costa-Broseta, Á., Perea-Resa, C., Castillo, M. C., Ruíz, M. F., Salinas, J., & León, J. (2018). Nitric Oxide Deficiency Reduces CBF Induction, ABA Signaling, Anthocyanin Synthesis and Cold Acclimation in Arabidopsis". The last three articles were submitted to Plant Physiology, The Plant Journal and Journal of Experimental Botany, respectively and were under revision when PhD Thesis writing was finished. All the results and figures that appear or are discussed here are derived from the work of the PhD student in collaboration with the other authors.

Plant responses to NO at the molecular level comprise three main different processes: alteration of gene expression, post-translational modification of proteins and metabolism reprogramming. Despite all the efforts to decipher the mechanisms underlying these responses in the last years, NO perception in plants remains mostly unknown. Moreover, researchers have been trying for decades to elucidate the way NO is synthesized and regulated in plants but the picture is still nowadays incomplete. The present work aims to shed light upon NO production and sensing although further research will be required for the full support of the presented hypothesis.

As a free radical gas, NO is very reactive and highly diffusible. This two features make difficult for NO molecules to reach distant locations from the spot they were synthetized in the cell, but, at the same time, very few structures are able to hamper its diffusion. Therefore, NO content inside cells should form sharp concentration gradients, with the highest concentrations around the microenvironment where it is produced. Thus, a short pulse of a high dose of exogenously applied gas represents an attempt to mimic conditions of high local accumulation of NO, similar to those that can occur under stress. On the other hand, the triple *nia1,2noa1-2* mutant plants, which are defective in NO production, represent an invaluable tool

to mimic conditions of NO shortage in plants (Fig. 4.42C). These plants are impaired not only in nitrate reductase-mediated but also in nitrate-independent NOA1-associated production of NO (Lozano-Juste & León, 2010). In this work, NO fumigation and *nia1,2noa1-2* were used as alternative experimental systems to investigate, at genome-wide scale, sensing and signaling associated to NO in plants by conducting two different transcriptomic analyses. First, early plant responses to NO were studied by analyzing changes in the transcriptome shortly after treating plants with a pulse of pure NO gas (Supplementary Tables S2, S3 and S4 in Castillo *et al.*, 2018). Second, the effects of NO endogenous deficit were investigated by comparing the transcriptomes of wild type and *nia1,2noa1-2* plants (GEO code GSE41958).

Endogenous NO has been reported to positively regulate the photomorphogenesis via the control of processes like the hypocotyl elongation (Lozano-Juste & Leon, 2011) and apical hook opening (Abbas et al., 2015) in etiolated seedlings. To identify the regulatory components implicated in NO sensing, the information obtained from the transcriptomic analysis of plants exposed to a NO pulse was combined with a screening of TPT transgenic lines conditionally expressing single TFs (Fig. 4.14, Coego et al., 2014) relying on a NO sensitivity assay that measures the inhibition of hypocotyl elongation after exposure of etiolated seedlings to NO. Despite the high reactivity of NO, most of the changes observed in the transcriptomic data occurred by one hour after the NO pulse (Fig. 4.13B), being consistent with the first significant altered pattern of NOrelated post-translational protein modifications, observed also one hour after exposure to NO (Fig. 4.24B), and the later extensive metabolic reprogramming detected six hours after treatment (Fig. 4.23A, B, Supplementary Table S1 in León et al., 2016). Due to the lack of a true NO receptor in plants, the way the plants sense NO would mainly depend on the NO-triggered PTMs in key signaling proteins. Modified proteins would then transmit the signal to other amplifying signaling proteins, thus modulating the transcriptome and eventually the metabolome. A strong evidence suggesting that hormone biosynthesis and signaling pathways are direct targets of NO action came from the characterization of the NO-responsive transcriptome (Supplementary Table S3 in Castillo et al., 2018). Moreover, the genetic approach based on the screening of TPT lines for NO sensitivity allowed confirming the NO-phytohormone connection.

The inability of ethylene-related mutants to properly sense NO (Fig. 4.16A) suggests a key role of ethylene signaling in hypocotyl NO sensing. Both perception and signaling of this hormone would be necessary for NO to trigger inhibition of hypocotyl elongation under darkness. Despite the complex and sometimes controversial interaction between ethylene and NO (Gniazdowska *et al.*, 2007; Montilla-Bascón *et al.*, 2017), it has been recently reported that the NO control on cell cycle progression requires the function of EIN2 in Arabidopsis cell cultures (Novikova *et al.*, 2017), thus suggesting this mechanism could be on the basis of the hypocotyl growth inhibition by NO. Ethylene involvement in NO sensing and

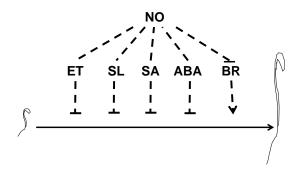
response is further supported by the identification of large number of ethylenerelated transcription factors in our screening of TPT lines. Among the identified TFs, the AP2-related and integrase-type ORA47 and RAP2.6L (Krishnaswamy et al., 2011; Chen et al., 2016) were found, as well as four additional integrase-type ERF TFs ERF014, ERF037, ERF056 and ERF113/RAP2.6L and the ethylene responsive element binding factor 1 (ATERF-1), all of them related to ethylene signaling. In addition, the inhibition of hypocotyl elongation by NO requires also the SL biosynthesis and signaling, as SL mutants were insensitive to NO (Fig. 4.18B). These data would be consistent with NO being involved in SL-exerted opposite regulatory effects in root and shoot growth as reported (Jia et al., 2014; Manoli et al., 2016; Sun et al., 2016). Nevertheless, the interaction between NO and SLs seems to be very complex as recently reviewed (Kolbert, 2018). Despite NO lightly induces some of the SL biosynthesis genes (Fig. 4.18C), whether NO can trigger SL production or not will require to measure SL levels in this plants. On the other hand, SA biosynthesis and accumulation seems to be also essential for hypocotyl NO sensing, as a SA-deficient plants were fully insensitive to NO-triggered inhibition of hypocotyl elongation (Fig. 4.18A). The complicated relation reported between the production and signaling of NO, SLs and SA (Tari et al., 2011; Bharti & Bhatla, 2015; Rozpadek et al., 2018) suggests the existence of a self-regulatory hormonal loop involved in ensuring the correct NO sensing under different conditions and/or organ/cellular locations.

A functional interaction between NO and ABA is also depicted by the NOtriggered up-regulation of a subset of ABA signaling genes (Supplementary Table S2 in Castillo *et al.*, 2018, Fig. 4.19A-C). Further support to this interaction comes from the partially insensitive phenotype displayed by the double ABA receptor *pyl6pyl7* mutant plants (Fig. 4.19D). Previously reported data suggest that NO modulates the activity and stability of the ABA receptors through PTMs (Castillo *et al.*, 2015), thus supporting a tight functional link between NO and ABA. Also the ABA involvement in NO sensing was supported by the identification of ABA-related TFs in the screening of TPT lines. Among those TFs, MYB-type HRS1 and MYB30 (Wu *et al.*, 2012; Lee & Seo, 2016) and NAC058 (Coego *et al.*, 2014) are functionally related to ABA homeostasis or signaling.

Lastly, the overlapping found between NO- and BR-responsive transcriptomes (Fig. 4.21) suggested also BRs could be involved in NO sensing. However, a complex connection between NO and BRs in the regulation of the hypocotyl elongation in etiolated seedlings seems to operate. The BR-deficient *det2-1* mutant despite displaying short hypocotyls was fully sensitive to NO (Fig. 4.22C), thus suggesting BR biosynthesis is not required for NO sensing. Besides, the gain-of-function *bes1-D* mutant in BES1/BZR2 BR signaling component was hypersensitive to NO (Fig. 4.22C). Because *BES1/BZR2* gene was found to be downregulated by NO in the transcriptomic analysis (Fig. 4.17C), this TF might represent a potential node for a NO-related regulatory loop.

It is noteworthy mentioning that despite a large amount of jasmonate-related genes were found to be NO-responsive (Supplementary Table S3 in Castillo *et al.*, 2018), none of the mutants in biosynthetic or signaling JA components tested were altered in hypocotyl NO sensing (Fig. 4.20B). These data suggest that JA is likely not implicated in sensing NO in etiolated hypocotyls but do not rule out the potential involvement of JA in NO sensing in other organs/tissues or plants under different conditions.

Potential hormone-related NO targets as described above are likely affected by NO-triggered PTMs. An in silico prediction for these types of post-translational modifications for those potential targets (Table A6) shows that all analyzed proteins may be potentially S-nitrosylated or nitrated. Among them, some residues were predicted to be more likely modified as prediction coincided in two different platforms. This is the case for the S-nitrosylation of C1063 and C1218 of EIN2, and C63 of MAX2, as well as for the nitration of Y783 of EIN2 and Y176 of PYL6 (Table A6). However, these are just predictions and none of these proteins have been identified yet as post-translationally modified. Further proteomic work will clarify whether the NO-related post-translational modifications of these signaling proteins could be important for the NO sensing mechanisms operating in etiolated hypocotyls. Altogether, NO sensing in Arabidopsis hypocotyls needs at least the biosynthesis and/or signaling of ethylene, SLs, SA and ABA, while the negative regulation of BR signaling genes by NO also contributes to the NO-triggered inhibition of hypocotyl elongation (Fig. 5.1). As endogenous levels of NO and hormones may change from one tissue to another, future work will clarify if this model of NO sensing may be extrapolated to other organs.



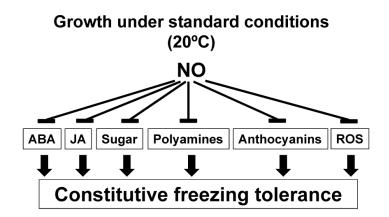


As a complementary approach to the analysis of the effects of an acute NO dose, the effects of constitutively low endogenous NO content in *nia1,2noa1-2* plants were also studied in this work. In the analysis of the differential transcriptome of NO-deficient plants (GEO code GSE41958), neither a master regulator of the NO-

triggered responses nor NO-specific gene markers were identified. These data support that regulatory functions of NO are exerted in coordination with other regulators through diverse signaling pathways, as in the above mentioned control over hypocotyl elongation. In agreement with this hypothesis, several genes that were up-regulated in the NO-deficient mutant compared to the wild type, coded for enzymes involved in JA and ABA biosynthesis (Table A8). Supporting these data, nia1,2noa1-2 plants contain indeed a significantly higher content of ABA and JA than wild-type plants (Fig. 4.39A). Interestingly, an overall analysis of the upregulated transcriptome in NO-deficient plants showed a significant over representation of genes implicated in cold responses and freezing tolerance (Table A8). ABA and JA have been reported to play a role in plant response to freezing stress (Llorente et al., 2000; Hu et al., 2013), so its higher contents in nia1,2noa1-2 plants should contribute to their enhanced constitutive freezing tolerance. In addition, a metabolomic analysis of nia1,2noa1-2 plants compared to Col-0 wild type plants revealed that mutant plants also accumulated high amounts of osmoprotective metabolites such as sugars, polyamines, and antioxidant metabolites, including anthocyanins and other flavonoids (Fig. 4.39B, C, D, Table S2 in Costa-Broseta et al., 2018), which, in all likelihood, limit the impact of the freezing-related damage. Sugars and polyamines have been reported to increase the freezing tolerance (Alet et al., 2011; Nägele et al., 2012) while flavonoids seem to exert a key antioxidant protection against the damage caused by the photoinhibition at low temperatures when the photosynthesis rate decreases (Schulz et al., 2016).

As suggested by the transcriptomic and metabolomic data, the survival assays to freezing conditions showed that indeed NO-deficient mutant plants were constitutively more tolerant to freezing than wild type plants (Fig. 4.42A). However this result comes into conflict with previous works with the double mutant nia1.2 that describe NO being required for constitutive freezing tolerance (Zhao et al., 2009; Cantrel et al., 2011). This discrepancy could be explained by the differences in the experimental conditions. Freezing assays with the double mutant in previous works were performed in Petri dishes with MS media supplemented with agar and sucrose, whereas in this work the assays were performed using plants grown on soil. These two experimental systems are quite different in terms of humidity and sugar availability, factors that affect freezing tolerance. Another factor that could explain this discrepancy is the difference in NO and proline content between the two mutants used. Due to the additive effect of the mutations, triple mutant has lower endogenous levels of NO than nia1,2 (Lozano-Juste & León, 2010). The proline content was found to be the same in *nia1,2noa1-2* and wild type plants (Table S2 in Costa-Broseta et al., 2018). It was previously reported an increase in proline in wild type plants exposed to cold but not in *nia1,2* plants (Zhao et al., 2009). However, despite the reported increases in proline content during cold exposure, it has been also proposed that there is no correlation with enhanced freezing tolerance (Zuther et al., 2012).

All these data together suggest that NO would function as a negative regulator of the constitutive freezing tolerance in Arabidopsis by altering hormone homeostasis and by reducing the synthesis of osmoprotective and antioxidant metabolites. Although the mechanisms by which NO exerts this function haven't been properly studied yet, it is very likely that it would regulate these processes by triggering PTMs on proteins with a relevant function in related metabolic routes. Previous work identified several proteins involved in metabolism of C, N and S to be nitrated in vivo in a proteomic assay (Lozano-Juste et al., 2011). Besides, it has been reported that several of the enzymes involved in antioxidant systems undergo NOtriggered PTMs (Begara-Morales et al., 2016). Although further investigation will clarify the real relevance of PTMs in the mechanisms underlying the regulation of freezing tolerance, the low levels of NO in the NO-deficient triple mutant should lead to a lower content in nitrated proteins which could explain the great differences between the molecular phenotypes of the wild type and mutant plants (Table S2 in Costa-Broseta et al., 2018). NO would scavenge ROS and suppress the metabolic changes needed for the increase of the content of polyamines, sugars, flavonoids, ABA and JA (Fig. 5.2).



**Figure 5.2. Model of NO involvement in the regulation of constitutive freezing tolerance.** Blunt ended and black solid arrows represent negative and positive regulation on freezing tolerance, respectively. ABA, JA and ROS mean Abscisic acid, Jasmonates and Reactive Oxygen Species, respectively.

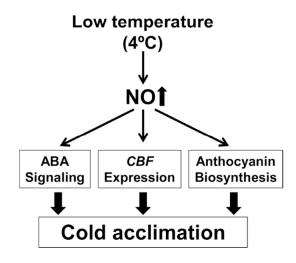
Given the improved constitutive freezing tolerance of *nia1,2noa1-2* plants, their cold acclimated-induced freezing tolerance was also tested and compared to wild type plants. Interestingly, it turned out that the triple mutant was impaired in its ability to cold acclimate (Fig. 4.45B), thus confirming previous research suggesting that NO is required for correct development of cold acclimation (Zhao *et al.*, 2009; Cantrel *et al.*, 2011; Puyaubert & Baudouin, 2014). Supporting this, the cold-induced large increase in the endogenous NO content was observed to be very slight in the NO-deficient mutant (Fig. 4.43) as was previously described for the double mutant *nia1,2* (Zhao *et al.*, 2009). However, it is noteworthy mentioning that

in this work the increase of NO levels during acclimation at 4°C was not observed after few hours of cold (Fig. 4.44) as previously reported (Zhao *et al.*, 2009; Cantrel *et al.*, 2011), but only after 7 days of cold treatment (Fig. 4.43). These long periods have been reported to be required for full cold-acclimation (Tähtiharjau & Palva, 2001; Kawamura & Uemura, 2003). Differences in humidity conditions between the experimental systems used as well as specific contributions of the *noa1-2* mutation to the cold-acclimation phenotype could be on the basis of the observed changes in the pattern of NO accumulation.

Three main factors have been found to explain the involvement of NO in the regulation of cold acclimation. In first place, as previously described by using the double mutant *nia1,2* (Cantrel et al., 2011), NO would induce the expression of CBFs and therefore their gene targets in response to low temperature (Fig. 4.47A,B), but also would control the process of cold acclimation through CBFindependent regulatory pathways. Second, NO was found to promote the induction of genes involved in the biosynthesis of anthocyanin and other flavonoids (Fig. 4.49B). Accordingly, after acclimation anthocyanin levels were strongly increased in wild type but not in *nia1,2noa1-2* plants (Fig. 4.49A). Lastly, a differential expression pattern observed for ABA signaling pathway-related genes under low temperature conditions between the triple mutant and the wild type plants (Fig. 4.48B) suggests that NO controls ABA perception and signaling but not its homeostasis during cold acclimation (Fig. 4.48A). The importance of flavonoids and ABA in cold acclimation and freezing tolerance has been discussed above, but it is important to highlight the role of flavonoids in preventing the accumulation of ROS under cold-induced photoinhibition of photosystems (Havaux & Kloppstech, 2001). Once again, the regulation exerted by NO over cold acclimation is proposed to be performed by triggering PTMs in proteins related to the aforementioned processes. In agreement with this hypothesis, flavonoid-related enzyme guercetin-3-O-methyltransferase 1 was found to be nitrated in vivo (Lozano-Juste et al., 2011) while NO has been reported to perform a negative regulation on ABA signaling by causing the nitration and inactivation of its receptors (Castillo et al., 2015).

However, despite all indications suggesting that the lower endogenous NO levels in *nia1,2noa1-2* are the reason for an impaired cold acclimation in this mutant, its defective nitrogen assimilation cannot be ruled out to be behind some of the effects observed. For instance, wild type plants accumulated higher levels of NO when growing in a medium with nitrate as a unique N source than when growing in a medium with nitrite or ammonium or subjected to N starvation, an effect that was not observed in *nia1,2* plants (Fig. 4.46). Therefore this enhanced NO production must be due to the NR activity, as the genes of the two NR enzymes of Arabidopsis are mutated in the triple mutant. Also it has been previously reported that anthocyanins accumulate under deficient nitrogen assimilation (Diaz *et al.*, 2006) but the NO-deficient mutant was unable to induce the anthocyanin biosynthetic genes and produce them during cold acclimation (Fig. 4.49), thus supporting the hypothesis of NO being essential for the cold-triggered accumulation of anthocyanins and thus for the acclimated-induced freezing tolerance.

All these data suggest that NO would regulate metabolism, hormone signaling and gene expression presumably through PTMs on key regulatory proteins, in such a way that during cold acclimation, NO levels would increase to allow the correct functioning of CBF-dependent and –independent regulatory pathways (Fig. 5.3). Therefore, NO is essential for the full cold-induction of *CBF* genes and flavonoid biosynthesis, as well as for regulating ABA perception and signaling.



### Figure 5.3. Scheme showing the endogenous and environmental factors involved in the positive regulation exerted by NO on cold acclimation-induced freezing tolerance.

A noteworthy issue is the apparently opposite roles of NO in constitutive freezing tolerance and cold acclimation. It is not the first time that a paradoxical function has been attributed to NO, which can exert anti-oxidant or pro-oxidant roles depending on acting in a chronic low or acute doses (Groß et al., 2013). Moreover, the highly reactive nature of NO makes it potentially capable of triggering changes in signaling pathways with different and even opposite regulatory effects. However, the function of NO as a repressor of constitutive freezing tolerance and as an inducer of cold acclimation can be seen as an adaptive mechanism of plants, consisting in a fine tuning of the regulation of the cell processes depending on the concentration of NO in response to the environmental temperature. Since in the absence of stressrelated stimuli the allocation of energy and resources to stress responses would entail a waste for the plant, in standard conditions of temperature the equilibrium between development and defense must be unbalanced towards the plant growth. Therefore in this situation NO acts as a brake to those unnecessary mechanisms that are involved in the development of the freezing tolerance. Nevertheless, under cold non-freezing temperatures plants will require the development of cold acclimation, thus anticipating future freezing temperatures. In this process NO is

accumulated and its higher concentrations promote responses that NO would usually be repressing, such as ABA signaling and anthocyanin biosynthesis, leading to a full cold acclimation.

Both aforementioned conducted transcriptomic analyses pointed to NO triggering large metabolic changes that would be responsible for the correct regulation of processes such as hypocotyl elongation, constitutive freezing tolerance and cold acclimation. These suspected metabolic alterations were confirmed in a metabolomic assay that compared the metabolomes of wild type and *nia1,2noa1-2* plants (Table S2 in Costa-Broseta *et al.*, 2018) which helped to figure out the role of NO in repressing freezing tolerance, as mentioned above. This analysis allowed to investigate the effects of a lack of NO in the plant metabolome, but another analysis was pending in order to understand the opposite situation. Therefore, a metabolomic analysis of plants collected at different times after a short pulse of pure NO gas was also performed (Supplementary Table S1 in León *et al.*, 2016). In this analysis a sharp and transient alteration in the metabolome was observed in NO-treated plants by 6 h after treatment, but this response almost disappeared by 24 h after treatment (Fig. 4.23A, B, Supplementary Table S1 in León *et al.*, 2016), showing the extraordinary plasticity of Arabidopsis metabolism.

One of the main NO-triggered effects observed in this analysis was the alleviation of oxidative stress. This response to NO must be understood to be strictly associated to high NO concentrations and would require further investigation to be extrapolated to specific cell events or tissues, as NO has been reported to possess both antioxidant and prooxidants effects (Groß et al., 2013). As observed in the freezing tolerance and cold acclimation, the effect of NO on oxidative stress constitutes another example of the sometimes paradoxical function of NO as a signaling molecule. It could be due to multiple factors including the relative cellular concentration, the location where it is synthetized and the complexity of the interacting microenvironment. When NO palliates oxidative stress, it can be by altering gene expression or by acting as a direct antioxidant. By scavenging ROS such as superoxide anion, NO will be transformed into peroxynitrite, which has been characterized as a potent tyrosine nitration-inducer (Holzmeister et al., 2015). The NO-related activation of processes involved in lowering oxidative stress in NOtreated plants was suggested by a significant increase in the production of polyamines and cysteine (Supplementary Table S1 in León et al., 2016), a reduction of superoxide anions (Fig. 4.24A) and the accumulation of nitrated proteins (Fig. 4.24B). Furthermore, the endogenous content of two important antioxidant molecules such as ascorbate and  $\alpha$ -tocopherol (Szarka *et al.*, 2012) were reduced in NO-treated plants (Supplementary Table S1 in León et al., 2016) while the content of threonate increased, suggesting NO would trigger the irreversible catabolism of ascorbate to the non-antioxidant threonate instead of inducing its metabolism through reversible oxidation (Parsons et al., 2011).

Another metabolic disturbance caused by NO was the alteration of central processes of primary metabolism, since several significant changes were observed in the categories of peptides, amino acids, carbohydrates and lipids (Fig. 4.23A). Among these, the changes in the lipidome are of special relevance, as its signaling potential has been reported (Hou *et al.*, 2016). Upon NO treatment, it was observed a significant increase in the content of phospholipids, lyso-lipids, PUFAs and oxylipins (Fig. 4.26 and Supplementary Table S1 in León *et al.*, 2016). The detection of elevated levels of C20 PUFAs could result from the lipase action on trace lipids, or from the enhanced elongase activity (Zhou *et al.*, 2014), while the rise in the oxylipins levels may be indicative of the activation of responses to stress (Pohl & Kock, 2014).

Promotion of cell death was also one of the stress responses that the data from the metabolomic analysis pointed out to be promoted by the NO treatment. The strong increase in pheophorbide a content (Fig. 4.28B) was also accompanied by an alteration in lipidic structures (Fig. 4.27) and metabolic changes associated to massive nucleic acid and protein degradation (Supplementary Table S1 in León et al., 2016), all these processes being usually related to cell death (Hirashima et al., 2009; Araújo et al., 2011; Sakamoto & Takami, 2014). Nevertheless, the absence of DNA ladders in the patterns of DNA degradation in NO-fumigated plants (Fig. 4.28A) suggests that NO would trigger necrotic cell death instead of apoptosis. Confirming all these hints, cell death was detected by Evans blue staining in NOtreated plants (Fig. 4.29C). Besides, other alterations observed in plants upon NO treatment that could be related to NO triggering cell death include an increased nucleic acid metabolism (Supplementary Table S1 in León et al., 2016), the aforementioned increment in the content of polyamines (Fig. 4.25) and a strong accumulation of nitration in tyrosine proteins (Fig. 4.24B). The metabolism of nucleic acids fits the observed DNA degradation (Fig. 4.28A), whereas polyamines have been reported to be able to delay cell death (Del Duca et al., 2014). Regarding protein nitration, as mentioned above, it has been described how this NO-triggered PTM is responsible for the inactivation and further degradation of ABA receptors (Castillo et al., 2015), so, although more work is required to elucidate the connection between NO, protein nitration and protein breakdown, the increase of NO-related PTMs in proteins could be one of the mechanisms leading to the characteristic protein turn-over that is associated with cell death (Araújo et al., 2011). Despite all the observed evidence in this work supporting the role of NO as a cell death inducer, it is noteworthy that the regulation of this process seems to be yet another scenario of paradoxical behavior of this molecule, since NO has been reported to have both promoting and suppressing effects on cell death in plants depending on cell type, redox status and NO concentration (Wang et al., 2010). However, notwithstanding the mentioned multiple metabolic and cellular symptoms of cell death, those were only detected by 6 h after NO treatment, and by 24 h plants recovered its standard metabolic status (Fig 4.23A) and were fully viable, as 7 days after treatment all plants survived and grew normally (Fig. 4.29C).

Therefore, under natural stress conditions, NO would only trigger cell death in a very discrete manner, only in those cell locations where its concentration surpasses a threshold level.

Lastly, a small but statistically significant increase in a wide array of dipeptides was also detected in NO-treated plants (Supplementary Table S1 in León *et al.*, 2016). This accumulation could be due to the N-terminal dipeptide release form target proteins or a plant response to ameliorate the combined effects of NO and O<sub>2</sub> in the cysteine branch of the N-terminal targeted proteolysis of certain protein substrates through the N-end rule pathway. Thus, NO would indirectly promote the accumulation of dipeptides in an attempt by the plant to protect some key proteins from specific N-terminal directed proteolysis, since dipeptides have been described to inhibit the N-end rule pathway proteolysis (Kitamura, 2016).

In brief, data from the metabolomic analysis of NO-treated plants suggest that NO triggers an array of responses to alleviate toxicity and cellular damage that includes massive but transient metabolic reprogramming involving metabolites of both primary and secondary metabolism (Fig. 5.4). As already discussed, this metabolic reprogramming was found to take place by 6 h after an NO pulse, while accordingly effects on the transcriptome and proteome were observed after 1 h (Fig. 4.13B and Fig. 4.24B). This timing would suggest that in order to promote such an extensive metabolic alteration, first NO would have to alter gene expression and cause PTMs in proteins.

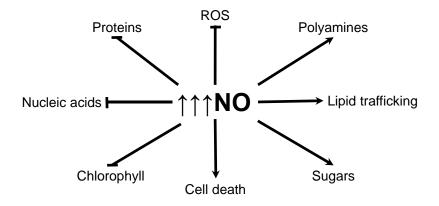


Figure 5.4. Scheme summarizing the regulation that high concentrations of NO exert over different metabolite categories and cell processes.

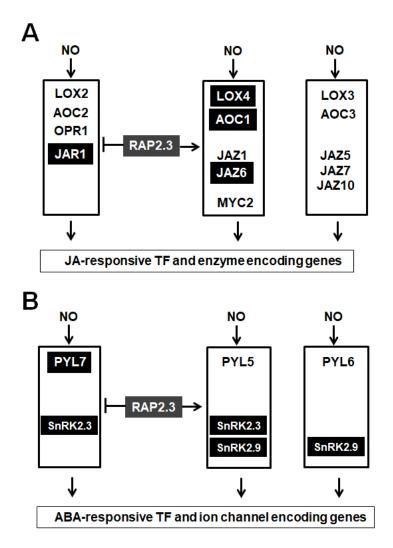
The results obtained through this metabolomic analysis were not a perfect mirror of those obtained from the metabolomic analysis performed with NO-deficient plants. Since the experimental systems behind these analyses aimed to mimic low and high NO concentration in plants, it could be expected to obtain directly opposing results. Strikingly, that was not the case, as almost the same metabolite categories were altered and in some cases very similar changes were detected in both analyses. ROS represent one of the few examples of opposing responses. NO-deficient plants displayed the effects of constitutive oxidative stress including the accumulation of ascorbate as a response to ameliorate the redox status, while NO-fumigated plants had its ROS levels decreased and were observed to have a decrease in ascorbate levels and an increase in threonate. By contrast, three metabolite categories that changed in the same direction in NO-treated plants and NO-deficient plants were polyamines, cysteine and cysteine production-related metabolites, and oxylipins, which increased in both cases. It is important to remark that nia1,2noa1-2 plants have very limited NR activity due to a mutation in the two NR enzymes of Arabidopsis (Wilkinson & Crawford, 1993), so some of the alterations observed may be related to a deficient N assimilation instead of being caused by low endogenous NO levels. Regarding the fumigation with NO, some of the effects observed upon NO treatment could be caused by NO<sub>2</sub> instead, given the rapid NO conversion into NO<sub>2</sub> in the presence of oxygen (Kasten et al., 2017). remarkably. the differential transcriptomes of NO-treated plants Also (Supplementary Tables S2, S3 and S4 in Castillo et al., 2018) and untreated NOdeficient *nia1,2noa1-2* plants (GEO code GSE41958), show a big overlapping, thus suggesting the possibility of NO acting as a repressor of its own biosynthesis when exogenously supplied at high concentrations. Further work will be required to clarify these unexpected results and the true behavior of NO in the regulation of transcriptome and metabolome.

One of the most intriguing metabolic changes observed in NO-fumigated plants was the accumulation of dipeptides. This response has been proposed to be a mechanism to inhibit the N-end rule pathway proteolytic degradation of key proteins in mammals (Baker & Varshavsky, 1991). In plants, the degradation of the ERFVII TFs through this pathway was described to be a mechanism of NO sensing in Arabidopsis (Gibbs et al., 2014a). Given the potential importance of the ERFVIIs in controlling plant responses to NO, an analysis on the modulation of NO homeostasis and signaling by the ERFVII RAP2.3 was performed. The results of the transcriptomic analysis of  $\beta$ -estradiol inducible RAP2.3 TPT transgenic lines (Fig. 4.35C, D) suggest the existence of a regulatory loop involving NO and RAP2.3, with a significant contribution of the transcription factor regulating the plant responses to NO. Specifically, RAP2.3 was found to act mostly as a repressor of NO-triggered responses both at physiological (Fig. 4.31 and 4.34) and molecular (Table A9) levels. RAP2.3 does not contain the Ethylene response factorassociated Amphiphilic Repression (EAR) motif (Ohta et al., 2001), which enable other AP2/ERF transcription factors to act as transcriptional repressors (Tsutsui et al., 2009), so its potential activity as repressor in NO-triggered responses should be due to the RAP2.3-activated expression of a true repressor . However, none of the genes that were up-regulated by NO in TPT-RAP2.3 plants only after  $\beta$ estradiol treatment and containing the putative RAP2.3 binding motif, codes for an EAR-containing transcription factor (Table A7), so the attenuated NO-triggered response at the transcriptome level could be the result of other non-EAR-containing type of repressor or alternatively of a NO-induced transcriptional activator that is down-regulated upon RAP2.3 overexpression.

Among these candidates, three genes coding for AP2/ERF transcription factors (ERF095/ESE1, RAP2.6 and ERF016) and several genes (NAC055/NAC3, JAR1 and *MYB113*) containing the putative RAP2.3 binding domain in their promoters were strongly activated by NO only when RAP2.3 was not overexpressed (Table A7 and A10). RAP2.6 has been reported to be activated by NO and be involved in its response in shoot elongation (Imran et al., 2018), while NAC055 has been reported to participate in JA signaling (Bu et al., 2008). Data summarized in Figure 5.5 suggest the existence of RAP2.3-dependent and -independent NO-responsive branch pathways of JA and ABA signaling. Data presented in Figure 4.36 indicate that NO induces the expression of a subset of JA-related genes coding for its biosynthesis and signaling through a process that was repressed by RAP2.3 (Fig. 5.5A). Three different branches of JA signaling can be depicted based on the regulatory role exerted by RAP2.3. A RAP2.3-repressed branch would involve LOX2, AOC2, OPR1 and JAR1, from which only JAR1 would be a direct RAP2.3 target (Fig. 5.5A). JAR1 plays a crucial role in the biosynthesis of the active hormone jasmonoyl-isoleucine (Staswick et al., 2002). A second RAP2.3-activated JA signaling branch would recruit LOX4, AOC1, JAZ1, JAZ6 and MYC2, being LOX4, AOC1 and JAZ6 potential direct targets of RAP2.3 (Fig. 5.5A). Finally, a third RAP2.3-independent branch would involve the participation of LOX3, AOC3, JAZ5, JAZ7 and JAZ10 (Fig. 5.5A). On the other hand, the NO-sensitive ABA signaling pathway could be also split in three branch pathways (Fig. 5.5B). One, repressed by RAP2.3, would involve PYL7 and SnRK2.3, both with potential RAP2.3 binding sites in their promoters (Table A7); the second, involving PYL5 and SnRK2.3 that would require RAP2.3 over-expression for the NO-trigger upregulation of PYL5 but RAP2.3 repressing the NO-induced up-regulation of SnRK2.3; and finally, a third branch would comprise the function of the PYL6 receptor and the SnRK2.9 kinase acting through a RAP2.3-independent mechanism. It seems RAP2.3 would control the expression and stability of SnRK2.3 by promoting NO production, since the encoding gene of PP2b11, which modulates ABA signaling by controlling SnRK2.3 degradation (Cheng et al., 2017), was up-regulated by NO only when RAP2.3 is not overexpressed (Table A9). Also it has been reported that the function of SnRK2.3 can be repressed by NOtriggered PTMs (Wang et al., 2015c).

According to all these data, NO would modulate its own biosynthesis and associated responses as well as JA and ABA signaling through RAP2.3-independent and RAP2.3-dependent pathways. In this scenario, RAP2.3 exerts its regulatory effects on plant responses to NO partially through its capacity to be degraded by the N-end rule proteolytic pathway, since the ability of plants to sense NO was reduced when this degradation was blocked, like in a *prt6* mutant or a plant expressing a non-degradable version of the protein (Fig. 4.34). Therefore, NO responses would be modulated by using RAP2.3 as a rheostat, a mechanism already described for other key processes in plants (Baxter-Burrell *et al.*, 2002; Williams *et al.*, 2015; Tischer *et al.*, 2017). Furthermore, RAP2.3 would also

function as a NO- and O<sub>2</sub>-modulated rheostat integrating environment-triggered changes in the endogenous levels of NO and oxygen-containing molecules, as well as some hormones such as JA and ABA.



**Figure 5.5. RAP2.3-dependent and –independent NO-regulated pathways.** A, JA and B, ABA branches of the biosynthesis and signaling pathways and the components involved. Highlighted in black those presumably considered as direct targets of RAP2.3 because contain putative RAP2.3 binding sites in their gene promoters.

The rheostat model for the NO-RAP2.3 regulatory loop constitutes another case of complex regulation exerted by NO. The ability of NO to modulate almost every signaling pathway suggests that the possibility of self-controlled biosynthesis is a highly convenient trait for the plant. In agreement, the similarity of the transcriptomes of NO-treated plants and untreated NO-deficient plants (Supplementary Tables S2, S3 and S4 in Castillo *et al.*, 2018 and GEO code GSE41958), pointed out to the possibility of NO repressing its own production. This

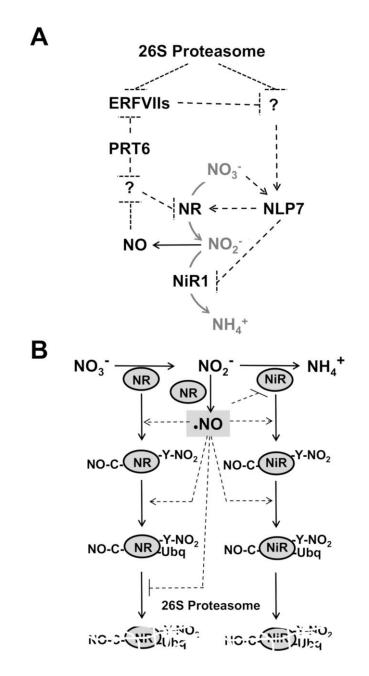
could represent an auto-regulatory mechanism for the plant to keep NO homeostasis under control, which would explain the absence of significant increase in the endogenous NO levels of seedlings treated with exogenous NO (Fig.4.29A).

Since it seems that NO production in plants is largely dependent on the activity of NRs, the balance between NR and NiR activities could be determinant for NO homeostasis, as both kinds of enzymes would compete for nitrite as a substrate for its reduction to NO or ammonium, respectively. Therefore, the accumulation of NO in plants with reduced NiR and enhanced NR activities can be expected, while the opposite situation would result in diminished NO levels. The absence of available T-DNA mutants with a loss-of-function mutation in NIR1 made necessary the generation of a *nir1-1* full knockout mutant through CRISPR-Cas9 technology. Together with its completely impaired capacity to reduce nitrite to ammonium, this mutant showed a strong increase in NO levels when grown on nitrate-containing media (Fig 4.5). This process is likely the result of the removal of toxic nitrite accumulated after nitrate reduction and incapacity to further reduce nitrite to ammonium. On the other hand, concomitantly, NO production in nia1,2noa1-2 plants is indeed reduced in both shoot (Fig. 4.42C) and root (Lozano-Juste & León, 2010). This triple mutant has a point mutation that does not truncate the open reading frame of NIA1 (Lozano-Juste & León, 2010), thus leading to synthesis of NIA1 with null activity.

As nitrate-dependent NO biosynthesis could be considered as a side branch of the nitrate assimilation metabolic pathway, it is expected that nitrate signaling pathway play a major regulatory role on NO production. Some TFs of the NLP family have been reported to act as master regulators in nitrate signaling (Konishi & Yanagisawa, 2013). Among them, NLP7 would regulate mainly NRs and NLP6 would regulate NIR1. NLP7 has been reported as a primary positive regulator of nitrate assimilation that binds to the promoter of NIA1, NIA2 and NIR1 to activate their expression (Marchive et al., 2013), but NIR1 protein levels and activity were found to be higher in *nlp7-1* plants (Fig. 4.2A, B). This could be due to a competition between NLP6 (Konishi & Yanagisawa, 2013) and NLP7 that would be unbalanced in the *nlp7-1* mutant. Most likely, NLP7 would be potentially connected to NO homeostasis control, but this relation seems to be quite complex, since nlp7-1prt6-1 plants, which carry also a mutation in the gene coding for the E3 ubiquitin ligase PRT6 that participates in the Arg/N-end rule pathway, display higher endogenous NO levels than wild type and their parental *nlp7-1* and *prt6-1* plants (Fig 4.2C). These data, together with the enhanced NR protein accumulation and activity observed in proteasome inhibitor-treated plants and the lack of significant effect regarding NIR1 (Fig. 4.1B, C), point to a connection between proteasomal degradation processes modulating NRs activity, the function of PRT6 and the NLP7 regulation of nitrate signaling. The NR protein content was increased in prt6-1 and gerfvii (with mutations in all ERFVII coding genes) plants treated with proteasome inhibitor, whereas NR levels decreased in *nlp7-1* plants (Fig. 4.6). These data suggest that NR stabilization requires NLP7 and the loss of PRT6 function independently of ERFVIIs. Interestingly, neither NIA1 nor NIA2 can be directly ubiquitylated by PRT6 as they are not MC-proteins. In turn, NLP7 fulfills this requirement and thus could be a potential substrate for the degradation mediated by the Arg/N-end rule pathway. Although this possibility should be further investigated, the functional link between NLP7 and PRT6 was supported by the appearance of plants expressing an N terminus-tagged NLP7 in *nlp7-1prt6-1* background, which rescued the pale green and small size phenotypes that characterize the leaves of *nlp7-1* and *prt6-1*, respectively (Fig. 4.3A). Furthermore, it is noteworthy that the overexpression of NLP7 in *nlp7-1* and *nlp7-1prt6-1* lead to enhanced NO levels (Fig. 4.3B). This functional link between NLP7-1 and PRT6 could be filled by a positive regulator of NLP7 regulated itself by the PRT6-mediated N-end rule pathway. Transcription factors ABR1 and WRKY14 are two candidates, being MC-proteins able to bind the ABRE-like box and the W-boxes present in *NLP7* promoter, respectively (Table A4 and Fig. A5) (Shen & Ho, 1995; Hobo *et al.*, 1999; Maeo *et al.*, 2001).

Besides the regulatory factors related to nitrate signaling and Arg/N-end rule proteolysic pathway, direct NO-related PTM-mediated effect in NR function and the subsequent NO production was suggested by new data. It has been described that phosphorylation and sumoylation can control NR function (Lillo *et al.*, 2004; Park *et al.*, 2011), but an analysis on the effect of NO-triggered PTMs on the NR activity has not been conducted yet. Above has been proposed that NO-triggered PTMs are responsible for the regulation NO exerts over processes such as constitutive freezing tolerance, cold acclimation and hypocotyl elongation, while its implication in ABA sensing has been already confirmed (Castillo *et al.*, 2015). Lysine ubiquitylation, tyrosine nitration and cysteine S-nitrosylation sites were identified *in planta* in NR1, NR2 and NIR1 by using transgenic plants overexpressing HA-tagged versions of these proteins after inmunopurification and LC-MS/MS proteomic analyses (Fig. 4.8, 4.9 and 4.10).

Regarding the NRs, the proteomic analyses showed a differential pattern of PTMs that could account for different regulation for each enzyme, but both were nitrated in two alternate tyrosine residues highly conserved in the central part of NR proteins (Fig. 4.8B) between the sumoylated lysine and the cytochrome b5 hemebinding domain (Fig. 4.9). Interestingly, nitration of those tyrosines was accompanied by ubiquitylation of two close consecutive lysines also highly conserved among plant nitrate reductases (Fig. 4.8B). Whether this cluster of nitration and ubiquitylation of NRs is associated to reduced function or degradation as in the case of ABA receptors (Castillo *et al.*, 2015) would require further work including enzyme assays after *in vitro* nitration of purified recombinant NR1 and NR2 proteins. Taking advantage of the availability of the 3D structural model of the corn NR cytochrome FAD- binding domain (PDB code 2cnd) and by amino acid sequence homology with Arabidopsis NRs, a nitrated tyrosine residue in NR1 (Y<sup>733</sup>) which corresponded to Y<sup>83</sup> very close to the FAD in the 3D model was located (Fig. 4.12). The introduction of a nitro group in that residue would largely perturb the



**Figure 5.6. Regulation of NRs, NiR1 and NO production.** A, The expression of *NRs* and *NiR1* genes and the stability of their corresponding proteins are regulated through NLP7-mediated nitrate signaling and proteasome-mediated degradation. B, NO-triggered post-translational modifications such as tyrosine nitration, cysteine S-nitrosylation, and lysine ubiquitylation control function and stability of NRs and NiR1 involved in NO production.

efficient binding of the flavin, thus likely leading to activity loss. However, we have not found the corresponding tyrosine nitrated in NR2, thus suggesting this could be a differential regulatory mechanism for both NRs. Despite the effect of PTMs in NRs function, the ubiquitylation is likely related to the control of their protein stabilities as treatment with proteasome inhibitor increased NR protein and activity levels, as mentioned above (Fig. 4.1B, C). Altogether, these data suggest that ubiquitylation of NRs is the default condition of NRs when NO-related PTMs affect them, thus triggering the proteolytic action of the proteasome. In the case of NIR1, among the identified PTMs, a heavy pattern of lysine ubiquitylation was found (Fig. 4.10), however as the inhibition of the proteasome did not affect the activity and stability of NIR1 (Fig. 4.1B, C) it is unlikely that these PTMs represent a relevant factor in controlling its function. On the other hand, the effect of NO-related PTMs on NIR1 activity appears to be decisive, as the cysteines involved in binding the 4Fe-4S cluster and the siroheme group were found to be S-nitrosylated (Fig. 4.10 and 4.11), which would in all likelihood impair the proper functioning of the enzyme. Therefore, NO could induce NIR1 inactivation through S-nitrosylation of these critical cysteine residues. This mechanism would allow NO to enhance its own biosynthesis, by preventing nitrite reduction to ammonium.

Altogether, the aforementioned data suggest that NO can control its own biosynthesis at two complementary levels, through a mechanism that regulates the activity, accumulation and stability of NRs and NiR1. The first level would implicate nitrate signaling and N-terminal guided proteolytic degradation acting on NO-producing enzymes or their upstream regulators (Fig. 5.6A). The other level is the post-translational regulation through nitration of Y residues of NRs and S-nitrosylation of C residues of NiR1 affecting FAD- and siroheme-binding, respectively (Fig. 5.6B). Therefore, NO-triggered control over NRs and NiR1, together with nitrate signaling and proteasomal degradation are thus potentially relevant to control NO production at the transcriptional, translational and post-translational levels.

This ability of the NO to regulate its own biosynthesis would explain the big overlapping of differential transcriptomes of NO-deficient plants and NO-fumigated plants (GEO code GSE41958, Supplementary Tables S2, S3 and S4 in Castillo et al., 2018), likely due to negative effect of NO on its production. As mentioned above, further work will confirm if the positive or negative regulation of NO on its homeostasis is dependent on its concentration (Fig. 5.7). This variable function of the NO resembles that observed for the modulation of cold stress-related plant resources, consisting on a switch from repressor to inducer of freezing tolerance (by enhancing cold acclimation) when cold triggers its accumulation (Fig. 5.7). Such a dramatic alteration in the plant signaling is possible given the capacity of NO to exert massive changes in the plant metabolome when its concentration surpasses a certain threshold (Fig. 5.7). However, it is important to remark that aside from its own concentration, the regulatory effect of NO on cell processes is subjected to multiple factors, including its location and the surrounding molecules. Thus, the highly complex interaction between NO and hormone signaling pathways implies that several hormone-related plant responses are regulated by NO while some NO-triggered responses are regulated by hormones (Fig. 5.7). At the same time, the N-end rule proteolytic pathway is regulated by NO but also is capable to modulate the function and homeostasis of NO (Fig. 5.7). Therefore, it can be concluded that, despite the great capacity of NO to exert extensive and diverse alterations in the plant physiology, unlike other signaling molecules, the regulatory role of NO is highly dependent on its interaction with a wide array of signaling pathways.

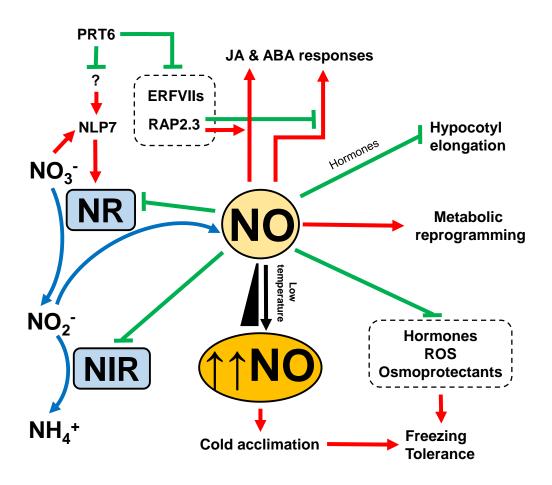


Figure 5.7. Scheme showing processes investigated in this work that regulate NO or are regulated by it. Green and red arrows represent negative and positive regulation, respectively; blue arrows represent catalysis, and the black arrow represents increase in concentration.

Discussion

## Conclusions

Conclusions

## 6. CONCLUSIONS

- 1) NO can control its own homeostasis in plants by regulating the function, accumulation and stability of NRs and NIR1 through nitrate signaling, N-end rule proteolytic pathway and post-translational modifications.
- NO sensing in Arabidopsis hypocotyls requires the biosynthesis and/or signaling of ethylene, strigolactones, salicylates and abscisic acid, and is enhanced by BES1-mediated brassinosteroid signaling.
- NO triggers an array of responses to alleviate toxicity and cellular damage that includes massive but transient reprogramming of primary and secondary metabolism.
- RAP2.3 negatively regulates NO biosynthesis and sensing through a NO- and O<sub>2</sub>-modulated rheostat-like mechanism in Arabidopsis that involves specific NO-related branches of jasmonate and abscisic acid signaling pathways.
- 5) NO acts as a sort of endogenous temperature sensor in plants preventing the development of freezing tolerance under non-stress temperature conditions, but triggering cold acclimation-induced freezing tolerance under low temperature stress.

Conclusions

## Bibliography

Bibliography

## 7. **BIBLIOGRAPHY**

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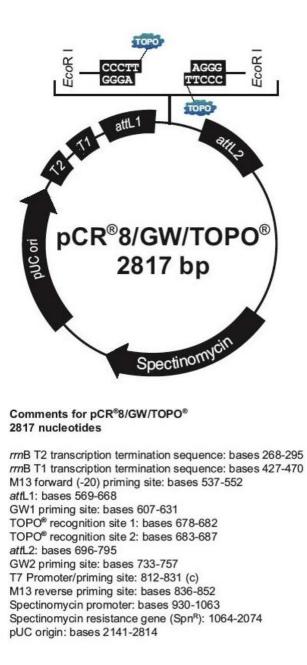
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# A. ANNEXES

Most of the figures and tables displayed in this section A come from the research articles: "León, J., Costa, Á., & Castillo, M. C. (2016). Nitric oxide triggers a transient metabolic reprogramming in Arabidopsis. Scientific Reports, 6, 37945", "Costa-Broseta, A., Perea-Resa, C., Castillo, M. C., Ruíz, M. F., Salinas, J., & León, J. (2018). Nitric Oxide Controls Constitutive Freezing Tolerance in Arabidopsis by Attenuating the Levels of Osmoprotectants, Stress-Related Hormones and Anthocyanins. Scientific Reports, 8(1), 9268", "Castillo, M. C., Coego, A., Costa-Broseta, Á., & León, J. (2018). Nitric oxide responses in Arabidopsis hypocotyls are mediated by diverse phytohormone pathways. Journal of experimental botany, 69(21), 5265-5278", "Costa-Broseta, A., Castillo, M. C., & León, J. (2018). Protein Stabilization and Post-translational Modifications Control NO Homeostasis in Arabidopsis", "León, J., Costa-Broseta, Á., & Castillo, M. C. (2018). RAP2.3 negatively regulates nitric oxide biosynthesis and sensing through a rheostat-like mechanism in Arabidopsis" and "Costa-Broseta, A., Perea-Resa, C., Castillo, M. C., Ruíz, M. F., Salinas, J., & León, J. (2018). Nitric Oxide Deficiency Reduces CBF Induction, ABA Signaling, Anthocyanin Synthesis and Cold Acclimation in Arabidopsis". The last three articles were submitted to Plant Physiology, The Plant Journal and Journal of Experimental Botany, respectively and were under revision when PhD Thesis writing was finished. All the figures and tables that appear here are derived from the work of the PhD student in collaboration with the other authors.



## Figure A1. Map of pCR8/GW/TOPO vector (Invitrogen).

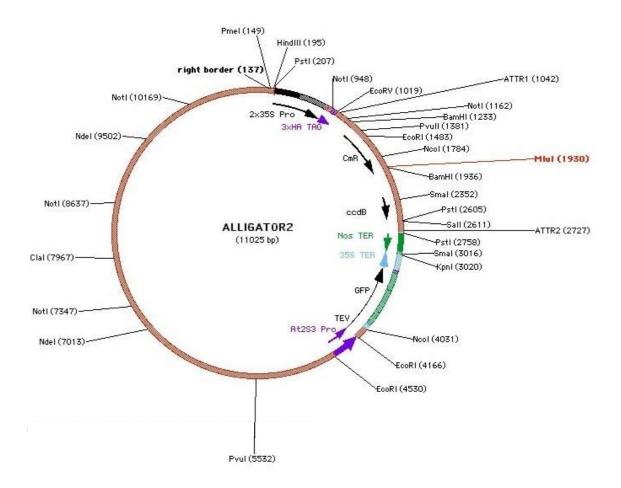


Figure A2. Map of pALLIGATOR2 vector.

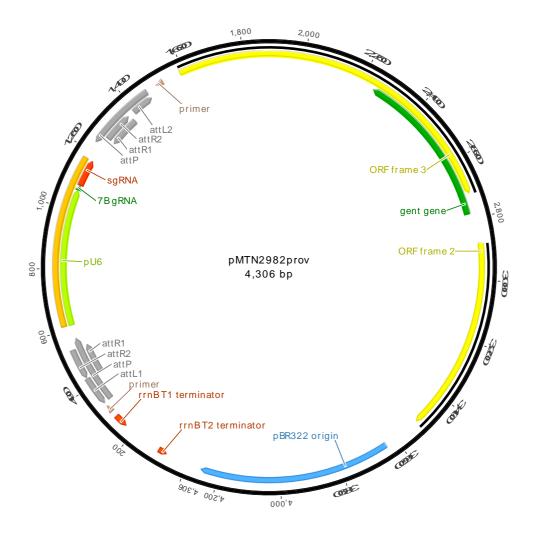


Figure A3. Map of pMTN2982 (pDONR207) vector.

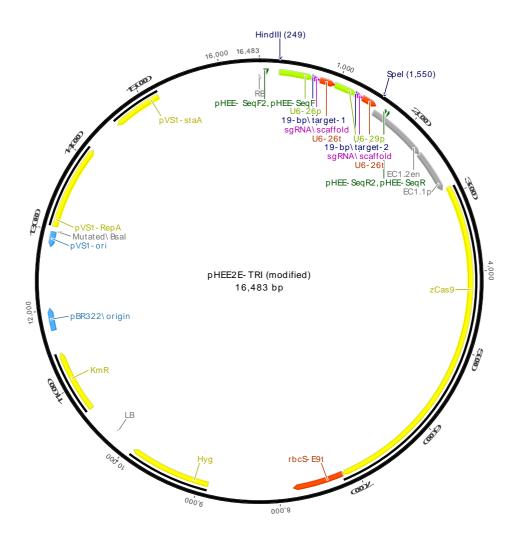


Figure A4. Map of pHEE2E-TRI vector.

gattattttaattctatgttaattccaggcattaaattttaagcgtaatttatagaa	agaagaaa
aaaaatctagttacattccatctaagataggggtacaaatccatacaagagtctatc	cttctgta
${\tt attcttagcaagttgaacatttcttaattttattttatt$	yttttctt
taatacaagtggcaaagatattctataacagttataatcatttatagttagggattt	ttttaatc
attttgtagtatttgtttttctcttttttcctaacaaagtttttggaacttttgat	<mark>catctct</mark> c
gattgaaagcgaacatataaataatttttactactgcaaatttttaaaaagttaata	attcgact
ttataaa <mark>agagatg</mark> agagtcaacaaaacaaaagcaactaattt <mark>tggacgtacg</mark> aaa	atgctttg
taaatacattgatatcacttatgtttataatatcactcatcccaagtgtt <mark>ggtcaa</mark> c	catttctt
tgataaaaattaaactattcaatagaacgaaaaccacaaaaaaattataaagaatta	attgataa
caactcttcatgttaatcttatatatttttttaaaaatatttgtttttaggttttaa	atacaaac
aacatgattctagtttattaaactaattaacgcaacttgatggattataaaaatata	agcttaac
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tgtcttttctccacttctcaatttccc <mark>attaat</mark> aatcccgaaaaggcattacgtaat	ta <mark>attaat</mark>
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gcagccgtcggacttgacaatgcaaaaatgctacataatctaaataattaactctcg	gcaataag
attcactaataataacatttttaacgacgatatataattttacaataagaaaaaaaa	atatgaat
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tttattattttgtagctgtcgaaactatggtgaataaaatctcgaggaatatattag	yaaaacaa
aaaagatctagaggaataataatattttaaatatttggggcacagttgtattgtgaa	agcagaga
${\tt atcggttctcaatatattatccgaaaatgtcttaattttaaattttgtttttgttt$	tgattctg
cgatattggaatcacacactctgccaat <mark>ttgacc</mark> tttctctctcgcttctctttctc	ctatccaa
tcggaaagagatcaaccaacgatagaggagaagaagaacttgcatacgcaaaaaaaa	ctttcccg
ggaaaattccagaaactgctttggaaaa <mark>ATG</mark>	

Site Name	<b>Position</b> -1072	Strand -	Matrix score. 9	sequence CGTACGTGCA	<b>function</b> cis-acting element involved in the abscisic acid responsiveness
Site Name	Position	Strand	Matrix score.	sequence	function
Box-W1	-128	+	6	TTGACC	fungal elicitor responsive element
Box-W1	-1002	-	6	TTGACC	fungal elicitor responsive element
Site Name	Position	Strand	Matrix score.	sequence	function
Site Name	Position	Strand +		sequence ATTAAT	<b>function</b> part of a conserved DNA module involved in light responsiveness
			score.	-	part of a conserved DNA module involved in light
Box4	-396	+	<b>score.</b> 6	ATTAAT	part of a conserved DNA module involved in light responsiveness part of a conserved DNA module involved in light
Box4 Box4	-396 -674	+ +	6 6	ATTAAT ATTAAT	part of a conserved DNA module involved in light responsiveness part of a conserved DNA module involved in light responsiveness part of a conserved DNA module involved in light
Box4 Box4 Box4	-396 -674 -705	+ + +	<b>score.</b> 6 6	ATTAAT ATTAAT ATTAAT	part of a conserved DNA module involved in light responsiveness part of a conserved DNA module involved in light responsiveness part of a conserved DNA module involved in light responsiveness
Box4 Box4 Box4 CATT-motif	-396 -674 -705 -578	+ + + +	<b>score.</b> 6 6 6 6	ATTAAT ATTAAT ATTAAT GCATTC	part of a conserved DNA module involved in light responsiveness part of a conserved DNA module involved in light responsiveness part of a conserved DNA module involved in light responsiveness part of a light responsive element

**Figure A5.** *In silico* **analyses of** *NLP7* **promoter.** Arabidopsis Gene Regulatory Information Server (AGRIS) tools were used to analyze the 1.5 kb *NLP7* promoter sequence upstream of the initiation codon. ABA and light responsiveness cis-acting elements as well as WRKY binding Box W1 consensus sequences are shown.

# Table A1. Abbreviations and acronyms.

2 NV	2 nitraturaning
3-NY A	3-nitrotyrosine acclimated
A. thaliana	Arabidopsis thaliana
A. tumefaciens	Agrobacterium tumefaciens
ABA	abscisic acid
acetyl-CoA ACT	acetyl coenzyme A Actin
ANOVA	analysis of variance
AO	Aldehyde Oxidase
AOC	Allene Oxide Cyclase
AOX	Alternative Oxidase
ARC Arg	Amidoxine Reducing Component arginine
aRNA	antisense RNA
ATE	Arg-tRNA Transferase
ATP	adenosine triphosphate
BASTA	glufosinate ammonium
BES1 bp	BRI1-EMS-Suppressor 1 base pair, unit of length of double stranded nucleic acids
BR	brassinosteroid
BSA	bovine serum albumin
BSTFA	bistrimethyl-silyl-triflouroacetamide
BZR2 C	Brassinazole-Resistant 2
C	cysteine control
CAPS	Cleaved Amplified Polymorphic Sequences
Cas9	CRISPR-associated 9
CBF/DREB1	C-repeat/dehydration-responsive element Binding Factor
cDNA CDS	complementary DNA
cGMP	coding DNA sequence cyclic guanosine monophosphate
CHS	Chalcone Synthase
СНХ	cicloheximide
Col-0	Columbia 0, wild type of <i>A. thaliana</i>
COR cPTIO	Cold-Responsive
CRISPR	2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide Clustered Regularly Interspaced Short Palindromic Repeats
Ct	threshold cycle of qPCR
CTAB	hexadecyltrimethylammonium bromide
Cys	cysteine
cyt Da	cytochrome dalton, unified atomic mass unit
DAF-FM DA	4-amino-5-methylamino-2',7'-difluorofluorescein diacetate
ddUTP	dideoxyuridine-5'-triphosphate
DEPC	diethyl pyrocarbonate
DET DFR	De-Etiolated Dihydroflavonol Reductase
dhJA	dihydrojasmonate
DIG	digoxigenin
DNA	deoxyribonucleic acid
dNTP	dinucleoside triphosphate
DTT E. coli	dithiothreitol Escherichia coli
EDTA	ethylenediaminetetraacetic acid
ERF/AP2	Ethylene Response Factors
ERFVIIs	group VII of ERFs
ESI EST	electrospray ionization
ET	β-estradiol ethylene
F	forward primer
FA	unsaturated fatty acid
FAD	flavin adenine dinucleotide
FC FDR	fold change false discovery rate
fed	ferredoxin
Fig.	Figure
FT	Fourier transform
FW	fresh weight
GA GABA	gibberellin y-aminobutyrate
GABA	Gibberellin Insensitive
GBH	γ -hydroxybutyrate

GC	Guanylate Cyclase
GC	gas chromatograhy
GCOS	genechip operating software
GEO	Gene Expression Omnibus
GFP	Green Fluorescent Protein
GPC	choline glycerophospholipid
GPE	ethanolamine glycerophospholipid
GS-GOGAT	Glutaminate Synthetase-Glutamine Oxoglutarate Aminotransferase
GSH	glutathione
GSNO	S-nitrosylated glutathione
GSNOR GSSG	GSNO Reductase
GSSG	glutathione disulfide
GTP	glutathione disulfide guanosine triphosphate
H₂O	water
HA	human influenza Hemagglutinin tag
HAI	Highly ABA-Induced
HB	hemoglobin
HF-PCR	high fidelity PCR
HLB	hydrophilic-lipophilic balance
HODE	hydroxyoctadecadienoic acid
HRE	Hypoxia Responsive ERF
HRP	horseradish peroxidase
Hyg	hygromycin
ICE1	Inducer of CBF Expression1
ICR	ion cyclotron resonance
ICS	Isochrorismate Synthase
JA	jasmonate
K	lysine
LB	lysogeny broth medium for bacteria
LB	left border
LC	liquid chromatography
LIMMA	linear model methods
LIT	linear ion-trap
LOO <sup>,</sup>	lipid peroxyl radical
LT₅₀ LTQ	temperature that causes 50% lethality
M	Linear Trap Quadrupole DNA ladder marker
MA-	methionine-alanine-
MA- MAP	Methionine Aminopeptidase
MC-	methionine-cysteine-
MES	2-(N-morpholino)ethanesulfonic acid
mETC	mitochondrial electron transport chain
MIAME	minimum information about a microarray experiment
Мосо	molybdenum cofactor
mRNA	messenger RNA
MS	Murashige & Skoog medium for plants
MS	mass spectrometer
MSE	β-estradiol-supplemented MS medium
MS-MES	MS medium with MES buffer
NAD	nicotinamide adenine dinucleotide
NBT	nitroblue tetrazolium
NH <sub>3</sub>	ammonia
	ammonium
Ni:NOR NIA1	Nitrite-Nitric Oxide Reductase Nitrate Reductase 1
NIA2 NIR	Nitrate Reductase 2
NIR1	Nitrite Reductase 1
NLP7	NIN-Like Protein 7
N-NEDA	N-(1-napthyl) ethylenediamine hydrochloride
NO	nitric oxide
NO <sub>2</sub> -	nitrite
NO <sub>2</sub> -FA	nitro-fatty acid
NO <sub>3</sub> -	nitrate
NOA1	Nitric Oxide Associated 1
NOFNIR	Nitric Oxide-Forming Nitrite Reductase
NOGC1	NO-dependent Guanylate Cyclase 1
NOS	Nitric Oxide Synthase
NOX	Mixture of NO and NO <sub>2</sub>
NR	nitrate reductase
0 <sub>2</sub>	oxygen superoxide apion
O <sub>2</sub> - oligo(dT)	superoxide anion short sequence of deoxy-thymidine
ONOO <sup>-</sup>	peroxynitrite
OPR	Oxophytodienoate Reductase
	• •

ORF	open reading frame
ох	overexpressed
р	promoter
PAM	protospacer adjacent motif
PAO	Pheophorbide a Oxidase
PC	phosphatidylcholine
PCA	Principal Component Analysis
PCO PCR	Plant Cysteine Oxidase
PDB	polymerase chain reaction potato dextrose broth
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PIF	Phytochrome Interacting Factor
PMSF	phenylmethylsulfonyl fluoride
PRE	before treatment
PRT6	Proteolysis6
PTM	post-translational modification
PUFA	polyunsaturated fatty acid
QC	quality control
qerfvii	rap2.12rap2.2rap2.3hre1hre2
qjaz	jaz10, jaz1,3,5,9,10
qPCR	quantitative PCR
R	reverse primer
RAP	Related to AP
RB	right border
RFLP	Restriction Fragment Length Polymorphism
RNA RNS	ribonucleic acid reactive nitrogen species
ROS	reactive oxygen species
RT	reverse transcription
rubisco	Ribulose-1,5-Bisphosphate Carboxylase / Oxygenase
SA	salicylic acid
SCC	Spearman's rank-order correlation coefficient
SD	standard deviation
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS polyacrylamide gel electrophoresis
SE	standard error
SIM	selected ion monitoring
SL	strigolactone
SLIM	Site-directed, Ligase-Independent Mutagenesis
SNO	S-nitrosothiol
SnRK	Sucrose non-fermenting 1-Related protein Kinase
SO T0	Sulfite Oxidase
T1	parental generation first generation
T2	second generation
TAE	Tris, acetic acid and EDTA buffer
TCA	tricarboxcylic acid
T-DNA	transfer DNA, plasmid of Agrobacterium
TE	Tris and EDTA buffer
ter	terminator
TF	transcription factor
THB1	Truncated Hemoglobin 1
TMT	Tandem Mass Tagged-S-nitrosylated protein
TPT	TRANSPLANTA
tRNA	transfer RNA
TT7/F3H	Flavonol 3-Hydroxylase
TTBS	Tris-buffered saline buffer with Tween 20
Tyr UHPLC	tyrosine ultra-high-performance liquid chromatography
UPLC	ultra-nign-performance liquid chromatography ultra erformance liquid chromatography
UV	ultraviolet light
xo	Xanthine Oxidase
Y	tyrosine
α-	anti-, before the antigen that gives name to an antibody

Buffer or solution	Composition (in MilliQ water)
Nutritive solution for plants	gFe (34.95 mg/l ), ZnSO₄·7H₂O (9.76 mg/l ), MnSO₄· H₂O (28.95 mg/l), Cu₂SO₄ (2.23 mg/l), H₃BO₃ (32.55 mg/l), (NH₄)₀Mo <sub>7</sub> O₂₄*4H₂O (1.51 mg/l), Ca(NO₃)₂·4H₂O (885 mg/l), KNO₃ (381 mg/l), KH₂PO₄ (102 mg/l), MgSO₄·7H₂O (367.5 mg/l), pH 6.5, CE 1.6 mS
Floral dipping transformation solution	Sucrose (5%), Silwet L-77 (0.02%)
TTBS	Tris pH 7.4 (10 mM), NaCl (150 μM), Tween 20 (0.1%)
TAE	Tris (40 mM), acetic acid (20 mM), EDTA (1 mM)
TE	Tris pH 8 (0.1 mM), EDTA (2 µM)
SDS-PAGE buffer	Tris (25 mM), Glycine (192 mM), SDS 10% (0,1%)
Semi-dry transference buffer	Tris (25 mM), Glycine (192 mM), methanol (20%)
H-buffer for hybridization	NaCl (300 mM), Tris-HCl pH 9 (50 mM), EDTA (20 mM)
DAF-FM DA staining buffer	MES-KOH pH 5.7 (5 mM), KCl (0.25 mM), CaCl <sub>2</sub> (1 mM)
Lugol's staining solution	lodine (5.7 mM), KI (43.4 mM), HCI (0.2 N)
DNA extraction buffer	Tris-HCl pH 7.5 (200 mM), NaCl (250 mM), EDTA (25 mM), SDS (0.5%)
Protein extraction buffer	Tris-HCl pH 8.0 (50 mM), NaCl (150 mM), glycerol (5%), EDTA (5 mM), Triton X-100 (0.05%), DTT (10 mM), protease inhibitor cocktail (1%), PMSF (1 mM)
NR activity assay extraction buffer	Tris-HCl pH 8.0 (250 mM), EDTA (1mM), Na₂MoO₄ (1 μM ), FAD (5 μM), DTT (3 mM), β-mercaptoethanol (12 mM), PMSF (250 μM)
NR activity reaction buffer	NaNO <sub>3</sub> (40 mM), K <sub>2</sub> HPO <sub>4</sub> (80 mM), KH <sub>2</sub> PO <sub>4</sub> (20 mM), NADH (0.2 mM), pH 7.5
NiR activity assay extraction buffer	Potassium phosphate buffer pH 7.5 (50 mM), EDTA (1 mM), β- mercaptoethanol (10 mM), PMSF (100 μM), PVP (1.25 mg/100 μl)
NiR activity reaction buffer	Potassium phosphate buffer pH 7.5 (33 mM), NaNO <sub>2</sub> (1 mM), methyl viologen (1 mM), sodium dithionite (11.11 mM)

# Table A3. Oligonucleotides used in this work.

Name	Sequence (5'to 3')	AGI	Application
M13-F	GTAAAACGACGGCCAG		Cloning
			pCR8/GW/TOPO
M13-R	CAGGAAACAGCTATGAC		Cloning
			pCR8/GW/TOPO
35S-seq	CCTTCGCAAGACCCTTCCTCTA		Cloning pAlligator2
NOS-term-rev	GCAAGACCGGCAACAGGATTCAATC		Cloning pAlligator2
NLP7-F	ATGTGCGAGCCCGATGATAATTCCGC	AT4G24020	Cloning NLP7
nostop-NLP7-R	CAATTCTCCAGTGCTCTCGCAGG	AT4G24020	Cloning NLP7
SeqNLP7_2R SeqNLP7_1F	GGTTAGGTGAATTATTGGTG ATAGTGAAAGTGACGTAGAG	AT4G24020 AT4G24020	Cloning NLP7 Cloning NLP7
SeqNLP7_3R	CGGTTCATCTAATTGGTTAC	AT4G24020	Cloning NLP7
NR1cds-F	ATGGCGACCTCCGTCGATAACCGCCATTATCCC	AT1G77760	Cloning NIA1
NR1cds-R	GAAGATTAAGAGATCCTCCTTCACGTTGTAACC	AT1G77760	Cloning NIA1
seqNR1_F	AGGAGATGGCGATGGATC	AT1G77760	Cloning NIA1
SeqNR1_3rev	GATCGAAGTCTTCTCAATG	AT1G77760	Cloning NIA1
SeqNR1_2fw	GAGCTAATCTCCGAGTTC	AT1G77760	Cloning NIA1
NR2cds-F	ATGGCGGCCTCTGTAGATAATCGCCAATACGCT	AT1G37130	Cloning NIA2
NR2cds-R seqNR2_F	GAATATCAAGAAATCCTCCTTGATGTTATATTG AGAACATGGTGAAGAAGTC	AT1G37130 AT1G37130	Cloning NIA2 Cloning NIA2
SeqNR2_2rev	CTAGAACCATATCCTCAAC	AT1G37130	Cloning NIA2
NIR1cds-F	ATGACTTCTTTCTCTCTCACTTTCACATCTCC	AT2G15620	Cloning NIR1
NIR1cds-R	ATCTTCATTCTCTTCTCTTCTCTAGGCACAG	AT2G15620	Cloning NIR1
seqNIR1_F	GTGGTATGGATAACGTGAG	AT2G15620	Cloning NIR1
SLIM_F	gttttagagctagaaatagcaag		Cloning NIR1guide
SLIM_R	caatcactacttcgactct		Cloning NIR1guide
SLIM_F_NiR1(1)	GCCGCTCAGACCACAGCTCgttttagagctagaaatagc	AT2G15620	Cloning NIR1guide
SLIM_R_NiR1(1)	aag GAGCTGTGGTCTGAGCGGCcaatcactacttcgactct	AT2G15620	Cloning NIR1guide
pDONR_F	TCGCGTTAACGCTAGCATGGATCTC	A12013020	Cloning NIR1guide
pDONR_R	GTAACATCAGAGATTTTGAGACAC		Cloning <i>NIR1guide</i>
P1b-HindIII	TTTTTTAAGCTTGTTTAAACAAGCTTTCGTTGAA		Cloning <i>NIR1guide</i>
	С		5 <b>5 5</b>
P4b-Spel	TTTTTTACTAGTGGTTTAAACAAAAAAAGCACCG		Cloning NIR1guide
pU6seqF	AGGCATCGAACCTTCAAGAATTTG		Cloning NIR1guide
pU6seqR	CTTCTCTTCTTCAGATTCC		Cloning NIR1guide
LB3 LBb1.3	TAGCATCTGAATTTCATAACCAATCTCGAT ATTTTGCCGATTTCGGAAC		Genotyping SAIL lines
CAPS_nr1-R	GGCTATAGATCCCGCATCGAC	AT1G77760	Genotyping SALK lines Genotyping <i>nia1</i>
CAPS_nr1-F	TACGACGACTCCTCAAGCGAC	AT1G77760	Genotyping nia1
NR2.1-RP	ACCTTCTTCGTCGGCGAGTTC	AT1G37130	Genotyping <i>nia</i> 2
NR2.1-LP	ACGGCGTGGTTCGTTCTTACA	AT1G37130	Genotyping nia2
AtNOS1.1-RP	GCACCTACACCACAGGCAAGC	AT3G47450	Genotyping noa1-2
AtNOS1.1-LP	CCAATTGGCAATGTTGGTCG	AT3G47450	Genotyping noa1-2
NIR1cds-F	ATGACTTCTTTCTCTCTCACATCTCC	AT2G15620	Genotyping nir1-1
seqnir1-1-R	GATCCCTTTTCGAAACTTC	AT2G15620	Genotyping <i>nir1-1</i>
SALK_026134C_LP SALK_026134C_RP	AAGAATCAACCGAACAACACG CTTCAAAATAGCAGGCCAAATG	AT4G24020 AT4G24020	Genotyping <i>nlp7-1</i> Genotyping <i>nlp7-1</i>
SALK_0201340_KP SAIL 1278 H11 LP3	AAAATTGATCCTTTCCATGCC	AT4G24020 AT5G02310	Genotyping <i>prt6-1</i>
SAIL_1278_H11_RP3	CAACATAAGAATCTGCGGGAG	AT5G02310	Genotyping prt6-1
Hyg-F	CGGCGAGTACTTCTACACAGC		Genotyping <i>NIR1</i> guide
Hyg-R	CTGATCGAAAAGTTCGACAGC		Genotyping NIR1 guide
qÅB1-F	TTCTCCGAAACCCAGATGGA	AT4G26080	qRT-PCR
qABI1-R	CGTTCTCGGAATCTTGATTTGAG	AT4G26080	qRT-PCR
qABI2-F	CTGCAGTCGCTGTTCCATTC	AT5G57050	qRT-PCR
qABI2-R	CACCGTTGCAATAGCCTCTAAGT	AT5G57050	qRT-PCR
qADH1-F	GCACCTGCTGAACATCAAGA AGGATGTGGGTGACCTTGTC	AT1G77120 AT1G77120	qRT-PCR qRT-PCR
qADH1-R qAHG1-F	CGAGGGAGGCTGCGATTT	AT5G51760	gRT-PCR
qAHG1-R	ACAATAATATGATCGTGCGTCAAAA	AT5G51760	gRT-PCR
qAHG3-F	TTGTTGCGGTGTTGTTGGA	AT3G11410	gRT-PCR
qAHG3-R	AGAGAAGCTCGAGAAGTTGAATCA A	AT3G11410	qRT-PCR
qAtNOA-2nd-F	CAATGAAGCAGAGCATGAGATACA	AT3G47450	qRT-PCR
qAtNOA-2nd-R	TGGCAATGTTGGTCGAAGAA	AT3G47450	qRT-PCR
qAXR5-F	TGGATGGCCTCCAGTGAGATCTAACC	AT4G14560	qRT-PCR
qAXR5-R	GGAGCTCCGTCCATACTCACTTTCAC	AT4G14560	qRT-PCR
qCBF1-F	GGAGACAATGTTTGGGATGC	AT4G25490	
qCBF1-R qCBF2-F	TTAGTAACTCCAAAGCGACACG GACGTGTCCTTATGGAGCTATTAAAA	AT4G25490 AT4G25470	qRT-PCR aRT-PCR
qCBF2-R qCBF2-R	TTACCATTTACATTCGTTTCTCACAAC	AT4G25470 AT4G25470	gRT-PCR
qCBF3-F	TTCCGTCCGTACAGTGGAAT	AT4G25480	gRT-PCR
qCBF3-R	AACTCCATAACGATACGTCGTC	AT4G25480	qRT-PCR
qCHI-F	ATGTCTTCATCCAACGCCTGCGCC	AT3G55120	qRT-PCR

qCHI-R	GACGGTGAAGATCACGAATTTACC	AT3G55120	qRT-PCR
qCHS-F	AGCTGATGGACCTGCAGGCATCTTGGC	AT5G13930	qRT-PCR
qCHS-R	TGCATGTGACGTTTCCGAATTGTCGAC	AT5G13930	qRT-PCR
qCYP707A3-F	TCTTTTGGGAATGAAGGGTCTG	AT5G45340	qRT-PCR
			•
qCYP707A3-R	TGATGTGCCAAAGGAGGACG	AT5G45340	qRT-PCR
qDFR-F	CGTGCCACCGTTCGAGATCC	AT5G42800	qRT-PCR
qDFR-R	GTGAGTAGCGTCTTGGCGTTTGGC	AT5G42800	qRT-PCR
•			
qF3H/TT7-F	ATGGCTCCAGGAACTTTGACTGAGCTA	AT3G51240	qRT-PCR
qF3H/TT7-R	GATCTGACGGCAGATCTCTCCTCTTT	AT3G51240	qRT-PCR
qHAB1-F	TCCCGCAGTTGCAATGACT	AT1G72770	qRT-PCR
qHAB1-R	CTGAGTGATCTCGACAGGTGATG	AT1G72770	qRT-PCR
qHAB2-F	CTGTTCAGCGGAGGTACATACTTC	AT1G17550	qRT-PCR
qHAB2-R	CCCACCAGCCGTTTATTCTC	AT1G17550	qRT-PCR
•			
qHAI1-F	ACGCGCATGGACATGGA	AT5G59220	qRT-PCR
gHAI1-R	CTCGCACCGGCATTTTG	AT5G59220	qRT-PCR
qHAI2-F	GCGACGGACGGGCTATG	AT1G07430	qRT-PCR
•			
qHAI2-R	ACACATGCGCACCATCGTA	AT1G07430	qRT-PCR
gHAI3-F	CCGTCGTCGGTTTATGAATCA	AT2G29380	qRT-PCR
qHAI3-R	TGCATCACCGTTTGGAATCTC	AT2G29380	qRT-PCR
qLEA4-5-F	GGAAAAGGCGGAGAAGATGA	AT5G06760	qRT-PCR
gLEA4-5-R	TTGTGCTGACGCGTTTCTCT	AT5G06760	qRT-PCR
	GTGAGACACAGAGGAAGTGAAGAG	AT1G52690	gRT-PCR
qLEA7-F			•
qLEA7-R	CTCACGAACGCAACAAACACTAATC	AT1G52690	qRT-PCR
qLTI65/RD29b-F	CTTGGCACCACCGTTGGGACTA	AT5G52300	qRT-PCR
qLTI65/RD29b-R	TCAGTTCCCAGAATCTTGAACT	AT5G52300	qRT-PCR
•			
q-NCED3-F	CGGTGGTTTACGACAAGAACAA	AT3G14440	qRT-PCR
q-NCED3-R	CAGAAGCAATCTGGAGCATCAA	AT3G14440	qRT-PCR
qNIA1-F	AGGTTTGGAAGGCGAATCG		
•		AT1G77760	qRT-PCR
qNIA1-R	TGGCTGCAACGCAAACTG	AT1G77760	qRT-PCR
qNIA2-F	CCCGTTGCACTACGTTCGTA	AT1G37130	qRT-PCR
			qRT-PCR
qNIA2-R	CGTCCATTCGGCCCATT	AT1G37130	
qP-ACT2-F	TTGTTCCAGCCCTCGTTTGT	AT3G18780	qRT-PCR
qP-ACT2-R	TGTCTCGTGGATTCCAGCAG	AT3G18780	qRT-PCR
qPAP1-F	GAGGGTTCGTCCAAAGGGCTGCG	AT1G56650	qRT-PCR
qPAP1-R	GCACCGGTTTAGCCCAGCTCTTAC	AT1G56650	qRT-PCR
gPYL1-F	GGCGAATTCAGAGTCCTCCTCC	AT5G46790	qRT-PCR
qPYL1-R	GGGAGAGTTGGGTGAATTCG	AT5G46790	qRT-PCR
qPYL2-F	CAGTACCGAGCGGCTTGAG	AT2G26040	qRT-PCR
gPYL2-R	GCCGACGACCCTGAAGCT	AT2G26040	qRT-PCR
qPYL3-F	CATCACTCATAGCACACCGTGTAG	AT1G73000	qRT-PCR
qPYL3-R	CGAAGTCGCGGACGAATCT	AT1G73000	qRT-PCR
qPYL4-F	GCTTGCCGTTCACCGTCCTTC	AT2G38310	qRT-PCR
qPYL4-R	CGCGATCATCGGGAATC	AT2G38310	qRT-PCR
qPYL5-F	GGTCACCGGTGCAACTCC	AT5G05440	qRT-PCR
qPYL5-R	CGCGTGGATCATCTGCACC	AT5G05440	qRT-PCR
qPYL6-F	CCAACGTCGATACAGTTTCAG	AT2G40330	gRT-PCR
qPYL6-R	CCTCCACGTCTTGTACCACG	AT2G40330	qRT-PCR
qPYL7-F	GATCGGAGGAGACGATACAGATACA	AT4G01026	gRT-PCR
qPYL7-R	AGTGGTGAAGATGACGCAACCT		qRT-PCR
		AT4G01026	
qPYL8-F	GGAAGCTAACGGGATTGAG	AT5G53160	qRT-PCR
gPYL8-R	CGGCTTATACTTCTGTGGC	AT5G53160	qRT-PCR
qPYL9-F	GATATGATGGACGGCGTTGAA	AT1G01360	gRT-PCR
qPYL9-R	ATGCGTCCGTACGTATTGCA	AT1G01360	qRT-PCR
qPYR1-F	GCCTTCGGAGTTAACACCAGAAG	AT4G17870	qRT-PCR
qPYR1-R	CGTCGTACGATTGACCAGACGAG	AT4G17870	qRT-PCR
qRAP2.12-F	TGCAGATTTCTCAGCGTCCCCATC	AT1G53910	qRT-PCR
qRAP2.12-R	GCTGCGGAAGGTTCAGTTTTTGGT	AT1G53910	qRT-PCR
qRAP2.3-F	CAAACTCCATCCCACCAACCAAGT	AT3G16770	qRT-PCR
qRAP2.3-R	TCTGTTGCCTGCTCCTTCTTCACT	AT3G16770	qRT-PCR
qSnRK2.10-F	TTCTATCACTTTGTGCCTTTTTTAGC	AT1G60940	qRT-PCR
gSnRK2.10-R	ACTACACAAAGTCACAAACCCAGAAA	AT1G60940	qRT-PCR
qSnRK2.1-F	TGCTCT CTGTTTCTCTTACTTTATTTCTTC	AT5G08590	qRT-PCR
qSnRK2.1-R	TGGCTTTGACAAATGTGTTTTTCT	AT5G08590	qRT-PCR
gSnRK2.2-F	GCAGATAATATCGGAGGCTACGA	AT3G50500	qRT-PCR
qSnRK2.2-R	TCAAGATTATCCGCCATGAAATC	AT3G50500	qRT-PCR
qSnRK2.3-F	TCGAATTTCTCTTTTTGTGATCAGA	AT5G66880	qRT-PCR
gSnRK2.3-R	ACTGTCGTGCATAATCGGCATA	AT5G66880	qRT-PCR
qSnRK2.4-F	CATCGTCATCTCTTTCCCTTT		•
		AT1G10940	qRT-PCR
qSnRK2.4-R	CAAAATCAAGGATGCGATTCC	AT1G10940	qRT-PCR
gSnRK2.5-F	CAACGAGAGATCGTGATCGTACTT	AT5G63650	qRT-PCR
qSnRK2.5-R	CAATACCTTCACAACCTCATACTTGT		qRT-PCR
		AT5G63650	
qSnRK2.7-F	TTTCCTCCGAGTGCAAGCAT	AT4G40010	qRT-PCR
gSnRK2.7-R	GTGCTTTTCGATTTCCGGTACA	AT4G40010	qRT-PCR
qSnRK2.8-F	GGCAACACATTTGGCGTTAGT	AT1G78290	gRT-PCR
qSnRK2.8-R	CGGCGCTGCAGATTCTTC	AT1G78290	qRT-PCR
qSnRK2.9-F	GGAGAAGTATGAGATGGTGAAGGATT	AT2G23030	qRT-PCR
•			

qSnRK2.9-R	CACAAGCTCGTTTGTTTGCTTATT	AT2G23030	qRT-PCR	
qTEM2-F	TGGTCCGAGAGAAAACCCG	AT1G68840	qRT-PCR	
qTEM2-R	TCAACTCCGAAAAGCCGAAC	AT1G68840	qRT-PCR	
qZF-F	TCATTTCCTCGTAACAATCCTTTATTC	AT4G29190	qRT-PCR	
qZF-R	CGGTGTTGTAGGCAGAGACTGA	AT4G29190	qRT-PCR	

Table A4. Proteins with the MC- N-terminal amino acid sequence in the Arabidopsis proteome. The Arabidopsis proteome (TAIR 10 protein database) was searched for proteins with MCX sequence starting in position 1 with the PatMatch tool. 247 proteins encoded by 206 genes were identified.

AGI code	Annotation	N-term seq	Transcript
T1G03106	unknown protein	MCG	AT1G03106.1
T1G04501	unknown protein	MCI	AT1G04501.1
T1G05280	Protein of unknown function (DUF604)	MCL	AT1G05280.1
T1G05550	Protein of unknown function (DUF295)	MCL	AT1G05550.2
T1G06350	Fatty acid desaturase family protein	MCD	AT1G06350.1
T1G06360	Fatty acid desaturase family protein	MCD	AT1G06360.1
AT1G06570	HPD_HPPD_PDS1phytoene desaturation 1	MCL	AT1G06570.1
T1G06570	HPD_HPPD_PDS1phytoene desaturation 1	MCL	AT1G06570.2
T1G09500	NAD(P)-binding Rossmann-fold superfamily protein	MCF	AT1G09500.2
T1G10657	Plant protein 1589 of unknown function	MCT	AT1G10657.1
T1G10657	Plant protein 1589 of unknown function	MCT	AT1G10657.2
T1G10657	Plant protein 1589 of unknown function	MCT	AT1G10657.3
T1G10657	Plant protein 1589 of unknown function	MCT	AT1G10657.4
T1G11362	Plant invertase/pectin methylesterase inhibitor superfamily protein	MCL	AT1G11362.1
T1G11970	Ubiquitin-like superfamily protein	MCH	AT1G11970.1
T1G14270	CAAX amino terminal protease family protein	MCG	AT1G14270.1
T1G17235	RTFL11ROTUNDIFOLIA like 11	MCI	AT1G17235.1
T1G20240	SWI-SNF-related chromatin binding protein	MCV	AT1G20240.1
T1G21738	unknown protein	MCV	AT1G20240.1 AT1G21738.1
T1G21738			
11622950	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	MCN	AT1G22950.1
T1G24380	unknown protein	MCI	AT1G24380.1
AT1G26770	ATEXP10_expansin A10	MCR	AT1G26770.2
T1G29010	unknown protein	MCS	AT1G29010.1
T1G29090	Cysteine proteinases superfamily protein	MCD	AT1G29090.1
T1G29951	CPuORF35conserved peptide upstream open reading frame 35	MCI	AT1G29951.1
T1G30650	AR411_ATWRKY14_WRKY DNA-binding protein 14	MCS	AT1G30650.1
T1G44090	ATGA20OX5_GA20OX5gibberellin 20-oxidase 5	MCI	AT1G44090.1
T1G49230	RING/U-box superfamily protein	MCT	AT1G49230.1
T1G52342	unknown protein	MCY	AT1G52342.1
T1G53625	unknown protein	MCR	AT1G53625.1
T1G53910	RAP2.12_related to AP2 12	MCG	AT1G53910.1
T1G53910	RAP2.12_related to AP2 12	MCG	AT1G53910.2
T1G53910	RAP2.12_related to AP2 12	MCG	AT1G53910.3
T1G55830	unknown protein	MCC	AT1G55830.1
T1G58340	BCD1_ZF14_ZRZMATE efflux family protein	MCN	AT1G58340.1
AT1G59535	unknown protein	MCS	AT1G59535.1
AT1G62150	Mitochondrial transcription termination factor family protein	MCS	AT1G62150.1
AT1G63200	Cystatin/monellin superfamily protein	MCD	AT1G63200.1
T1G63535	Defensin-like (DEFL) family protein	MCL	AT1G63535.1
	unknown protein	MCS	
AT1G63610			AT1G63610.1
T1G63610	unknown protein	MCS	AT1G63610.2
T1G65520	ATECI1_ECHIC_ECI1_PEC11delta(3), delta(2)- enoyl CoA isomerase 1	MCS	AT1G65520.1
T1G67520	lectin protein kinase family protein	MCS	AT1G67520.1
T1G68810	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	MCA	AT1G68810.1
T1G69580	Homeodomain-like superfamily protein	MCL	AT1G69580.1
T1G69580	Homeodomain-like superfamily protein	MCL	AT1G69580.2
T1G69710	Regulator of chromosome condensation (RCC1) family with FYVE	MCI	AT1G69710.1
T1C70550	zinc finger Brotoin of Lloknown Euroction (DLIE220)	MCI	AT1070550 4
AT1G70550 AT1G72360	Protein of Unknown Function (DUF239) AtERF73_ERF73_HRE1Integrase-type DNA-binding	MCL MCG	AT1G70550.1 AT1G72360.2
T1G72360	superfamily protein AtERF73_ERF73_HRE1Integrase-type DNA-binding	MCG	
11972300	superfamily protein	MCG	AT1G72360.3
T1G75810	unknown protein	MCL	AT1G75810.1
T1G75910	EXL4_extracellular lipase 4	MCS	AT1G75910.1
T1G80220	Protein of unknown function (DUF1644)	MCK	AT1G80220.1
T1G80640	Protein kinase superfamily protein Protein kinase superfamily protein	MCL	AT1G80640.1
AT1G80640	Protein kinase superfamily protein Protein kinase superfamily	MCL	AT1G80640.2
T2C04470	protein	MCV	AT2C04470 0
T2G01170	BAT1bidirectional amino acid transporter 1	MCV	AT2G01170.2
T2G02240	MEE66F-box family protein	MCG	AT2G02240.1
T2G03667	Asparagine synthase family protein	MCG	AT2G03667.1
T2G05185	unknown protein	MCK	AT2G05185.1
TOCOLADE	unknown protein	MCK	AT2G05185.2
AT2G05185 AT2G07362	unknown protein	MCS	AT2G07362.1

AT2G07640			
	NAD(P)-binding Rossmann-fold superfamily protein	MCH	AT2G07640.1
AT2G10450	14-3-3 family protein	MCC	AT2G10450.1
AT2G10560	unknown protein	MCE	AT2G10560.1
AT2G11405		MCW	AT2G11405.1
	unknown protein		
AT2G11620	unknown protein	MCN	AT2G11620.1
AT2G14288	unknown protein	MCW	AT2G14288.1
AT2G22802	unknown protein	MCR	AT2G22802.1
AT2G23118	unknown protein	MCL	AT2G23118.1
AT2G26050	Protein of unknown function (DUF1644)	MCE	AT2G26050.1
AT2G26110	Protein of unknown function (DUF761)	MCF	AT2G26110.1
AT2G26470	unknown protein	MCG	AT2G26470.1
AT2G26610	Transducin family protein / WD-40 repeat family protein	MCS	AT2G26610.1
AT2G27110	FRS3_FAR1-related sequence 3	MCW	AT2G27110.3
AT2G29263	unknown protein	MCC	AT2G29263.1
AT2G31090	unknown protein	MCD	AT2G31090.1
		MCQ	
AT2G31700	unknown protein		AT2G31700.1
AT2G33230	YUC7_YUCCA 7	MCN	AT2G33230.1
AT2G34010	unknown protein	MCS	AT2G34010.1
	•		
AT2G34238	unknown protein	MCL	AT2G34238.1
AT2G38570	CONTAINS InterPro DOMAIN/s: PRC-barrel-like	MCN	AT2G38570.1
AT2G39590	Ribosomal protein S8 family protein	MCV	AT2G39590.1
AT2G40140	ATSZF2_CZF1_SZF2_ZFAR1zinc finger (CCCH-type) family	MCG	AT2G40140.1
	protein		
AT2G40140	ATSZF2_CZF1_SZF2_ZFAR1zinc finger (CCCH-type) family	MCG	AT2G40140.2
	protein		
AT2G41240	BHLH100basic helix-loop-helix protein 100	MCA	AT2G41240.1
AT2G41240	BHLH100 basic helix-loop-helix protein 100	MCA	AT2G41240.2
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AT2G41470	unknown protein	MCN	AT2G41470.1
AT2G41900	OXS2 CCCH-type zinc finger protein with ARM repeat domain	MCC	AT2G41900.1
AT2G43340	Protein of unknown function (DUF1685)	MCD	AT2G43340.1
AT2G43490	Ypt/Rab-GAP domain of gyp1p superfamily protein	MCS	AT2G43490.1
AT2G43490	Ypt/Rab-GAP domain of gyp1p superfamily protein	MCS	AT2G43490.2
AT2G43490	Ypt/Rab-GAP domain of gyp1p superfamily protein	MCS	AT2G43490.3
AT2G43490	Ypt/Rab-GAP domain of gyp1p superfamily protein	MCS	AT2G43490.4
AT2G43490	Ypt/Rab-GAP domain of gyp1p superfamily protein	MCS	AT2G43490.5
AT2G43490	Ypt/Rab-GAP domain of gyp1p superfamily protein	MCS	AT2G43490.6
AT2G44304	unknown protein	MCE	AT2G44304.1
AT2G45380	unknown protein	MCM	AT2G45380.2
		-	
AT2G47520	AtERF71_ERF71_HRE2Integrase-type DNA-binding	MCG	AT2G47520.1
	superfamily protein		
AT2C01020		MCI	AT2C01020 1
AT3G01930	Major facilitator superfamily protein		AT3G01930.1
AT3G02250	O-fucosyltransferase family protein	MCK	AT3G02250.1
AT3G02800	AtPFA-DSP3_PFA-DSP3Tyrosine phosphatase family protein	MCL	AT3G02800.1
AT3G03720	CAT4cationic amino acid transporter 4	MCL	AT3G03720.1
AT3G03773	HSP20-like chaperones superfamily protein	MCD	AT3G03773.2
AT3G04732	unknown protein	MCI	AT3G04732.1
AT3G04750	Tetratricopeptide repeat (TPR)-like superfamily protein	MCF	AT3G04750.1
AT3G06019	unknown protein	MCK	AT3G06019.1
AT3G09870	SAUR-like auxin-responsive protein family	MCK	AT3G09870.1
	DCD (Development and Cell Death) domain protein	MCV	
AT3G11000			AT3G11000.1
AT0044770	Thioredoxin superfamily protein		
AT3G11773		MCS	AT3G11773.1
AT3G12600	atnudt16_NUDT16nudix hydrolase homolog 16	MCD	AT3G12600.1
	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein		
AT3G12600 AT3G13275	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein	MCD MCC	AT3G12600.1 AT3G13275.1
AT3G12600 AT3G13275 AT3G14230	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2	MCD MCC MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2	MCD MCC MCG MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2
AT3G12600 AT3G13275 AT3G14230 AT3G14230	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2	MCD MCC MCG MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2	MCD MCC MCG MCG MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3
AT3G12600 AT3G13275 AT3G14230 AT3G14230	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element	MCD MCC MCG MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2	MCD MCC MCG MCG MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein	MCD MCC MCG MCG MCG MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family	MCD MCC MCG MCG MCG MCG MCV	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein	MCD MCC MCG MCG MCG MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein	MCD MCC MCG MCG MCG MCG MCV MCV	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16970 AT3G16970 AT3G19560 AT3G21890	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein	MCD MCC MCG MCG MCG MCG MCV MCM MCR	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein	MCD MCC MCG MCG MCG MCC MCM MCM	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein	MCD MCC MCG MCG MCG MCC MCM MCM	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G23680	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein	MCD MCC MCG MCG MCG MCC MCM MCR MCR MCR	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G22680.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G23680 AT3G224090	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase	MCD MCC MCG MCG MCG MCC MCM MCM MCR MCM MCR MCR MCR MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G23680 AT3G224090	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase	MCD MCC MCG MCG MCG MCC MCM MCM MCR MCM MCR MCR MCR MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G22680 AT3G224090 AT3G26100	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCR MCR MCR MCR	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G26100.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G23680 AT3G224090	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily	MCD MCC MCG MCG MCG MCC MCM MCM MCR MCM MCR MCR MCR MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G22680 AT3G224090 AT3G26100	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCR MCR MCR MCR	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G26100.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G23680 AT3G24090 AT3G26100 AT3G27130	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein	MCD MCG MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCR MCR MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16970.1 AT3G16970.1 AT3G1890.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G221130.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G224090 AT3G224090 AT3G22130 AT3G227130	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein	MCD MCG MCG MCG MCG MCV MCM MCR MCM MCR MCR MCR MCG MCF	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16970.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G22409.1 AT3G24090.1 AT3G24090.1 AT3G221130.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G23680 AT3G24090 AT3G26100 AT3G27130	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein	MCD MCG MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCR MCR MCG	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16970.1 AT3G16970.1 AT3G1890.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G221130.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G224090 AT3G224090 AT3G226100 AT3G227130 AT3G28193 AT3G28530	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases	MCD MCG MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCR MCG MCF MCR	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16970.1 AT3G16970.1 AT3G21890.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G27130.1 AT3G28193.1 AT3G28193.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G23680 AT3G24090 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases unknown protein	MCD MCG MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCG MCF MCR MCR MCR MCS	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G22680.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G27130.1 AT3G28193.1 AT3G28193.1 AT3G28530.1 AT3G42780.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G224090 AT3G224090 AT3G226100 AT3G227130 AT3G28193 AT3G28530	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases	MCD MCG MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCR MCG MCF MCR	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16970.1 AT3G16970.1 AT3G21890.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G27130.1 AT3G28193.1 AT3G28193.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G23680 AT3G24090 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780 AT3G44713	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases unknown protein unknown protein	MCD MCG MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCG MCF MCR MCS MCL	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G24090.1 AT3G26100.1 AT3G28193.1 AT3G28193.1 AT3G28530.1 AT3G42780.1 AT3G44713.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G22415 AT3G26100 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780 AT3G44713 AT3G46980	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein UDP-glucose 4-epimerases unknown protein unknown protein PHT4;3phosphate transporter 4;3	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCS MCF MCR MCS MCL MCY	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G28193.1 AT3G28193.1 AT3G28530.1 AT3G42780.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G23680 AT3G24090 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780 AT3G44713	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases unknown protein unknown protein	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCG MCF MCF MCR MCS MCL MCY MCY	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G24090.1 AT3G24090.1 AT3G26100.1 AT3G28193.1 AT3G28193.1 AT3G28530.1 AT3G42780.1 AT3G44713.1
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G21890 AT3G22415 AT3G22415 AT3G22415 AT3G224090 AT3G26100 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780 AT3G44713 AT3G46980 AT3G46980	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein UDP-glucose 4-epimerases unknown protein UDP-glucose 4-epimerases unknown protein PHT4;3phosphate transporter 4;3 PHT4;3phosphate transporter 4;3	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCG MCF MCF MCR MCS MCL MCY MCY	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G28193.1 AT3G28193.1 AT3G28530.1 AT3G42780.1 AT3G44713.1 AT3G46980.1 AT3G46980.3
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G22415 AT3G224100 AT3G224190 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780 AT3G44713 AT3G46980 AT3G46980 AT3G46980	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases unknown protein PHT4;3phosphate transporter 4;3 PHT4;3phosphate transporter 4;3	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCG MCF MCF MCF MCS MCL MCY MCY MCY	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G28193.1 AT3G28530.1 AT3G28530.1 AT3G46980.1 AT3G46980.2
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G21890 AT3G22415 AT3G22415 AT3G22415 AT3G224090 AT3G26100 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780 AT3G44713 AT3G46980 AT3G46980	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases unknown protein PHT4;3phosphate transporter 4;3 PHT4;3phosphate transporter 4;3 ASN1_AT-ASN1_DIN6glutamine-dependent asparagine	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCG MCF MCF MCR MCS MCL MCY MCY	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G28193.1 AT3G28193.1 AT3G28530.1 AT3G42780.1 AT3G44713.1 AT3G46980.1 AT3G46980.3
AT3G12600 AT3G13275 AT3G14230 AT3G14230 AT3G14230 AT3G14230 AT3G16770 AT3G16970 AT3G19560 AT3G21890 AT3G22415 AT3G22415 AT3G22415 AT3G224100 AT3G224190 AT3G26100 AT3G27130 AT3G28193 AT3G28530 AT3G42780 AT3G44713 AT3G46980 AT3G46980 AT3G46980	atnudt16_NUDT16nudix hydrolase homolog 16 unknown protein RAP2.2related to AP2 2 RAP2.2related to AP2 2 RAP2.2related to AP2 2 ATEBP_EBP_ERF72_RAP2.3ethylene-responsive element binding protein Plant self-incompatibility protein S1 family F-box family protein BBX31, B-box type zinc finger family protein unknown protein F-box associated ubiquitination effector family protein glutamine-fructose-6-phosphate transaminase Regulator of chromosome condensation (RCC1) family protein P-loop containing nucleoside triphosphate hydrolases superfamily protein unknown protein UDP-glucose 4-epimerases unknown protein PHT4;3phosphate transporter 4;3 PHT4;3phosphate transporter 4;3	MCD MCC MCG MCG MCG MCV MCM MCR MCR MCR MCR MCR MCG MCF MCF MCF MCS MCL MCY MCY MCY	AT3G12600.1 AT3G13275.1 AT3G14230.1 AT3G14230.2 AT3G14230.3 AT3G16770.1 AT3G16970.1 AT3G19560.1 AT3G21890.1 AT3G22415.1 AT3G224090.1 AT3G224090.1 AT3G24090.1 AT3G26100.1 AT3G28193.1 AT3G28530.1 AT3G28530.1 AT3G46980.1 AT3G46980.2

AT3G47340	ASN1_AT-ASN1_DIN6glutamine-dependent asparagine	MCG	AT3G47340.2
	synthase 1		
AT3G47340	ASN1_AT-ASN1_DIN6glutamine-dependent asparagine	MCG	AT3G47340.3
	synthase 1		
AT3G48346	unknown protein	MCA	AT3G48346.1
AT3G49770	unknown protein	MCK	AT3G49770.1
AT3G50440	ATMES10_MES10methyl esterase 10	MCI	AT3G50440.1
AT3G51640	unknown protein	MCI	AT3G51640.1
AT3G51650	unknown protein	MCI	AT3G51650.1
AT3G53160	UGT73C7_UDP-glucosyl transferase 73C7	MCS	AT3G53160.1
AT3G54510	Early-responsive to dehydration stress protein (ERD4)	MCR	AT3G54510.1
AT3G55980	ATSZF1_SZF1salt-inducible zinc finger 1	MCS	AT3G55980.1
AT3G56550	Pentatricopeptide repeat (PPR) superfamily protein	MCE MCA	AT3G56550.1
AT3G56970	BHLH038_ORG2basic helix-loop-helix (bHLH) DNA-binding superfamily protein	MCA	AT3G56970.1
AT3G56980	BHLH039_ORG3basic helix-loop-helix (bHLH) DNA-binding	MCA	AT3G56980.1
A13G30900	superfamily protein	MCA	A13030900.1
AT3G59680	unknown protein	MCE	AT3G59680.1
AT3G60260	ELMO/CED-12 family protein	MCV	AT3G60260.3
AT3G60890	ZPR2protein binding ZPR2protein binding	MCL	AT3G60890.1
AT3G60890	ZPR2protein binding ZPR2protein binding	MCL	AT3G60890.2
AT3G61763	unknown protein	MCP	AT3G61763.1
AT3G63220	Galactose oxidase/kelch repeat superfamily protein	MCY	AT3G63220.2
AT4G00180	YAB3 Plant-specific transcription factor YABBY family protein	MCD	AT4G00180.2
AT4G00416	MBD3nethyl-CPG-binding domain 3	MCV	AT4G00416.1
AT4G02860	Phenazine biosynthesis PhzC/PhzF protein	MCD	AT4G02860.1
AT4G04650	RNA-directed DNA polymerase (reverse transcriptase)- related	MCK	AT4G04650.1
	family protein		
AT4G05553	zinc knuckle (CCHC-type) family protein	MCG	AT4G05553.1
AT4G09320	NDPK1Nucleoside diphosphate kinase family protein	MCG	AT4G09320.1
AT4G11470	CRK31 cysteine-rich RLK (RECEPTOR-like protein kinase) 31	MCL	AT4G11470.1
AT4G11480	CRK32cysteine-rich RLK (RECEPTOR-like protein kinase) 32	MCL	AT4G11480.1
AT4G11910	unknown protein	MCS	AT4G11910.1
AT4G11911	unknown protein	MCS	AT4G11911.1
AT4G12170	Thioredoxin superfamily protein	MCS	AT4G12170.1
AT4G12382	F-box family protein F-box family protein	MCK	AT4G12382.1
AT4G12382	F-box family protein F-box family protein	MCK	AT4G12382.2
AT4G13395	DVL10_RTFL12ROTUNDIFOLIA like 12	MCL	AT4G13395.1
AT4G14430	ATECI2_ECHIB_ECI2_IBR10_PEC12indole-3- butyric acid	MCT	AT4G14430.1
	response 10		
AT4G14440	ATECI3_ECI3_HCD13-hydroxyacyl-CoA dehydratase 1	MCT	AT4G14440.1
AT4G15248	BBX30, B-box type zinc finger family protein	MCR	AT4G15248.1
AT4G16845	VRN2VEFS-Box of polycomb protein	MCR	AT4G16845.1
AT4G20900	MS5_TDM1Tetratricopeptide repeat (TPR)-like superfamily	MCP	AT4G20900.1
	protein		
AT4G22190	unknown protein	MCY	AT4G22190.1
AT4G22920	ATNYE1_NYE1non-yellowing 1	MCS	AT4G22920.1
AT4G24020	NLP7NIN like protein 7	MCE	AT4G24020.1
AT4G24110	unknown protein	MCR	AT4G24110.1
AT4G24973	Plant self-incompatibility protein S1 family	MCP	AT4G24973.1
AT4G28775	unknown protein	MCV	AT4G28775.1
AT4G28820	HIT-type Zinc finger family protein HIT-type Zinc finger family	MCP	AT4G28820.1
AT4C20020	protein HIT-type Zinc finger family protein HIT-type Zinc finger family	MCP	AT4C20020.2
AT4G28820	protein	NCF	AT4G28820.2
AT4G29140	ADS1MATE efflux family protein	MCN	AT4G29140.1
AT4G29905	unknown protein	MCL	AT4G29140.1 AT4G29905.1
AT4G30760	Putative endonuclease or glycosyl hydrolase	MCA	AT4G30760.1
AT4G30760	Putative endonuclease or glycosyl hydrolase	MCA	AT4G30760.2
AT4G38260	Protein of unknown function (DUF833)	MCI	AT4G38260.1
AT4G38420	sks9SKU5 similar 9	MCW	AT4G38420.1
AT4G38960	BBX19, B-box type zinc finger family protein	MCN	AT4G38960.2
AT4G39470	Tetratricopeptide repeat (TPR)-like superfamily protein	MCS	AT4G39470.1
AT5G03210	AtDIP2_DIP2_unknown protein	MCF	AT5G03210.1
AT5G07640	RING/U-box superfamily protein	MCV	AT5G07640.1
AT5G10240	ASN3_asparagine synthetase 3	MCG	AT5G10240.1
AT5G10240	ASN3_asparagine synthetase 3	MCG	AT5G10240.2
AT5G10695	unknown protein	MCL	AT5G10695.1
AT5G10695	unknown protein	MCL	AT5G10695.2
AT5G12323	unknown protein	MCE	AT5G12323.1
AT5G12850	CCCH-type zinc finger protein with ARM repeat domain	MCG	AT5G12850.1
AT5G13917	unknown protein	MCS	AT5G13917.1
AT5G14370	CCT motif family protein	MCS	AT5G14370.1
AT5G14602	unknown protein	MCP	AT5G14602.1
AT5G14650	Pectin lyase-like superfamily protein	MCR	AT5G14650.1
AT5G15740	O-fucosyltransferase family protein	MCK	AT5G15740.1

AT5G18150	Methyltransferase-related protein	MCP	AT5G18150.
AT5G19729	unknown protein	MCV	AT5G19729.
AT5G20450	unknown protein	MCL	AT5G20450.
AT5G22545	unknown protein	MCR	AT5G225450.
	1	MCK	
AT5G22608	unknown protein		AT5G22608.
AT5G22608	unknown protein	MCK	AT5G22608.
AT5G22608	unknown protein	MCK	AT5G22608.
AT5G22870	Late embryogenesis abundant (LEA) hydroxyproline-rich	MCH	AT5G22870.
	glycoprotein family		
AT5G23640	unknown protein	MCV	AT5G23640.
AT5G24080	Protein kinase superfamily protein	MCL	AT5G24080.
AT5G26940	DPD1Polynucleotidyl transferase, ribonuclease H-like	MCI	AT5G26940.
	superfamily protein		
AT5G26940	DPD1Polynucleotidyl transferase, ribonuclease H-like	MCI	AT5G26940.
/110020010	superfamily protein	mor	/110020010.
AT5G26940	DPD1Polynucleotidyl transferase, ribonuclease H-like	MCI	AT5G26940.
A15G20940		NICI	A15G20940.
ATE000040	superfamily protein		475000040
AT5G26940	DPD1Polynucleotidyl transferase, ribonuclease H-like	MCI	AT5G26940.
	superfamily protein		
AT5G29070	unknown protein	MCY	AT5G29070.
AT5G37478	TPX2 (targeting protein for Xklp2) protein family	MCT	AT5G37478.
AT5G39720	AIG2L_avirulence induced gene 2 like protein	MCS	AT5G39720.
AT5G39770	Restriction endonuclease, type II-like superfamily protein	MCP	AT5G39770.
AT5G42210	Major facilitator superfamily protein	MCI	AT5G42210.
AT5G44310	Late embryogenesis abundant protein (LEA) family protein	MCE	AT5G44310.
AT5G44680	DNA glycosylase superfamily protein	MCS	AT5G44680.
AT5G44830	Pectin lyase-like superfamily protein	MCG	AT5G44830.
	Disease resistance protein (TIR-NBS-LRR class) family	MCS	
AT5G46270			AT5G46270.
AT5G48180	NSP5nitrile specifier protein 5	MCP	AT5G48180.
AT5G49480	ATCP1_CP1Ca2+-binding protein 1	MCP	AT5G49480.
AT5G50361	unknown protein	MCL	AT5G50361
AT5G54830	Dopamine beta-monooxygenase N-terminal domain-containing	MCD	AT5G54830.
	protein		
AT5G55508	unknown protein	MCN	AT5G55508.
AT5G55600	agenet domain-containing protein / bromo-adjacent homology	MCE	AT5G55600.
	(BAH) domain-containing protein		
AT5G55600	agenet domain-containing protein / bromo-adjacent homology	MCE	AT5G55600.
	(BAH) domain-containing protein		
AT5G55600	agenet domain-containing protein / bromo-adjacent homology	MCE	AT5G55600.
A10000000	(BAH) domain-containing protein	MOL	A1000000
	PM-ANTMitochondrial substrate carrier family protein	MCI	ATECEG AEO
AT5G56450		-	AT5G56450.
AT5G56720	c-NAD-MDH3_Lactate/malate dehydrogenase family protein	MCN	AT5G56720.
AT5G57123	unknown protein	MCL	AT5G57123.
AT5G57340	unknown protein	MCF	AT5G57340.
AT5G58375	Methyltransferase-related protein	MCP	AT5G58375.
AT5G59300	ATUBC7_UBC7ubiquitin carrier protein 7	MCS	AT5G59300.
AT5G59990	CCT motif family protein CCT motif family protein	MCG	AT5G59990.
AT5G59990	CCT motif family protein CCT motif family protein	MCG	AT5G59990.
AT5G60100	APRR3_PRR3_pseudo-response regulator 3	MCF	AT5G60100
AT5G60100	APRR3_PRR3pseudo-response regulator 3	MCF	AT5G60100.
AT5G60100	APRR3_PRR3pseudo-response regulator 3	MCF	AT5G60100
AT5G64343	CPuORF38conserved peptide upstream open reading frame 38	MCI	AT5G64343
AT5G64750	ABR1_Integrase-type DNA-binding superfamily protein	MCV	AT5G64750
AT5G65010	ASN2_asparagine synthetase 2 ASN2_asparagine synthetase 2	MCG	AT5G65010
AT5G65010	ASN2_asparagine synthetase 2 ASN2_asparagine synthetase 2	MCG	AT5G65010.
AT5G65080	AGL68_MAF5_K-box region and MADS-box transcription factor	MCR	AT5G65080.
	family protein		
AT5G65495	unknown protein	MCY	AT5G65495.
	MATKmaturase K	MCH	ATCG00040

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**Table A5. Genotyping of T1 generation of** *pU6::gNIR1-1.* A total of 172 hygromycin resistant T1 seedlings transformed with *pHEE2E-gNIR1* (carrying both the Cas9 gene under the control of the Egg Cell Promoter and the RNA scaffold with the RNA guide for *NIR1* under the control of the U6 promoter) were genotyped by sequencing the region around the RNA guide target in the *NIR1* locus. The table shows the found mutations.

Genotype	Number of individuals	Percentage
	172	100%
Wild type	88	51%
Homozygous mutant	0	0%
Heterozygous mutant:	84	49%
<ul> <li>~10bp deletion</li> </ul>	5	3%
<ul> <li>1bp insertion:</li> </ul>	79	46%
– G insertion	1	0.6%
<ul> <li>T insertion</li> </ul>	11	6%
<ul> <li>A insertion</li> </ul>	67	39%

			GPS S YN		iNitro -Tyr	iSNO- PseAAC
Protein	Position	Peptide	Score	Cutoff	Δ	
P49333,	4	****MEV <u>C</u> NCIEPQW	4.142	1.484		
ETR1	65					$(\cdot)$
	65		2 5 4 0	0.440		(+)
	99 572		3.549	2.443		(.)
	573	WIESDGLGKGCTAIFDVKLGI				(+)
	634	VTKGLLVHLGCEVTTVSSNEE	0.000	0.440		(+)
	661		2.832	2.443	0.550	
	195	GLELQLS <u>Y</u> TLRHQHP	0.700	0.54	0.556	
000044	735		2.766	2.51		
Q9S814, EIN2	344	RILAVAPALY <u>C</u> VWTSGADGIY				(+)
	370	TQVLVAMMLPCSVIPLFRIAS				(+)
	729	SFGKDISSGYCMSPTAKGMDS				(+)
	1063	LLQSFRH <u>C</u> ILKLIKL	3.505	2.443		
	1063	EAKLLQSFRH <u>C</u> ILKLIKLEGS				(+)
	1141	ADLIVSFGVWCIHRVLDLSLM				(+)
	1218	AKPAKGK <u>C</u> TTAVTLL	2.897	2.443		
	1218	PPAAKPAKGK <u>C</u> TTAVTLLDLI				(+)
	32	LLVSVG <u>Y</u> IDPGKWV			0.072	
	525	TSVTSSV <u>Y</u> DLPENIL	1.116	0.725		
	746	SQMTSSL <u>Y</u> DSLKQQR	0.946	0.725		
	783	RMQMLGA <u>Y</u> GNTTNNN	1.264	0.828		
	783	RMQMLGA <u>Y</u> GNTTNNN			0.548	
	828	HGYQMKS <u>Y</u> VDNLAKE	1.674	1.065		
	859				0.012	
	901	RQSERSY <u>Y</u> GVPSSGN	0.757	0.725		
	984	RVGVPST <u>Y</u> DDISQSR	1.114	0.828		
	1099				0.587	
	1161				0.542	
B9DFU2, MAX1	467	FIPFGIGPRACVGQRFALQEI				(+)
	147				0.066	
	316	KNIFTSD <u>Y</u> ISAVTYE	1.14	1.065		
	341				0.291	
	409	KEVEIGG <u>Y</u> LLPKGTW	1.4	1.065		
	504				0.322	
Q9SIM9, MAX2	63	DLSLVPD <u>C</u> FRSISHL	2.511	2.443		
	63	NARDLSLVPD <u>C</u> FRSISHLDLS				(+)
	215	SSEIVSITKSCPNLKTFRVAC				(+)
	317	GVALEALNSKCKKLRVLKLGQ				(+)
	332	VLKLGQFQGVCSATEWRRLDG				(+)
	346	EWRRLDGVALCGGLQSLSIKN				(+)
	372	DMGLVAIGRGCCKLTTFEIQG				(+)
	373	MGLVAIGRGCCKLTTFEIQGC				(+)
	410	KTLTDVRISCCKNLDTAASLK				(+)
	426	AASLKAIEPICDRIKRLHIDC				(+)
	471	DDGYERSQKRCKYSFEEEHCS				(+)
	536	EEIRIKIEGDCRGKRRPAEPE				(+)
	551	RPAEPEFGLSCLALYPKLSKM				(+)
	565	YPKLSKMQLDCGDTIGFALTA				(+)

# Table A6. Prediction of S-nitrosylation and nitration sites of potential NO targets.

	111	FVESLNV <u>Y</u> TRSPSSL	0.865	0.828		
	169				1.363	
	464	HEEEDDG <u>Y</u> ERSQKRC	1.189	0.725		
	500				0.415	
Q8VY26,	-	-	-	-		
MAX4						
	79	EKEKVEGERRCHVAWTSVQQE				(+)
	166	YKAAKKHNRLCYREFSETPKS				(+)
	217	VIKLGDGRVMCLTETQKGSIL				(+)
	395	GKATVIIADCCEHNADTRILD				(+)
	474	LGQKYRYVYACGAQRPCNFPN				(+)
	480	YVYACGAQRPCNFPNALSKVD				(+)
	565	PYGLPYGLHGCWIPKDXXXXX				(+)
	167				0.461	
	313				0.342	
	368				1.265	
	463				1.023	
	566				0.060	
Q8S8E3,	61	HVVGPSQ <u>C</u> FSVVVQD	4.196	2.443		
PYL6						
	96	PQAYKHFVKSCHVVIGDGREV				(+)
	199	TCSFADTIVRCNLQSLAKLAE				(+)
	89				0.187	
	176	RTRVVES <u>Y</u> VVDVPAG	1.4	1.065		
	176	_			0.739	
Q1ECF1,	36	HHCRENQ <u>C</u> TSVLVKY	3.549	2.443		
PYL7						
	170	TCYFVESLIKCNLKSLACVSE				(+)
	177	LIKCNLKSLACVSERLAAQDI				(+)
	195	QDITNSIATFCNASNGYREKN				(+)
	14	DDTDTEM <u>Y</u> GALVTAQ	1.405	0.725		
	162				0.527	
Q9LN63,	62	AQGNYNLPKH <u>C</u> DNNEVLKALC				(+)
BES1/BZR2						
	-	-	-	-		
	-	-			-	

Patmatch	
Hits found:	7994
Sequences	6186
with hits:	
Sequences	33602
searched:	
Bytes	33600250
searched:	100000101
Pattern:	MGCCGYM
Dataset	TAIR10 Loci Upstream Sequences 1000 bp (DNA)
searched:	
188 genes w	ith MGCCGYM motif and UP-regulated by NO only in TPT_RAP2.3 –EST
At3g09520	ATEXO70H4_EXO70H4exocyst subunit exo70 family protein H4
At1g03800	ATERF10_ERF10ERF domain protein 10
At5g06865	other RNA
At1g21120	IGMT2_O-methyltransferase family protein
At1g18570 At3g14590	AtMYB51_BW51A_BW51B_HIG1_MYB51myb domain protein 51 NTMC2T6.2_NTMC2TYPE6.2Calcium-dependent lipid-binding (CaLB domain) family protein
At5g58787	RING/U-box superfamily protein
At1g43160	RAP2.6 related to AP2 6
At1g79245	
At5g05140	Transcription elongation factor (TFIIS) family protein
At1g67810	SUFE2_sulfur E2
At1g07500	SMR5
At4g34135	UGT73B2UDP-glucosyltransferase 73B2
At4g13110	BSD domain-containing protein
At4g40065	other RNA
At4g21410	CRK29cysteine-rich RLK (RECEPTOR-like protein kinase) 29
At1g78895	Reticulon family protein ATVSP2_VSP2_vegetative storage protein 2
At5g24770 At3g55940	PLC7_Phosphoinositide-specific phospholipase C family protein
At2g40095	Alpha/beta hydrolase related protein
At1g73805	SARD1Calmodulin binding protein-like
At3g50030	ARM-repeat/Tetratricopeptide repeat (TPR)-like protein
At1g27770	ACA1_PEA1autoinhibited Ca2+-ATPase 1
At5g14700	NAD(P)-binding Rossmann-fold superfamily protein
At3g25790	myb-like transcription factor family protein
At5g27380	AtGSH2_GSH2_GSHBglutathione synthetase 2
At2g45360	Protein of unknown function (DUF1442)
At4g31980 At5g63790	ANAC102 NAC102 NAC domain containing protein 102
At1g33560	ADR1Disease resistance protein (CC-NBS-LRR class) family
At2g31865	PARG2_poly(ADP-ribose) glycohydrolase 2
At1g19770	ATPUP14_PUP14_purine permease 14
At4g05010	AtFBS3_FBS3_F-box family protein
At5g20190	Tetratricopeptide repeat (TPR)-like superfamily protein
At3g21781	other RNA
At3g47960	AtNPF2.10_GTR1_NPF2.10Major facilitator superfamily protein
At1g56140	Leucine-rich repeat transmembrane protein kinase
At4g39890	AtRABH1c_RABH1cRAB GTPase homolog H1C
At3g15500	ANAC055_ATNAC3_NAC055_NAC3NAC domain containing protein 3
At3g25760 At2g46370	AOC1_ERD12allene oxide cyclase 1 AtGH3.11_FIN219_JAR1Auxin-responsive GH3 family protein
At3g30405	transposable element gene
At4g27970	SLAH2_SLAC1 homologue 2
At2g21500	RING/U-box superfamily protein
At1g19570	ATDHAR1_DHAR1_DHAR5dehydroascorbate reductase
At3g21780	UGT71B6_UDP-glucosyl transferase 71B6
At5g61810	APC1Mitochondrial substrate carrier family protein
At4g03510	ATRMA1_RMA1RING membrane-anchor 1
At2g24100	ASG1
At1g27130	ATGSTU13_GST12_GSTU13glutathione S-transferase tau 13
At1g15430	Protein of unknown function (DUF1644)
At5g35735	Auxin-responsive family protein alpha/beta-Hydrolases superfamily protein
At2g05260 At1g21000	PLATZ transcription factor family protein
At2g18750	Calmodulin-binding protein
At1g19670	ATCLH1_ATHCOR1_CLH1_CORI1chlorophyllase 1
At4g18197	ATPUP7_PEX17_PUP7_purine permease 7
At1g53280	AtDJ1B_DJ-1b_DJ1BClass I glutamine amidotransferase-like superfamily protein
At1g79310	AtMC7_AtMCP2a_MC7_MCP2ametacaspase 7

Table A7. In silico genome-wide analysis of putative *RAP2.3*-binding motifs and the intersection with NO-regulated genes.

At4g01026	PYL7_RCAR2PYR1-like 7
At3g14660	CYP72A13cytochrome P450, family 72, subfamily A, polypeptide 13
At1q72850	Disease resistance protein (TIR-NBS class)
At2g17705	
At1g22360	AtUGT85A2_UGT85A2UDP-glucosyl transferase 85A2
At1g26730	EXS (ERD1/XPR1/SYG1) family protein
-	
At5g27420	ATL31_CNI1carbon/nitrogen insensitive 1
At5g66880	SNRK2-3_SNRK2.3_SRK2Isucrose nonfermenting 1(SNF1)-related protein kinase 2.3
At5g49015	Expressed protein
At3g27420	
At4g06529	transposable element gene
At1g50740	Transmembrane proteins 14C
At4g19390	Uncharacterised protein family (UPF0114)
0	
At2g44370	Cysteine/Histidine-rich C1 domain family protein
At1g78410	VQ motif-containing protein
At3g21070	ATNADK-1_NADK1NAD kinase 1
At3g25070	RIN4RPM1 interacting protein 4
At4g22690	CYP706A1cytochrome P450, family 706, subfamily A, polypeptide 1
At2g34930	disease resistance family protein / LRR family protein
At5g20250	DIN10_RS6Raffinose synthase family protein
· · · · · · · · · · · · · · · · · · ·	
At5g18540	
At4g11650	ATOSM34_OSM34osmotin 34
At1g66370	AtMYB113_MYB113myb domain protein 113
At1g61370	S-locus lectin protein kinase family protein
At1g01260	JAM2basic helix-loop-helix (bHLH) DNA-binding superfamily protein
At2g42760	
At3g57760	Protein kinase superfamily protein
At3g57210	Protein of unknown function (DUF626)
At2g43520	ATTI2_TI2_trypsin inhibitor protein 2
At4g36030	ARO3_armadillo repeat only 3
At3g14990	AtDJ1A_DJ-1a_DJ1AClass I glutamine amidotransferase-like superfamily protein
At4g12005	
At4g39940	AKN2_APK2APS-kinase 2
At5g10695	
	ketose-bisphosphate aldolase class-II family protein
At1g18270	
At1g60260	BGLU5beta glucosidase 5
At5g13550	SULTR4;1sulfate transporter 4.1
At4g36930	SPTbasic helix-loop-helix (bHLH) DNA-binding superfamily protein
At5g58400	Peroxidase superfamily protein
At2g18200	
At1g78850	D-mannose binding lectin protein with Apple-like carbohydrate-binding domain
	FBD-like domain family protein
At2g27505	
At5g04020	calmodulin binding
At1g21680	DPP6 N-terminal domain-like protein
At1g13390	
At1g20490	AMP-dependent synthetase and ligase family protein
At1g23850	
At5g07440	GDH2glutamate dehydrogenase 2
At5g21960	Integrase-type DNA-binding superfamily protein
At3g23220	ESE1_Integrase-type DNA-binding superfamily protein
At5g59730	ATEXO70H7_EXO70H7exocyst subunit exo70 family protein H7
At5g25210	
At2g44790	UCC2_uclacyanin 2
At1g14860	NUDT18_atnudt18nudix hydrolase homolog 18
At3g43250	Family of unknown function (DUF572)
At5g56490	AtGulLO4_GulLO4_D-arabinono-1,4-lactone oxidase family protein
At3g47340	ASN1_AT-ASN1_DIN6glutamine-dependent asparagine synthase 1
•	
At5g10830	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
At1g77360	APPR6Tetratricopeptide repeat (TPR)-like superfamily protein
At2g34580	
At4g36140	disease resistance protein (TIR-NBS-LRR class), putative
At4g31875	
At5g14730	
At5g25450	Cytochrome bd ubiquinol oxidase, 14kDa subunit
At3g47210	Plant protein of unknown function (DUF247)
-	
At3g44190	FAD/NAD(P)-binding oxidoreductase family protein
At1g21110	IGMT3O-methyltransferase family protein
At5g10820	Major facilitator superfamily protein
At2g34355	Major facilitator superfamily protein
At1g69920	ATGSTU12_GSTU12glutathione S-transferase TAU 12
At1g57990	ATPUP18_PUP18_purine permease 18
At5g01760	ENTH/VHS/GAT family protein
At2g30870	ATGSTF10_ATGSTF4_ERD13_GSTF10glutathione S-transferase PHI 10
	ABCC7_ATMRP7_MRP7multidrug resistance-associated protein 7
At3g13100	
At4g34131	UGT73B3_UDP-glucosyl transferase 73B3
At3g26470	Powdery mildew resistance protein, RPW8 domain

	At1g22470	
	At5g67410	
	At5g66050	Wound-responsive family protein
	At2g43550	Scorpion toxin-like knottin superfamily protein
	At4g38580	ATFP6_FP6_HIPP26farnesylated protein 6
	At1g66870	Carbohydrate-binding X8 domain superfamily protein
	At1g10140	Uncharacterised conserved protein UCP031279
	At3g01290	AtHIR2_HIR2_SPFH/Band 7/PHB domain-containing membrane-associated protein family
	At1g75000	GNS1/SUR4 membrane protein family
	At3g14200	Chaperone DnaJ-domain superfamily protein
	At3g56710	SIB1_sigma factor binding protein 1
	At4g39675	
	At5g63320	NPX1nuclear protein X1
	At1g67365	other RNA
	At3g45638	other RNA
	At2g44390	Cysteine/Histidine-rich C1 domain family protein
	At3g26840	PES2Esterase/lipase/thioesterase family protein
	At5g07890	myosin heavy chain-related
	At4g13820	Leucine-rich repeat (LRR) family protein
	At5g07460	ATMSRA2_PMSR2peptidemethionine sulfoxide reductase 2
	At5g55460	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
	At4g25030	
	At3g50970	LTI30_XERO2dehydrin family protein
	At5g40348	other RNA
	At3g25500	AFH1_AHF1_ATFH1_FH1formin homology 1
	At1g28380	NSL1MAC/Perforin domain-containing protein
	At4g14680	APS3Pseudouridine synthase/archaeosine transglycosylase-like family protein
	At5g18470	Curculin-like (mannose-binding) lectin family protein
	At1g15125	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
	At1g55850	ATCSLE1_CSLE1cellulose synthase like E1
	At4g13310	CYP71A20cytochrome P450, family 71, subfamily A, polypeptide 20
	At1g73480	alpha/beta-Hydrolases superfamily protein
	At4g22710	CYP706A2cytochrome P450, family 706, subfamily A, polypeptide 2
	At1g22890	
	At4g28400	Protein phosphatase 2C family protein
	At3g60140	BGLU30_DIN2_SRG2Glycosyl hydrolase superfamily protein
	At3g15356	Legume lectin family protein
	At1g07890	APX1_ATAPX01_ATAPX1_CS1_MEE6ascorbate peroxidase 1
	At3g14060	
	At3g25882	NIMIN-2NIM1-interacting 2
	At5g37250	RING/U-box superfamily protein
	At2g45760	BAL_BAP2_BON association protein 2
	At1g06000	UDP-Glycosyltransferase superfamily protein
	At1g77450	NAC032_anac032NAC domain containing protein 32
	At3g58980	F-box family protein
	At4g18510	CLE2CLAVATA3/ESR-related 2
	At1g49650	alpha/beta-Hydrolases superfamily protein
	At2g31810	ACT domain-containing small subunit of acetolactate synthase protein
	At3g26600	ARO4_armadillo repeat only 4
	At2g39420	alpha/beta-Hydrolases superfamily protein
	At2g40200	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
	At4g38470	STY46ACT-like protein tyrosine kinase family protein
	At1g53440	Leucine-rich repeat transmembrane protein kinase
-		MGCCGYM motif and UP-regulated by NO only in TPT_RAP2.3 plants +EST
	At3g53180	NodGSglutamate-ammonia ligases;catalytics;glutamate-ammonia ligases
	At2g41640	Glycosyltransferase family 61 protein
	At1g64160	AtDIR5_DIR5_Disease resistance-responsive (dirigent-like protein) family protein
	At4a21870	HSP20-like chaperones superfamily protein

At4g21870 At3g19830 At4g33467 At1g32460

## 52 genes with MGCCGYM motif and UP-regulated by NO in TPT\_RAP2.3 plants both -EST & +EST

HSP20-like chaperones superfamily protein NTMC2T5.2\_NTMC2TYPE5.2\_Calcium-dependent lipid-binding (CaLB domain) family protein

At1g76600	
At4g18340	Glycosyl hydrolase superfamily protein
At3g03020	
At1g61340	AtFBS1_FBS1F-box family protein
At3g46640	LUX_PCL1Homeodomain-like superfamily protein
At4g12720	AtNUDT7_GFG1_NUDT7MutT/nudix family protein
At1g51780	ILL5_IAA-leucine resistant (ILR)-like gene 5
At2g22880	VQ motif-containing protein
At1g19210	Integrase-type DNA-binding superfamily protein
At5g54940	Translation initiation factor SUI1 family protein
At4g18205	Nucleotide-sugar transporter family protein
At1g28050	BBX13B-box type zinc finger protein with CCT domain

At1g49520	SWIB complex BAF60b domain-containing protein
At1g59870	ABCG36_ATABCG36_ATPDR8_PDR8_PEN3ABC-2 and Plant PDR ABC-type transporter family
0	protein
At2g44810	DAD1alpha/beta-Hydrolases superfamily protein
At4g30350	SMXL2 Double Clp-N motif-containing P-loop nucleoside triphosphate hydrolases superfamily protein
At5g04760	Duplicated homeodomain-like superfamily protein
At5g11650	alpha/beta-Hydrolases superfamily protein
At5g13190	AtGILP_GILP_
At1g30135	JAZ8_TIFY5Ajasmonate-zim-domain protein 8
At3g11020	DREB2_DREB2BDRE/CRT-binding protein 2B
At3q53600	C2H2-type zinc finger family protein
0	Ankyrin repeat family protein
At1g14480	
At5g25440	Protein kinase superfamily protein
At2g41100	ATCAL4_TCH3Calcium-binding EF hand family protein
At1g72450	JAZ6_TIFY11B_jasmonate-zim-domain protein 6
At2g23030	SNRK2-9_SNRK2.9_SNF1-related protein kinase 2.9
At4g36150	Disease resistance protein (TIR-NBS-LRR class) family
At5g24590	ANAC091_TIPTCV-interacting protein
At1g06620	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
At1g73500	ATMKK9_MKK9_MAP kinase kinase 9
At3g11840	PUB24plant U-box 24
At5g52882	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At3g56880	VQ motif-containing protein
At1g72520	ATLOX4_LOX4_PLAT/LH2 domain-containing lipoxygenase family protein
At4g06746	DEAR5_RAP2.9_related to AP2 9
At3g15440	
At1g53430	Leucine-rich repeat transmembrane protein kinase
At3g51450	Calcium-dependent phosphotriesterase superfamily protein
At1g76590	PLATZ transcription factor family protein
At3g29000	Calcium-binding EF-hand family protein
At1g32640	ATMYC2_JAI1_JIN1_MYC2_RD22BP1_ZBF1Basic helix-loop-helix (bHLH) DNA-binding family
•	protein
At4g11360	RHA1B_RING-H2 finger A1B
At5g16410	HXXXD-type acyl-transferase family protein
At1g74930	ORA47_Integrase-type DNA-binding superfamily protein
At5g05490	AtREC8_DIF1_REC8_SYN1Rad21/Rec8-like family protein
At4g17500	ATERF-1_ERF-1ethylene responsive element binding factor 1
At4g35480	RHA3BRING-H2 finger A3B
At3g24500	ATMBF1C_MBF1Cmultiprotein bridging factor 1C
At1g51760	IAR3_JR3_ peptidase M20/M25/M40 family protein
At5g67480	ATBT4_BT4_BTB and TAZ domain protein 4
At5g06570	alpha/beta-Hydrolases superfamily protein
Ū	
97 genes wit	h MGCCGYM motif and DOWN-regulated by NO only in TPT_RAP2.3 plants -EST
At5g47630	mtACP3_mitochondrial acyl carrier protein 3
At5g47630 At5g66460	
•	mtACP3mitochondrial acyl carrier protein 3
At5g66460 At1g64370 At3g20240	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein
At5g66460 At1g64370	mtACP3mitochondrial acyl carrier protein 3 AtMAN7_MAN7Glycosyl hydrolase superfamily protein
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2
At5g66460 At1g64370 At3g20240 At1g70120	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At2g26080	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At2g26080 At3g08600	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2glycine decarboxylase P-protein 2 Protein of unknown function (DUF1191)
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At2g26080 At3g08600 At4g12320	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2 Protein of unknown function (DUF1191) CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At2g26080 At3g08600 At4g12320 At1g07490	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2 Protein of unknown function (DUF1191) CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At2g26080 At3g08600 At4g12320 At1g07490 At3g56870	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2 Protein of unknown function (DUF1191) CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6 DVL9_RTFL3_ROTUNDIFOLIA like 3
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At2g26080 At3g08600 At4g12320 At1g07490 At3g56870 At2g36690	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2 Protein of unknown function (DUF1191) CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6 DVL9_RTFL3_ROTUNDIFOLIA like 3
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At2g26080 At3g08600 At4g12320 At1g07490 At3g56870 At2g36690 At1g48460	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2 Protein of unknown function (DUF1191) CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6 DVL9_RTFL3_ROTUNDIFOLIA like 3
At5g66460 At1g64370 At3g20240 At1g70120 At2g37560 At4g22240 At3g08600 At3g08600 At4g12320 At1g07490 At3g56870 At3g56870 At3g56870 At3g56690 At1g48460 At1g69160	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glycine decarboxylase P-protein 2 Protein of unknown function (DUF1191) CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6 DVL9_RTFL3_ROTUNDIFOLIA like 3 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
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At5g66460 At1g64370 At3g20240 At3g20240 At3g20240 At2g37560 At4g22240 At3g08600 At3g08600 At3g08600 At3g08600 At3g08600 At3g08690 At3g56870 At2g36690 At1g48460 At1g69160 At1g69160 At2g43110 At2g24820 At5g16500 At5g43080 At2g32290 At4g27030 At4g09970 At3g55800 At3g19270 At3g61550 At3g61550 At3g61550 At1g17920 At1g16489 At4g26790 At1g22850	mtACP3_mitochondrial acyl carrier protein 3         AtMAN7_MAN7_Glycosyl hydrolase superfamily protein         Mitochondrial substrate carrier family protein         Protein of unknown function (DUF1163)         ATORC2_ORC2_origin recognition complex second largest subunit 2         Plastid-lipid associated protein PAP / fibrillin family protein         AtGLDP2_GLDP2_glycine decarboxylase P-protein 2         Protein of unknown function (DUF1191)         CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6         DVL9_RTFL3_ROTUNDIFOLIA like 3         2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein         Ubiquitin carboxyl-terminal hydrolase family protein         AtTic55_TIC55-II_Tic55_translocon at the inner envelope membrane of chloroplasts 55-II         LIP1_Protein kinase superfamily protein         CYCA3;1_Cyclin A3;1         BAM6_BMY5_beta-amylase 6         FAD4_FADA_fatty acid desaturase A         SBPASE_sedoheptulose-bisphosphatase         CYP707A4_cytochrome P450, family 707, subfamily A, polypeptide 4         ATINS1_NAS1_nicotianamine synthase 1         RING/U-box superfamily protein         HDG12_homeodomain GLABROUS 12         other RNA         GDSL-like Lipase/Acylhydrolase superfamily protein         SNARE associated Golgi protein family
At5g66460 At1g64370 At3g20240 At3g20240 At2g37560 At4g22240 At2g26080 At3g08600 At3g08600 At3g08600 At3g08600 At3g56870 At2g36690 At1g48460 At1g69160 At1g69160 At2g43110 At2g24820 At5g16500 At5g43080 At2g32290 At4g27030 At4g09970 At3g55800 At3g19270 At3g61550 At3g61550 At3g61550 At1g17920 At1g16489 At4g26790	mtACP3_mitochondrial acyl carrier protein 3 AtMAN7_MAN7_Glycosyl hydrolase superfamily protein Mitochondrial substrate carrier family protein Protein of unknown function (DUF1163) ATORC2_ORC2_origin recognition complex second largest subunit 2 Plastid-lipid associated protein PAP / fibrillin family protein AtGLDP2_GLDP2_glyCine decarboxylase P-protein 2 Protein of unknown function (DUF1191) CYP706A6_cytochrome P450, family 706, subfamily A, polypeptide 6 DVL9_RTFL3_ROTUNDIFOLIA like 3 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein Ubiquitin carboxyl-terminal hydrolase family protein AtTic55_TIC55-II_Tic55_translocon at the inner envelope membrane of chloroplasts 55-II LIP1_Protein kinase superfamily protein CYCA3;1_Cyclin A3;1 BAM6_BMY5_beta-amylase 6 FAD4_FADA_fatty acid desaturase A SBPASE_sedoheptulose-bisphosphatase CYP707A4_cytochrome P450, family 707, subfamily A, polypeptide 4 ATNAS1_NAS1_nicotianamine synthase 1 RING/U-box superfamily protein HDG12_homeodomain GLABROUS 12 other RNA GDSL-like Lipase/Acylhydrolase superfamily protein

At5g18407 Defensin-like (DEFL) family protein At4q07725 transposable element gene Leucine-rich repeat protein kinase family protein At5g67200 At1g10370 ATGSTU17\_ERD9\_GST30\_GST30B\_GSTU17\_\_Glutathione S-transferase family protein At5g51110 Transcriptional coactivator/pterin dehydratase At3q01060 At1g79890 RAD3-like DNA-binding helicase protein At5g23060 CaS\_calcium sensing receptor At3g16870 GATA17\_\_GATA transcription factor 17 At2g31660 EMA1\_SAD2\_URM9\_\_ARM repeat superfamily protein At4g31600 UTr7\_UDP-N-acetylglucosamine (UAA) transporter family At3g48160 DEL1\_E2FE\_E2L3\_\_DP-E2F-like 1 At3g14740 RING/FYVE/PHD zinc finger superfamily protein At5g61000 ATRPA70D\_RPA1D\_RPA70D\_Replication factor-A protein 1-related At1g73445 transposable element gene At5g01410 ATPDX1\_ATPDX1.3\_PDX1\_PDX1.3\_RSR4\_\_Aldolase-type TIM barrel family protein At5g55040 DNA-binding bromodomain-containing protein At5g65850 F-box and associated interaction domains-containing protein At4g31330 Protein of unknown function, DUF599 At2q32610 ATCSLB01 ATCSLB1 CSLB01 cellulose synthase-like B1 At4g01037 AtWTF1\_WTF1\_Ubiquitin carboxyl-terminal hydrolase family protein ATNUC-L2\_NUC2\_PARLL1\_nucleolin like 2 At3g18610 At4g14790 ATSUV3\_EDA15\_\_ATP-dependent RNA helicase, mitochondrial (SUV3) At2g28050 Pentatricopeptide repeat (PPR) superfamily protein FLN2\_\_fructokinase-like 2 At1g69200 At2g32220 Ribosomal L27e protein family At5q64630 FAS2 MUB3.9 NFB01 NFB1 Transducin/WD40 repeat-like superfamily protein At1g14430 glyoxal oxidase-related protein At4g28310 At1g62250 At5g43370 APT1\_PHT1;2\_PHT2\_\_phosphate transporter 2 At5g04230 ATPAL3\_PAL3\_phenyl alanine ammonia-lyase 3 At5g42445 At5g22100 RNA cyclase family protein At1g45201 ATTLL1\_TLL1\_triacylglycerol lipase-like 1 At5g23300 PYRD\_\_pyrimidine d At1g78090 ATTPPB\_TPPB\_trehalose-6-phosphate phosphatase At1g68570 AtNPF3.1\_NPF3.1\_\_Major facilitator superfamily protein At2q37240 Thioredoxin superfamily protein At1g08280 GALT29A\_\_Glycosyltransferase family 29 (sialyltransferase) family protein At5g38100 S-adenosyl-L-methionine-dependent methyltransferases superfamily protein At2g47490 ATNDT1 NDT1 NAD+ transporter 1 At4g13020 MHK\_\_Protein kinase superfamily protein At3g14900 EMB3120 At5q02180 Transmembrane amino acid transporter family protein NOP56\_homolog of nucleolar protein NOP56 Protein of unknown function (DUF1005) At1g56110 At4g29310 At2g20724 At4g28630 ABCB23 ATATM1 ATM1 ABC transporter of the mitochondrion 1 At4g15810 P-loop containing nucleoside triphosphate hydrolases superfamily protein At2g38920 SPX (SYG1/Pho81/XPR1) domain-containing protein / zinc finger (C3HC4-type RING finger) proteinrelated At1g27480 At1g03020 alpha/beta-Hydrolases superfamily protein Thioredoxin superfamily protein At5g26790 At2g12462 At4g33560 Wound-responsive family protein At1g07010 AtSLP1\_SLP1\_Calcineurin-like metallo-phosphoesterase superfamily protein At4g28230 At3g46870 Pentatricopeptide repeat (PPR) superfamily protein At4g05410 YAO\_\_Transducin/WD40 repeat-like superfamily protein At1g16730 UP6\_\_unknown protein 6 At3g56290 At5q55720 Pectin lyase-like superfamily protein At1g09890 Rhamnogalacturonate lyase family protein At1g66250 O-Glycosyl hydrolases family 17 protein 4 genes with MGCCGYM motif and DOWN-regulated by NO only in TPT\_RAP2.3 plants +EST At1g48630 RACK1B\_RACK1B\_AT\_\_receptor for activated C kinase 1B At1g29460 SAUR65 SAUR-like auxin-responsive protein family At3g14570 ATGSL04\_GSL04\_GSL4\_atgsl4\_\_glucan synthase-like 4 At4g04790 Tetratricopeptide repeat (TPR)-like superfamily protein

#### 7 genes with MGCCGYM motif and DOWN-regulated by NO in TPT\_RAP2.3 plants both -EST & +EST

At2g27840	HD2D_HDA13_HDT04_HDT4histone deacetylase-related / HD-related
At4g13710	Pectin lyase-like superfamily protein

At2g46040	ARID/BRIGHT DNA-binding domain;ELM2 domain protein
At3g47430	PEX11B_peroxin 11B
At1g14345	NAD(P)-linked oxidoreductase superfamily protein
At1g14280	PKS2phytochrome kinase substrate 2
At1g14440	AtHB31_FTM2_HB31_ZHD4homeobox protein 31

# Table A8. Genes that were up-regulated in non-acclimated *nia1,2noa1-2* plants and were reported to be related to cold-triggered responses

Fold	FDR	ProbelD	GI ID	Annotation	
Change	(LiMMA)	TIODEID	Anno		
11,99	0,00006474	262128_at	At1g52690		
10,01	0,00000076	263486_at	At2g22200	family	
9,62	0,00001112	247047_at	At5g66650		
8,3	0,00001112	258347_at	At3g17520	Late embryogenesis abundant protein (LEA) family protein	
7,72	0,00000449	255479_at	At4g02380	AtLEA5_SAG21senescence-associated gene 21	
7,61	0,00000108	254634_at	At4g18650	•	
7,41	0,00002258		At3g02480		
6,48	0,00001112	_	-	ADC2_ATADC2_SPE2_arginine decarboxylase 2	
6,16	0,00002258	—	At3g17609		
5,1	0,00007264	_	At1g77120	, ,	
4,53	0,00596634		At3g50930	_, ,	
4,43	0,0006744	246099_at	At5g20230		
4,24		253268_s_at	0		
4,17 4	0,00010364 0,00023961	_	At5g64260		
4 3,94	0,00023981	252367_at 262113_at	At3g48360 At1g02820		
3,94 3,85	0,0007264	_	At3g21420		
5,05	0,00007204	250010_at	Alog2 1420	superfamily protein	
3,83	0,00004115	260410_at	At1g69870		
3,83	0,00004038	259681_at	At1g77760	GNR1_NIA1_NR1nitrate reductase 1	
3,74	0,00041234	253104_at	At4g36010	Pathogenesis-related thaumatin superfamily protein	
3,65	0,00001112	256891_at	At3g19030	unknown protein	
3,57	0,00053704	_	At2g41280	ATM10_M10_late embryogenesis abundant protein (M10) / LEA protein M10	
3,56	0,00056934		At1g77450	•••	
3,48	0,00034439	260856_at	At1g21910		
3,45	0,00017589	259365_at	At1g13300		
3,45	0,00050337	255524_at	At4g02330	AtPME41_ATPMEPCRB_PME41Plant invertase/pectin methylesterase inhibitor superfamily	
3,33	0,00008324	254663_at	At4g18290		
3,3	0,01604548	249527_at	At5g38710	Methylenetetrahydrofolate reductase family protein	
3,28	0,00099033	252123_at	At3g51240	F3'H_F3H_TT6flavanone 3-hydroxylase	
3,25	0,01709944	261648_at	At1g27730	STZ_ZAT10salt tolerance zinc finger	
3,08	0,00136951	250207_at	At5g13930	protein	
3,06	0,00045948 0,00004871	_	At2g16060	AHB1_ARATH GLB1_ATGLB1_GLB1_HB1_NSHB1hemoglobin 1 BH01:H1EXS_(ERD1/VBB1(SYC1) formily protein	
3,06 3	0,00004871	_		PHO1;H1EXS (ERD1/XPR1/SYG1) family protein 5PTASE2_AT5PTASE2_IP5PIImyo-inositol polyphosphate 5-	
5	0,00300030	254707_at	Al+910010	phosphatase 2	
2,99	0,00014127	262644_at	At1g62710	BETA-VPE_BETAVPEbeta vacuolar processing enzyme	
2,96	0,00206871		At5g49480		
2,86	0,00057889		At5g59220		
2,84	0,01284149		At2g02990		
2,81	0,00003968		At4g10040	,	
2,8	0,00035228	261192_at	At1g32870		
2,78	0,00129584	266313_at	At2g26980	•••	
2,75	0,00450314 0,00055768	—	At1g56650	pigment 1	
2,72		_	At5g06760		
2,72 2,67	0,00326299	247925_at 255543_at	At5g57560 At4g01870	TCH4_XTH22Xyloglucan endotransglucosylase/hydrolase family protein toIB protein-related	
2,67 2,65	0,01728287		At4g01870 At5g19875		
2,65 2,64	0,00054792		At4g39670		
2,64 2,63	0,00751802	266832_at	At2g30040		
2,03 2,6	0,00024078	262262_at	At1g70780		
2,6	0,00133771	246310_at	At3g51895		
2,56	0,00881697	256576_at	At3g28210		
,	.,				

2,54	0,00928913	266544_at	At2g35300	AtLEA4-2_LEA18_LEA4-2Late embryogenesis abundant protein, group 1 protein	
2,53	0.00038051	264217_at	At1a60190	AtPUB19_PUB19ARM repeat superfamily protein	
2,51	0,0000475	267254_at	-	SNRK2-9_SNRK2.9_SNF1-related protein kinase 2.9	
2,51	0,0003961	253559_at	-	0 O-Glycosyl hydrolases family 17 protein	
2,5	0,01480833	264000_at	•	ATPUMP5_DIC1_UCP5uncoupling protein 5	
2,45	0,00072723		-	ATFD3_FD3ferredoxin 3	
2,45	0,01481085	256442_at	At3q10930	unknown protein	
2,44	0.02386155	261892_at	At1g80840	ATWRKY40_WRKY40_WRKY DNA-binding protein 40	
2,42	0,00161733	265119_at	At1g62570	FMO GS-OX4flavin-monooxygenase glucosinolate S-	
2,36	0,00409642	263823_s_at	At2g40340	oxygenase 4 AtERF48_DREB2CIntegrase-type DNA-binding superfamily protein	
2,35	0,00376141	267246_at	At2g30250	ATWRKY25_WRKY25WRKY DNA-binding protein 25	
2,33	0,00002778	260357 at	-	AFP1ABI5 binding protein	
2,33	0,00009348	263473_at	0	UGT74D1UDP-glucosyl transferase 74D1	
2,32	0,00227904	_	•	Protein kinase superfamily protein	
2,31	0,00206124		-	AtCLO3_CLO-3_CLO3_RD20Caleosin-related family protein	
2,31	0,00067342	_	-	LTI65_RD29BCAP160 protein	
2,24	0,00071909		-	ATDI21_DI21_drought-induced 21	
2,21	0,00482667		-	PLATZ transcription factor family protein	
2,2	0,00663873	256017_at	-	JAZ1_TIFY10Ajasmonate-zim-domain protein 1	
2,2	0,00706728	262050_at	-	Tetratricopeptide repeat (TPR)-like superfamily protein	
2,2	0,00008759	253245_at	At4g34590	ATB2_AtbZIP11_BZIP11_GBF6G-box binding factor 6	
2,2	0,00313122	245711_at	At5g04340	C2H2_CZF2_ZAT6zinc finger of Arabidopsis thaliana 6	
2,2	0,00129607	248381_at	At5g51830	pfkB-like carbohydrate kinase family protein	
2,18	0,00102094	265670_s_at	At2g32190	unknown protein	
2,17	0,00022176	264436_at	At1g10370	ATGSTU17_ERD9_GST30_GST30B_GSTU17Glutathione S- transferase family protein	
2,17	0,00072928	253097_at	At4g37320	CYP81D5_cytochrome P450, family 81, subfamily D, polypeptide 5	
2,12	0,00343103	266545_at	At2g35290	unknown protein	
2,1	0,0101275	264758_at	At1g61340	F-box family protein	
2,07	0,00372371	256296_at	At1g69480	EXS (ERD1/XPR1/SYG1) family protein	
2,07	0,00030772	246495_at	At5g16200	50S ribosomal protein-related	
2,05	0,00626975	254432_at	-	FAD-binding Berberine family protein	
2,05	0,00057688	253841_at	At4g27830	BGLU10beta glucosidase 10	
2,04	0,00123699	251827_at	At3g55120	A11_CFI_TT5Chalcone-flavanone isomerase family protein	
2,04	0,00971052	267080_at	At2g41190	Transmembrane amino acid transporter family protein	
2,04	0,00575895	258805_at	At3g04010		
2,04	0,00551881	253485_at	-	ATWRKY18_WRKY18WRKY DNA-binding protein 18	
2,02	0,00872669	250777_at	At5g05440	PYL5_RCAR8Polyketide cyclase/dehydrase and lipid transport superfamily protein	

(Table A9 is only available in the digital version of the manuscript)

Table A9. Differential transcript levels in TPT-RAP2.3 lines not-treated with β-estradiol. Levels at 1h after treatment with a NO pulse (C1)

versus those at 0 time (C0). Description of microarray experiments according to MIAME

Investigation Design Fo			
Investigation title	IBMCP JLeon lab NO-exposed (60 min after pulse of 300 ppm NO during 5 min) TPT-RAP2.3 plants .		
Experimental designs	NO-treated (1) vs untreated (0) TPT-RAP2.3 plants in the absence (C) or presence (E) of -estradiol inducer.		
Person Last Name	León		
Person First Name	José		
E-mail	jleon@ibmcp.upv.es		
Telephone	(+)3496387782		
Affiliation & Address	IBMCP (CSIC-UPV), CPI Edificio 8E, Ingeniero Fausto Elio s/n, 46022 Valencia (Spain)		
Person role	Investigator, submitter		
Replicate types	3 independent biological replicates per genotype		
Experiment description	<ol> <li>Type of experiment: Compared analysis of the transcriptomes of 10-day old seedlings treated with a NO pulse for 5 min .</li> <li>Experimental factors: Samples were harvested by 1 h after NO exposure at 6 h after dawn</li> </ol>		
	of day 10. Seedlings were grown under long days (16 h light / 8 h darkness) photoperiodic conditions.		
	3. Number of hybridizations to Arabidopsis Agilent microarrays):		
	12 distributed as 3 independent biological replicates of C0, C1, E0 and E1.		
	4. Goals of proposed experiments:		
	Identification of the No-regulated transcriptome affected by RAP2.3		

Sample and Data Relationship Format (SDRF)						
1. Hybridization design:						
# Sample	Label	Genotype	Growth conditions	Tissue		
1	B C0	TPT-RAP2.3 transgenic line B (Coego et al., 2014)	MS media plus 1 % sucrose, 10 days	Seedlings		

2	B C1	TPT-RAP2.3 transgenic line B (Coego et al., 2014)	MS media plus 1 % sucrose, 10 days	Seedlings
3	B E0	TPT-RAP2.3 transgenic line B (Coego et al., 2014)		Seedlings
4	B E1	TPT-RAP2.3 transgenic line B (Coego et al., 2014)		Seedlings
5	G C0	TPT-RAP2.3 transgenic line G (Coego et al., 2014)		Seedlings
6	G C1	TPT-RAP2.3 transgenic line G (Coego et al., 2014)		Seedlings
7	G E0	TPT-RAP2.3 transgenic line G (Coego et al., 2014)		Seedlings
8	G E1	TPT-RAP2.3 transgenic line G (Coego et al., 2014)		Seedlings
9	н Со	TPT-RAP2.3 transgenic line H (Coego et al., 2014)		Seedlings
10	Н С1	TPT-RAP2.3 transgenic line H (Coego et al., 2014)	MS media plus 1 % sucrose, 10 days	Seedlings
11	H E0	TPT-RAP2.3 transgenic line H (Coego et al., 2014)	MS media plus 1 % sucrose, 10 days	Seedlings
12	H E1	TPT-RAP2.3 transgenic line H (Coego et al., 2014)	MS media plus 1 % sucrose, 10 days	Seedlings
2 RNA extraction:		1		<u>I</u>

#### 2. RNA extraction:

Total RNA from wild type and NO-treated plants was isolated and purified by the Micro-to-Midi Total RNA Purification System from Invitrogen (Carlsbad, CA, USA).

#### 3. Quality controls:

RNAs from every genotype were checked by RT-PCR for mRNA levels of the *RAP2.3 and ACT2* genes. Moreover, total RNAs used for further preparation of hybridization probes were analysed to check integrity and purity by nanocapilar electrophoresis in Bioanalyzer Agilent 2100.

## 4. Labeling and hybridization protocols:

Sample RNA (0.5 µg) was amplified and labeled with the Agilent Low Input Quick Amp Labeling Kit. An Agilent Spike-In Kit was used to assess the labeling and hybridization efficiencies. Hybridization an slide washing were performed with the Gene Expression Hybridization Kit and Gene Expression Wash Buffers, respectively. Three biological replicates of C0, E0, C1 and E1 were hybridized to Arabidposis (V4) Gene Expression Microarray 4x44K, which contained 43,803 probes (60-mer oligonucleotides) and was used in a one-color experimental design. Chip 1 was hybridized with samples 1,3,2 and 4. Chip 2 was hybridized with samples 8,5,7 and 6. Chipm 3 was hybridixed with samples 10,12,9 and 11. After washing and drying, slides were scanned in an Agilent G2565AA microarray scanner, at 5 µm resolution and using the double scanning, as recommended. Image files were analyzed with the Feature Extraction software 9.5.1. Interarray analyses were performed with the GeneSpring 11.5 software.

5. Sample comparisons:

C1 vs C0 and E1 vs E0 for the three TPT-RAP2.3 incependent transgenic lines.

6. Statistical analysis:

Linear model methods (LiMMA) were used for determining differentially expressed genes. To control the false-discovery rate, P-values were corrected using the method of Benjamini and Hochberg (1995). Criteria for selection of genes were fold value >1.5 and false-discovery rate $\leq$  0.05. Statistical analysis and graphical visualization of data were performed with the interactive tool fiesta (http://bioinfogp.cnb.csic.es/tools/FIESTA/).

Differential transcript levels in TPT-RAP2.3 lines not-treated with estradiol. Levels at 1h after treatment with a NO pulse (C1) versus those at 0 time (C0).

ProbeName	p (Corr) p		FC	Log FC AGI	Symbol	Corrected annotation
A_84_P13535	0,02403887		447,5877		Cymbol	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
A 84 P11046	0.03807803	0,0020636		7,82355 AT4G34410	RRTF1	RRTF1 redox responsive transcription factor 1
A_84_P18492	0,00775991	,	,	7,213783 AT3G44870		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P10609	0,01106583			7,124415 AT2G24850	TAT. TAT3	TAT_TAT3tyrosine aminotransferase 3TAT_TAT3tyrosine aminotransferase 3
A_84_P595141	0.01066107	,	,	7,026993 AT1G30135	JAZ8, TIFY5A	JAZ8_TIFY5A_jasmonate-zim-domain protein 8
A_84_P21769	0,00178308	1,41E-07	,	7,011652 AT1G61120		GES_TPS04_TPS4terpene synthase 04
A 84 P761749	0,00461552			6,791906 AT3G22275	010, 00 ., 0	
A_84_P17546	0.00764839	'	107,4723	6,74782 AT3G44860	FAMT	FAMTfarnesoic acid carboxyl-O-methyltransferase
A_84_P521598	,			6,600599 AT2G22760		basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A 84 P544235	0.00387638			6,224372 AT5G28237		Pyridoxal-5'-phosphate-dependent enzyme family protein
A_84_P17584	0,00466105	,	,	6,208581 AT1G17380	JAZ5. TIFY11A	JAZ5_TIFY11A_jasmonate-zim-domain protein 5
A_84_P21573	0,00387638			6,199612 AT5G38120		4CL8_AMP-dependent synthetase and ligase family protein
A_84_P611898	0,00466105			6,176848 AT5G59580		UGT76E1_UDP-glucosyl transferase 76E1
A_84_P18113	0,00772467			5,862449 AT1G76640		CML39_Calcium-binding EF-hand family protein
A 84 P91639	0,0352222			5,765584 AT2G39030	NATA1	NATA1_Acyl-CoA N-acyltransferases (NAT) superfamily protein
	0,00574999		-	5,750817 AT3G44860;		
A 84 P762988	0.00894727	3,22E-05		5,742693 AT4G36950		MAPKKK21mitogen-activated protein kinase kinase kinase 21
A 84 P14748	0,00477309	,		5,628436 AT1G17420		ATLOX3_LOX3_lipoxygenase 3
A_84_P18289	0,00772467	,	,	5,557467 AT2G44810	,	DAD1alpha/beta-Hydrolases superfamily protein
A_84_P524680				5,474251 AT5G13220		JAS1_JAZ10_TIFY9_jasmonate-zim-domain protein 10
A_84_P502791				5,416363 AT1G32910	, ,	HXXXD-type acyl-transferase family protein
A 84 P851247				5,392831 AT5G28237		Pyridoxal-5'-phosphate-dependent enzyme family protein
A_84_P785475	0,00571375	5,56E-06	40,4212	5,33704 AT1G17420	ATLOX3. LOX3	ATLOX3_LOX3_lipoxygenase 3
A_84_P19302	0,00935958			5,317832 AT3G23220		ESE1_Integrase-type DNA-binding superfamily protein
A 84 P788408		1,49E-05		5,300226 AT2G22760	-	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P813757	0,00764839	,		5,200539 AT3G44860	FAMT	FAMTfarnesoic acid carboxyI-O-methyltransferase
A 84 P17343	0,01766588	2,59E-04				ATERF13_EREBP_ERF13ethylene-responsive element binding factor 13
A_84_P21194	0,01163687	,		5,153216 AT3G23240		ATERF1_ERF1_ethylene response factor 1
A_84_P12324	0,0266426			5,052535 AT1G70130	,	LecRK-V.2 Concanavalin A-like lectin protein kinase family protein
A 84 P16173	0,00877275			5,051905 AT1G28480	GRX480. roxv19	GRX480_roxy19_Thioredoxin superfamily protein
A_84_P13172	0,02931333	0,0010869		5,026 AT5G67080		MAPKKK19mitogen-activated protein kinase kinase kinase 19
	0,00901326			-		JAS1_JAZ10_TIFY9_ jasmonate-zim-domain protein 10
A 84 P610481	0.00894727			4,977179 AT2G34600		JAZ7_TIFY5B_jasmonate-zim-domain protein 7
A_84_P16121	0,00387638	1,24E-06		4,965738 AT1G43160		RAP2.6_related to AP2 6
A_84_P18072	0,03349368			4,878201 AT1G72520		ATLOX4_LOX4_PLAT/LH2 domain-containing lipoxygenase family protein
A_84_P14331	0,01432252	,	,	4,829273 AT1G76650	,	CML38calmodulin-like 38
A_84_P816405	0,02342488			4,814133 AT5G62360		Plant invertase/pectin methylesterase inhibitor superfamily protein
A_84_P20323	0,04056462	0,0024299	27,65687	4,789566 AT3G49620	DIN11	DIN11_2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
A_84_P23712	0,04917615		-	4,716649 AT1G59860		HSP20-like chaperones superfamily protein
A_84_P16247	0,00952359		24,47912	4,61348 AT1G12610	DDF1	DDF1_Integrase-type DNA-binding superfamily protein
A_84_P20976	0,00900545			4,573487 AT1G19210		Integrase-type DNA-binding superfamily protein
A_84_P10863	0,00633665			4,570359 AT3G48520	CYP94B3	CYP94B3cytochrome P450, family 94, subfamily B, polypeptide 3
A_84_P544827	0,01341196	1,14E-04	23,61297	4,561507 AT3G25760	AOC1, ERD12	AOC1_ERD12allene oxide cyclase 1AOC1_ERD12allene oxide cyclase 1
A_84_P223429	0,00894727			4,551025 AT5G43650		BHLH92 basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P20120	0,00124154			4,542597 AT2G27690		CYP94C1cytochrome P450, family 94, subfamily C, polypeptide 1
A_84_P768692	0,02248672			4,451829 AT5G40348		other RNA
A_84_P23179	0,00775991	1,58E-05		4,443474 AT3G53600		C2H2-type zinc finger family protein
A_84_P13031	0,01063389	5,23E-05	21,00736	4,392823 AT5G19110		Eukaryotic aspartyl protease family protein
	,	-,	,	,		

A 84 P79105 0.0114343 6.36E-05 20.38735 4.349603 AT1G66860 Class I glutamine amidotransferase-like superfamily protein A 84 P592948 0.00736156 1,04E-05 20,13802 4,33185 AT4G11911 A 84 P15993 0,01220927 8,85E-05 19,97414 4,320062 AT5G63450 CYP94B1 CYP94B1\_\_cytochrome P450, family 94, subfamily B, polypeptide 1 A\_84\_P19845 ATMYB21, ATMYB ATMYB21\_ATMYB3\_MYB21\_\_myb domain protein 21 0,01774115 2,74E-04 19,45943 4,282397 AT3G27810 0,003957 19,42797 4,280063 AT5G05220 A 84 P786477 0,04961063 A\_84\_P786499 0,01163687 7,08E-05 18,72404 4,22682 AT3G25760 AOC1, ERD12 AOC1\_ERD12\_allene oxide cyclase 1AOC1\_ERD12\_allene oxide cyclase 1 A\_84\_P572473 0,03484798 0,0016811 18,15437 4,182245 AT4G21920 A\_84\_P815994 0,03807803 0,0020632 17,93385 4,164613 AT1G54020 GDSL-like Lipase/Acylhydrolase superfamily protein 17,75253 4,149953 AT4G31800 ATWRKY18, WRKY ATWRKY18 WRKY18 WRKY DNA-binding protein 18 A 84 P232439 0,00772467 1,38E-05 A\_84\_P853664 0,00464882 3,01E-06 17,52594 4,13142 AT2G06050 AtOPR3, DDE1, OP AtOPR3\_DDE1\_OPR3\_\_oxophytodienoate-reductase 3 A 84 P15340 0,00412491 1,43E-06 17,36241 4,117895 AT2G06050 AtOPR3, DDE1, OP AtOPR3 DDE1 OPR3 oxophytodienoate-reductase 3 A 84 P272600 0,01785415 2,84E-04 17,31235 4,11373 AT5G21960 ERF016 Integrase-type DNA-binding superfamily protein GDSL-like Lipase/Acvlhvdrolase superfamily protein A\_84\_P15194 0,00894727 3,06E-05 17,05285 4,091941 AT1G54020 A\_84\_P215028 0,02308556 5.66E-04 16,78474 4,069078 AT3G25770 AOC2 AOC2 allene oxide cvclase 2 AtERF53 ERF53 Integrase-type DNA-binding superfamily protein A 84 P10627 0,02277204 5,49E-04 16,25142 4,022494 AT2G20880 AtERF53, ERF53 A\_84\_P611334 0,02676006 8,71E-04 16,00674 4,000608 AT2G22880 VQ motif-containing protein A 84 P604601 0.04572882 0,0032095 15,74719 3,977023 AT4G37140 ATMES20, MEE69, ATMES20\_MEE69\_MES20\_alpha/beta-Hydrolases superfamily protein A 84 P537220 0.02728939 9,07E-04 15,22946 3,928793 AT5G57510 A 84 P862159 0.01539257 1.76E-04 15.10558 3,91701 AT2G24850 TAT. TAT3 TAT TAT3 tyrosine aminotransferase 3TAT TAT3 tyrosine aminotransferase 3 A\_84\_P20248 0,03608721 0,001822 15,09034 3,915553 AT3G23230 AtERF98 AtTDR1\_ERF98\_TDR1\_\_Integrase-type DNA-binding superfamily protein A 84 P812170 0.00950448 4,18E-05 15,02068 3,908878 AT3G45140 ATLOX2, LOX2 ATLOX2 LOX2 lipoxygenase 2ATLOX2 LOX2 lipoxygenase 2 A\_84\_P12788 0,01930832 3,49E-04 14,58847 3,866756 AT3G55970 ATJRG21, JRG21 ATJRG21\_JRG21\_jasmonate-regulated gene 21 A 84 P14985 0.00894727 2,76E-05 14,52517 3,860483 AT5G47220 ATERF2, ATERF-2 ATERF-2 ATERF2 ERF2 ethylene responsive element binding factor 2 A 84 P23517 0,00894727 2,90E-05 14,32943 3,840909 AT5G52320 CYP96A4 CYP96A4 cytochrome P450, family 96, subfamily A, polypeptide 4 A\_84\_P786287 0,03636191 0,0018611 14,03171 3,810619 AT5G05410 DREB2, DREB2A DREB2\_DREB2A\_DRE-binding protein 2 7,10E-05 13,89037 3,796013 AT3G23250 ATMYB15, ATY19, ATMYB15\_ATY19\_MYB15\_myb domain protein 15 A\_84\_P22140 0,01163687 A 84 P847119 0.01564355 1,85E-04 13,77601 3,784086 AT5G62360 Plant invertase/pectin methylesterase inhibitor superfamily protein 0 A\_84\_P558681 0,00877048 2.08E-05 13,6393 3,769698 AT1G53885; A\_84\_P757973 0,01785415 2,84E-04 13,60557 3,766125 AT2G44578 RING/U-box superfamily protein A 84 P785932 0.0325659 0.0014245 13,55619 3,76088 AT1G25400 A\_84\_P164303 0,02449762 6,96E-04 13,4704 3,751721 AT5G58680 ARM repeat superfamily proteinARM repeat superfamily protein 4,29E-04 13,43344 3,747757 AT1G28480 GRX480, roxy19 A\_84\_P812834 0.02060719 GRX480 roxv19 Thioredoxin superfamily protein A 84 P11822 0,00764839 1,21E-05 13,39123 3,743217 AT3G51450 Calcium-dependent phosphotriesterase superfamily protein NAKR1 NPCC6 Heavy metal transport/detoxification superfamily protein A\_84\_P852628 0,01065349 5.39E-05 13,3837 3,742405 AT5G02600 NAKR1. NPCC6 A\_84\_P870349 0,01163687 6,89E-05 13,26385 3,729427 AT3G23250 ATMYB15, ATY19, ATMYB15\_ATY19\_MYB15\_\_myb domain protein 15 A 84 P515468 0,0145606 1,52E-04 13,24495 3,727371 AT2G45940 Protein of unknown function (DUF295) A\_84\_P17881 9,62E-05 Plant invertase/pectin methylesterase inhibitor superfamily protein 0,01246891 13,1251 3,714256 AT5G62360 13,10179 3,711692 AT1G80840 ATWRKY40, WRKY ATWRKY40 WRKY40 WRKY DNA-binding protein 40 A 84 P13271 0.04963833 0.0039626 A\_84\_P287970 0,00466105 3,46E-06 13,01229 3,701803 AT5G65690 PCK2, PEPCK PCK2\_PEPCK\_\_phosphoenolpyruvate carboxykinase 2 A 84 P172281 0,02271562 5,43E-04 12,98571 3,698853 AT5G48850 ATSDI1 ATSDI1 Tetratricopeptide repeat (TPR)-like superfamily protein A\_84\_P67084 0,04636379 0,0033147 12,84307 3,682919 AT1G25400 A 84 P21501 0.03484798 0.0016798 12,79647 3,677674 AT5G05410 DREB2, DREB2A DREB2 DREB2A DRE-binding protein 2 A\_84\_P832673 0,01375994 1,23E-04 12,505 3,644433 AT3G20340 A\_84\_P19584 0.01961223 3.70E-04 12.49937 3.643784 AT4G15975 RING/U-box superfamily protein A\_84\_P793001 0,04398336 0,0028868 12,48152 3,641722 AT1G17420 ATLOX3, LOX3 ATLOX3\_LOX3\_\_lipoxygenase 3 A 84 P11724 0,01163687 6,92E-05 12,47795 3,641309 AT3G16150 ASPGB1 ASPGB1 N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein A\_84\_P529724 0,01785415 2,85E-04 12,32328 3,623315 AT3G20340 A 84 P829373 0,04674462 12,30838 3,621569 AT5G67080 MAPKKK19 MAPKKK19 mitogen-activated protein kinase kinase kinase 19 0,0034026 A 84 P242975 0,04741487 0,0034965 12,10399 3,597411 AT1G78410 VQ motif-containing protein A\_84\_P704033 other RNA 0,00764839 1,17E-05 12,06141 3,592327 AT1G11185 A 84 P15304 0.00606654 6.89E-06 11.94813 3.578713 AT1G65390 ATPP2-A5. PP2-A5 ATPP2-A5 PP2-A5 phloem protein 2 A5

A\_84\_P13216 0.00894727 3,19E-05 11,93524 3,577156 AT1G20510 OPCL1 OPCL1\_OPC-8:0 CoA ligase1 A 84 P553370 0,04036312 0,0024108 11,85587 3,56753 AT1G56240 AtPP2-B13, PP2-B1 AtPP2-B13 PP2-B13 phloem protein 2-B13 A\_84\_P11789 0,04372359 0,0028553 11,62336 3,538955 AT3G43250 Family of unknown function (DUF572) A\_84\_P16877 NUDT8\_atnudt8\_\_nudix hydrolase homolog 8 0,00877275 2,21E-05 11,49364 3,522764 AT5G47240 atnudt8, NUDT8 0,01163687 S-locus lectin protein kinase family protein A 84 P18925 7,07E-05 11,45863 3,518363 AT1G61610 A\_84\_P19028 0,01066107 5,66E-05 11,13812 3,477434 AT1G74930 ORA47 ORA47\_Integrase-type DNA-binding superfamily protein A\_84\_P191974 0,00894727 2,73E-05 10,98623 3,457625 AT5G03210 AtDIP2, DIP2 AtDIP2\_DIP2\_ A\_84\_P762569 0,0495561 0,0039515 10,96403 3,454706 AT3G27809 A 84 P209868 0.01372322 1,20E-04 10,89406 3,44547 AT2G32130 Plant protein of unknown function (DUF641) A\_84\_P789698 0,00772467 1,50E-05 10,78722 3,431252 AT1G72416 Chaperone DnaJ-domain superfamily proteinChaperone DnaJ-domain superfamily protein A 84 P11721 0,03914714 0,0022026 10,63132 3,410249 AT3G15500 ANAC055, ATNAC3 ANAC055\_ATNAC3\_NAC055\_NAC3\_NAC domain containing protein 3 A 84 P20831 0.01647879 2,16E-04 10,63009 3,410082 AT1G61340 AtFBS1 FBS1 F-box family protein A\_84\_P217688 0,0188704 3,32E-04 10,47759 3,389236 AT1G76600 A\_84\_P16963 0,03439173 0,0016258 10,37977 3,375703 AT5G16960 Zinc-binding dehydrogenase family protein A 84 P216248 0,01163687 7,22E-05 10,01018 3,323397 AT1G69890 Protein of unknown function (DUF569) A\_84\_P193424 0,00464882 2,97E-06 9,970627 3,317684 AT1G19180 JAZ1, TIFY10A AtJAZ1\_JAZ1\_TIFY10A\_\_jasmonate-zim-domain protein 1 A\_84\_P785249 0,00682995 8,93E-06 9,916223 3,309791 AT4G31800 ATWRKY18, WRKY ATWRKY18\_WRKY18\_WRKY DNA-binding protein 18 A 84 P769350 0.04737008 0,003488 9,885389 3,305298 AT5G54165 0,0033429 9,874924 3,30377 AT5G63250 A\_84\_P196714 0,04641205 Carbohydrate-binding X8 domain superfamily protein 8,01E-05 9,798193 3,292516 AT1G72416 Chaperone DnaJ-domain superfamily proteinChaperone DnaJ-domain superfamily protein A\_84\_P715317 0,01177365 A 84 P586962 0,01624931 2,07E-04 9,748003 3,285107 AT5G56880 A\_84\_P503102 0,03707299 0,0019504 9,668864 3,273346 AT3G15440 A 84 P22519 0,01393426 1,35E-04 9,607289 3,264129 AT5G38130 HXXXD-type acyl-transferase family protein A 84 P15826 0,02751414 9,30E-04 9,599502 3,26296 AT5G05600 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein A\_84\_P823879 0,00178308 1,70E-07 9,584101 3,260643 AT5G47240 atnudt8, NUDT8 NUDT8\_atnudt8\_\_nudix hydrolase homolog 8NUDT8\_atnudt8\_\_nudix hydrolase homolog 8 A\_84\_P575998 0,02568428 7,98E-04 9,582057 3,260335 AT4G14365 XBAT34 XBAT34\_\_XB3 ortholog 4 in Arabidopsis thaliana A 84 P549255 0,04647869 0,0033487 9,528822 3,252298 AT3G28600 P-loop containing nucleoside triphosphate hydrolases superfamily protein A\_84\_P65264 0,02353903 5,90E-04 9,117328 3,188611 AT3G15356 Legume lectin family protein A\_84\_P17140 0,00461552 2,11E-06 9,010446 3,171598 AT1G32640 ATMYC2, JAI1, JIN ATMYC2\_JAI1\_JIN1\_MYC2\_RD22BP1\_ZBF1\_Basic helix-loop-helix (bHLH) DNA-binding family protein A 84 P11613 0.04806924 0,0036151 8,917522 3,156643 AT2G34930 disease resistance family protein / LRR family protein A\_84\_P820417 0,04572882 0,0032087 8,908025 3,155106 AT4G17490 ATERF6, ERF6, ER ATERF6\_ERF-6-6\_ERF6\_ethylene responsive element binding factor 6 A\_84\_P860087 0,01715813 2,39E-04 8,857978 3,146977 AT1G27020 A 84 P300030 0.00877275 2,31E-05 8,842048 3,144381 AT5G08790 anac081, ATAF2 ATAF2 anac081 NAC (No Apical Meristem) domain transcriptional regulator superfamily protein ATLOX3\_LOX3\_lipoxygenase 3 A\_84\_P833808 0,00894727 3,13E-05 8,464248 3,081382 AT1G17420 ATLOX3, LOX3 A\_84\_P847302 0,04831925 0,0037243 8,443587 3,077856 AT3G60140 BGLU30, DIN2, SR BGLU30\_DIN2\_SRG2\_Glycosyl hydrolase superfamily protein A 84 P22274 0.02921359 0.0010751 8.397223 3.069912 AT3G63350 AT-HSFA7B, HSFA AT-HSFA7B HSFA7B winged-helix DNA-binding transcription factor family protein A\_84\_P21275 0,01872867 3,26E-04 8,37904 3,066785 AT3G50930 BCS1 BCS1\_cytochrome BC1 synthesis A 84 P20535 0.01457646 1.52E-04 8.298972 3.052933 AT4G17500 ATERF-1. ERF-1 ATERF-1 ERF-1 ethylene responsive element binding factor 1 A\_84\_P826417 0,04511397 0,0030989 8,288296 3,051076 AT5G24110 ATWRKY30, WRKY ATWRKY30\_WRKY30\_WRKY DNA-binding protein 30 A 84 P283560 0.04851664 0.0037686 8,27021 3,047924 AT2G24600 Ankyrin repeat family protein 1,06E-04 8,269592 3,047816 AT1G19530 A\_84\_P840621 0,01313344 A\_84\_P869292 0,01785633 2,87E-04 8,249113 3,044239 AT4G17500 ATERF-1, ERF-1 ATERF-1 ERF-1 ethylene responsive element binding factor 1 A\_84\_P594805 0,01375994 1,23E-04 8,234659 3,041709 AT4G36820 Protein of unknown function (DUF607) A\_84\_P306860 0.03025034 0.0011889 8.159666 3.02851 AT5G10695 A\_84\_P18211 0,01830757 3,07E-04 8,049125 3,008832 AT2G26530 AR781 AR781\_Protein of unknown function (DUF1645)AR781\_Protein of unknown function (DUF1645) A 84 P159795 0.00532816 4,94E-06 8,029499 3,00531 AT1G19530 A\_84\_P840907 0.016924 2,32E-04 8,029396 3,005292 AT5G56880 A 84 P786720 0,00894727 2,93E-05 8,004184 3,000754 AT5G08790 anac081, ATAF2 ATAF2 anac081 NAC (No Apical Meristem) domain transcriptional regulator superfamily protein A\_84\_P857687 0,01304823 1,03E-04 8,001444 3,00026 AT1G73500 ATMKK9, MKK9 ATMKK9 MKK9 MAP kinase kinase 9 AR781\_Protein of unknown function (DUF1645) A\_84\_P813646 0,01618926 2,04E-04 7,927353 2,986839 AT2G26530 AR781 A 84 P18100 0.04077161 0.0024607 7.915859 2.984746 AT1G69930 ATGSTU11. GSTU ATGSTU11 GSTU11 glutathione S-transferase TAU 11

A\_84\_P21851 0,01331538 1.13E-04 7.896535 2,98122 AT1G66370 AtMYB113, MYB11 AtMYB113\_MYB113\_myb domain protein 113 A 84 P542646 0,00900545 3,43E-05 7,629397 2,931569 AT1G15040 GAT GAT1 2.1 Class I glutamine amidotransferase-like superfamily protein A 84 P23284 0.01769782 2,62E-04 7,628701 2,931438 AT4G21680 NRT1.8 AtNPF7.2 NPF7.2 NRT1.8 NITRATE TRANSPORTER 1.8 A\_84\_P586644 0,01525796 1,72E-04 7,590999 2,92429 AT3G53232 DVL20, RTFL1 DVL20\_RTFL1\_\_ROTUNDIFOLIA like 1 A 84 P573393 0.01830216 3,01E-04 7,587153 2,923559 AT4G27654 A\_84\_P106016 0,04822512 0,0036633 7,542984 2,915135 AT1G15125 S-adenosyl-L-methionine-dependent methyltransferases superfamily protein A 84\_P16821 7,84E-06 7,536729 2,913939 AT5G24470 APRR5, PRR5 APRR5 PRR5 pseudo-response regulator 5 0.00640993 A\_84\_P10039 0,02240153 5,27E-04 7,531903 2,913014 AT1G27020 A 84 P17934 0.01961223 3,65E-04 7,526135 2,911909 AT5G04340 C2H2, CZF2, ZAT6 C2H2 CZF2 ZAT6 zinc finger of Arabidopsis thaliana 6 A\_84\_P562774 0,01492891 1,61E-04 7,518686 2,910481 AT5G12340 A 84 P784449 0,0497107 0,0039859 7,514286 2,909636 AT4G23220 CRK14 CRK14 cysteine-rich RLK (RECEPTOR-like protein kinase) 14 A\_84\_P802615 0,03187662 0,0013502 7,506802 2,908198 AT5G62360 Plant invertase/pectin methylesterase inhibitor superfamily proteinsuperfamily protein A 84 P826766 0.02884718 0,0010294 7,487862 2,904554 AT5G58680 ARM repeat superfamily proteinARM repeat superfamily protein A\_84\_P10495 0.00461552 2,41E-06 7,478293 2,902709 AT1G65890 AAE12 AAE12\_acyl activating enzyme 12 A 84 P282640 0.01066107 5,68E-05 7,461075 2,899384 AT2G23170 GH3.3 GH3.3 Auxin-responsive GH3 family protein A\_84\_P15720 0,01961223 3,71E-04 7,409588 2,889393 AT4G22620 SAUR34\_\_SAUR-like auxin-responsive protein family A 84 P818172 0,0145606 1,48E-04 7,380822 2,883781 AT1G15040 GAT GAT1 2.1 Class I glutamine amidotransferase-like superfamily protein A 84 P288904 0.01539895 1,77E-04 7,374735 2,882591 AT3G49570 LSU3 LSU3 response to low sulfur 3 Serine protease inhibitor, potato inhibitor I-type family protein A 84 P511454 0.02850118 9.95E-04 7.361979 2.880094 AT5G43570 A\_84\_P245475 0,00668285 8,37E-06 7,354634 2,878654 AT2G25460 A 84 P259470 0.01375994 1,27E-04 7,336154 2,875024 AT1G61890 MATE efflux family protein A\_84\_P19511 0,0282192 9,78E-04 7,325701 2,872967 AT4G24380 A 84 P20466 0.03367831 0,0015599 7,296392 2,867183 AT4G26260 MIOX4 MIOX4 myo-inositol oxygenase 4 A 84 P286390 0.00877048 2,11E-05 7,261025 2,860173 AT3G25780 AOC3 AOC3 allene oxide cyclase 3 A\_84\_P870348 0,04465805 0,0030167 7,216152 2,85123 AT2G41100 ATCAL4, TCH3 ATCAL4\_TCH3\_Calcium-binding EF hand family protein Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein A\_84\_P559351 0,01305407 1,04E-04 7,170948 2,842164 AT5G55460 A 84 P759481 0,00772467 1,32E-05 7,119588 2,831794 AT3G12145 FLOR1, FLR1 FLOR1 FLR1 FTM4 Leucine-rich repeat (LRR) family protein A\_84\_P85269 0,00877275 2,28E-05 7,004758 2,808335 AT1G24909; ASB1,TRP4,WEI7 A\_84\_P10611 0,00903297 3,59E-05 6,996785 2,806692 AT2G43530 Scorpion toxin-like knottin superfamily protein A 84 P836406 0.01504028 1,66E-04 6,991565 2,805615 AT1G15125 S-adenosyl-L-methionine-dependent methyltransferases superfamily protein A\_84\_P609369 0,01177365 7,92E-05 6,945716 2,796124 AT1G31550 GDSL-like Lipase/Acylhydrolase superfamily protein A 84\_P137619 PBP1 pinoid-binding protein 1 0.030758 0,0012295 6,944105 2,795789 AT5G54490 PBP1 A 84 P10561 0.00464882 2,73E-06 6,928264 2,792494 AT1G51780 ILL5 ILL5 IAA-leucine resistant (ILR)-like gene 5 A\_84\_P302360 0,04465805 ARM repeat superfamily protein 0,0030186 6,918314 2,790421 AT5G37490 A\_84\_P14490 0,02312214 5,67E-04 6,915822 2,789901 AT2G34180 ATWL2, CIPK13, S ATWL2\_CIPK13\_SnRK3.7\_WL2\_CBL-interacting protein kinase 13 A 84 P21931 0.01961223 3,77E-04 6,88339 2,783119 AT1G28370 ATERF11, ERF11 ATERF11 ERF11 ERF domain protein 11 A\_84\_P14882 0,00466105 3,99E-06 6,854181 2,776984 AT1G06620 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein HSP20-like chaperones superfamily protein A 84 P18032 0.03926466 0.0022285 6.777144 2.760677 AT1G54050 A\_84\_P23070 0.01257935 9,79E-05 6,751513 2,755211 AT3G22740 HMT3 HMT3\_homocysteine S-methyltransferase 3 A 84 P811729 0.02663922 8,62E-04 6,750648 2,755026 AT2G33830 AtDRM2 DRM2 Dormancy/auxin associated family protein A\_84\_P169283 0,01911949 3,43E-04 6,718439 2,748126 AT5G06570 alpha/beta-Hydrolases superfamily protein A 84 P101246 0.03864233 0.0021259 6.699562 2.744067 AT5G22530 A\_84\_P595805 0,02403887 6,53E-04 6,649183 2,733177 AT5G36925 A 84 P811808 0.00461552 2.28E-06 6,61285 2,725272 AT3G12145 FLOR1, FLR1 FLOR1\_FLR1\_FTM4\_\_Leucine-rich repeat (LRR) family protein A\_84\_P602187 0,03358237 0,0015229 6,596125 2,721619 AT1G05575 A 84 P548213 0,0293697 0.0010921 6,595964 2,721583 AT2G31945 0,00772467 A\_84\_P13376 1,32E-05 6,538024 2,708855 AT1G74430 ATMYB95, ATMYB ATMYB95\_ATMYBCP66\_MYB95\_myb domain protein 95 A 84 P230699 0.02150118 4,76E-04 6,491094 2,698462 AT5G05530 RING/U-box superfamily protein A 84 P504336 0.01017677 4,71E-05 6,487664 2,697699 AT5G37250 RING/U-box superfamily protein A\_84\_P13607 Plant invertase/pectin methylesterase inhibitor superfamily 0,0121042 8,72E-05 6,451693 2,689678 AT3G06830 7,40E-05 6,437172 2,686427 AT1G73500 ATMKK9. MKK9 A 84 P22892 0.01163687 ATMKK9 MKK9 MAP kinase kinase 9

				<b>-</b> - <b>-</b>		
A_84_P267120				2,678295 AT1G74950		JAZ2_TIFY10BTIFY domain/Divergent CCT motif family protein
A_84_P530732	0,0161733	2,03E-04	,	2,676017 AT4G06746	<i>'</i>	DEAR5_RAP2.9_related to AP2 9
A_84_P10171	0,00877048					ATMES5_AtHNL_HNL_MES5methyl esterase 5
A_84_P290124	0,02400674	,	,	2,670646 AT2G22860	ATPSK2, PSK2	ATPSK2_PSK2phytosulfokine 2 precursor
A_84_P97916	0,03700301			2,659602 AT4G29780		
A_84_P19191	0,00894727		6,298683		ATCLH1, ATHCOR	ATCLH1_ATHCOR1_CLH1_CORI1chlorophyllase 1
A_84_P761897	0,02745852	,	,	2,647131 AT3G19615		
A_84_P24073	0,02830536	9,83E-04	6,261674	2,646548 AT3G28740		CYP81D11Cytochrome P450 superfamily protein
A_84_P10992	0,01526442	,	6,249454		APR3, ATAPR3, PF	R APR3_ATAPR3_PRH-26_PRH26APS reductase 3
A_84_P141619	0,01842527	3,13E-04	6,208693	2,63429 AT1G66870		Carbohydrate-binding X8 domain superfamily protein
A_84_P11272	0,01156977	6,67E-05	6,187967	2,629466 AT1G52390		
A_84_P522895	0,04360947	0,0028168	6,178401	2,627234 AT5G10210		
A_84_P290684	0,03913938	0,0021977	6,153355	2,621373 AT4G17490	ATERF6, ERF6, EF	R ATERF6_ERF-6-6_ERF6ethylene responsive element binding factor 6
A_84_P605140	0,00877048	2,06E-05	6,138272	2,617833 AT2G28920		RING/U-box superfamily protein
A_84_P12419	0,01132469	6,27E-05	6,114624	2,612264 AT1G51760	IAR3, JR3	IAR3_JR3peptidase M20/M25/M40 family protein
A_84_P516067	0,03206014	0,001375	6,113522	2,612004 AT1G17345		SAUR77SAUR-like auxin-responsive protein family
A_84_P15269	0,01961223	3,70E-04	6,102159	2,60932 AT1G52890	ANAC019, NAC019	ANAC019_NAC019_NAC domain containing protein 19
A_84_P765109	0,04371872	0,002852	6,090106	2,606467 AT4G36052		other RNA
A_84_P15450	0,02054225	4,25E-04	6,062175	2,599835 AT2G32120	HSP70T-2	HSP70T-2_heat-shock protein 70T-2
A_84_P17787	0,02452861	7,06E-04	6,043014	2,595268 AT5G37670		HSP20-like chaperones superfamily protein
A 84 P101906			6,038961	2,5943 AT1G70700	JAZ9, TIFY7	JAZ9_TIFY7TIFY domain/Divergent CCT motif family protein
A_84_P712636	0,02524157	7,71E-04	6,017644	2,589199 AT3G27415		0 ,1
A_84_P811806	0,01403995			2,579022 AT3G12145	FLOR1, FLR1	FLOR1_FLR1_FTM4Leucine-rich repeat (LRR) family protein
A 84 P16803	0,00466105			2,567864 AT5G14700	,	NAD(P)-binding Rossmann-fold superfamily protein
A_84_P10330	0,04812316			2,567231 AT5G65600		LecRK-IX.2_Concanavalin A-like lectin protein kinase family protein
A_84_P18976	0,0265841			2,563709 AT1G30730		FAD-binding Berberine family protein
A_84_P22732	0,01526442			2,561751 AT1G08860	BON3	BON3_Calcium-dependent phospholipid-binding Copine family protein
A_84_P159915	0,01507741			2,558968 AT4G27970		SLAH2_SLAC1 homologue 2
A 84 P23204	0,02612251	8,34E-04		2,555429 AT3G59480	02.112	pfkB-like carbohydrate kinase family protein
A_84_P21864	0,03959642	,	,	2,544045 AT1G73805	SARD1	SARD1Calmodulin binding protein-like
A_84_P17644	0,03879603	0,002141		2,543974 AT4G29710	0/11/01	Alkaline-phosphatase-like family protein
A_84_P582920	-	,	,	2,541161 AT2G45760	BAL BAP2	BAL_BAP2_BON association protein 2
A_84_P787617	0,0127527			2,536467 AT5G03270		LOG6_lysine decarboxylase family protein
A_84_P811724	,	0.0013546		2,53387 AT2G33830	2000	AtDRM2_DRM2Dormancy/auxin associated family protein
A_84_P756822	0,029931	,		2,527347 AT2G07731;		ALDININZ_DININZDormancy/adxin associated family protein
A 84 P606233	0,00918073	3,86E-05		2,526424 AT3G56260	NADU -	
A_84_P76184	0,02626428	,				ATWRKY30_WRKY30WRKY DNA-binding protein 30
A 84 P11239	0,01368821	-		2,521725 AT5G56840		myb-like transcription factor family protein
A_84_P789539	,	,	,	,		
				2,506323 AT4G06746		DEAR5_RAP2.9_related to AP2 9DEAR5_RAP2.9_related to AP2 9 ATOPR1_OPR1_12-oxophytodienoate reductase 1
A_84_P15276	0,0288939			2,487063 AT1G76680		,
A_84_P812177	,	0,0013988		2,479495 AT3G45140		ATLOX2_LOX2_lipoxygenase 2ATLOX2_LOX2_lipoxygenase 2
A_84_P767439				2,47612 AT5G37230;	0	
A_84_P826608		-		2,471307 AT5G66650		Protein of unknown function (DUF607)Protein of unknown function (DUF607)
A_84_P803770			5,529426		AIUPKI, UPKI	ATOPR1_OPR1_12-oxophytodienoate reductase 1
A_84_P19710	0,0278461	-		2,448763 AT5G46040		Major facilitator superfamily protein
A_84_P236643	,	6,29E-06	5,45451	2,44745 AT1G44350		ILL6_IAA-leucine resistant (ILR)-like gene 6
A_84_P255510				2,446687 AT1G72450	JAZ6, TIFY11B	JAZ6_TIFY11B_jasmonate-zim-domain protein 6
A_84_P858725				2,438685 AT1G72450	JAZ6, TIFY11B	JAZ6_TIFY11B_jasmonate-zim-domain protein 6
A_84_P501308				2,418945 AT3G14260		Protein of unknown function (DUF567)
A_84_P241449		-		2,417769 AT1G28190		
A_84_P193944	0,0227833	5,50E-04	5,3431	2,417677 AT5G66650		Protein of unknown function (DUF607)Protein of unknown function (DUF607)

A\_84\_P14417 0.03359263 0,0015462 5,326117 2,413084 AT2G20560 DNAJ heat shock family proteinDNAJ heat shock family protein A 84 P23398 0,00894727 3,26E-05 5,318908 2,41113 AT5G06870 ATPGIP2, PGIP2 ATPGIP2 PGIP2 polygalacturonase inhibiting protein 2 A\_84\_P12686 0.04864829 0,003789 5,30064 2,406167 AT3G25180 CYP82G1 CYP82G1 cytochrome P450, family 82, subfamily G, polypeptide 1 A\_84\_P16831 ATL31\_CNI1\_\_carbon/nitrogen insensitive 1 0,0390369 0,0021726 5,282131 2,40112 AT5G27420 ATL31, CNI1 2,86E-05 5,194599 2,377012 AT1G76680 ATOPR1, OPR1 ATOPR1 OPR1 12-oxophytodienoate reductase 1 A 84 P855161 0,00894727 A\_84\_P154615 0,01785633 2,86E-04 5,181008 2,373233 AT1G76590 PLATZ transcription factor family protein A\_84\_P224809 0,02047567 4,17E-04 5,179902 2,372925 AT2G38790 A\_84\_P827393 0,0307965 0,0012415 5,178978 2,372667 AT3G48650 A 84 P19561 0.01176281 7,82E-05 5,163301 2,368294 AT4G35480 RHA3B RHA3B RING-H2 finger A3B A\_84\_P829388 2,18E-05 5,150688 2,364765 AT1G07150 MAPKKK13 MAPKKK13\_\_\_mitogen-activated protein kinase kinase kinase 13 0,00877275 A 84 P12418 0.01742975 2,51E-04 5,134507 2,360226 AT1G49530 GGPS6 GGPS6\_\_geranylgeranyl pyrophosphate synthase 6 A 84 P23308 0.01751097 2,55E-04 5,101237 2,350847 AT4G27140 AT2S1, SESA1 AT2S1 SESA1 seed storage albumin 1 A\_84\_P18198 0.0378567 0,0020381 5,091441 2,348074 AT2G16660 Major facilitator superfamily protein A\_84\_P572494 0,00900545 3,50E-05 5,081011 2,345116 AT4G28703 RmIC-like cupins superfamily proteinRmIC-like cupins superfamily protein A\_84\_P579612 0,01166756 7,46E-05 5,044079 2,334591 AT4G27652 A\_84\_P289964 0,04324035 0,0027782 5,010316 2,324902 AT1G19020 A 84 P761787 0.03308225 0,0014794 4,998734 2,321563 AT3G14185 other RNA A 84 P19375 0,01718834 2,40E-04 4,984381 2,317414 AT3G49110 ATPCA, ATPRX33, ATPCA ATPRX33 PRX33 PRXCA peroxidase CA RmIC-like cupins superfamily proteinRmIC-like cupins superfamily protein A\_84\_P787406 0.00772467 1,48E-05 4,974677 2,314603 AT4G28703 A\_84\_P13315 0.02300682 5,63E-04 4,970052 2,313261 AT1G16370 OCT6, ATOCT6 ATOCT6\_OCT6\_organic cation/carnitine transporter 6 A 84 P299810 0,0246688 7,24E-04 4,933648 2,302655 AT1G11925 Stigma-specific Stig1 family protein ZAT11\_\_C2H2 and C2HC zinc fingers superfamily protein A\_84\_P22991 0,0339728 0,0015838 4,92892 2,301271 AT2G37430 A 84 P786490 0.0378567 0.0020384 4.92617 2,300467 AT2G46400 ATWRKY46, WRKY ATWRKY46 WRKY46 WRKY DNA-binding protein 46 A 84 P18334 0,01066107 5,52E-05 4,920482 2.2988 AT3G06490 AtMYB108, BOS1, AtMYB108 BOS1 MYB108 myb domain protein 108 A\_84\_P10711 0,0013819 4,912672 2,296508 AT2G39330 JAL23 JAL23\_jacalin-related lectin 23 0,0321314 4,89059 2,290009 AT3G56400 ATWRKY70, WRKY ATWRKY70\_WRKY70\_WRKY DNA-binding protein 70 A\_84\_P16571 0,03927104 0,0022318 A 84 P211588 0,02991109 0,0011472 4,877779 2,286224 AT4G24110 A\_84\_P16037 0,00857212 1,85E-05 4,875406 2,285523 AT1G56650 ATMYB75, MYB75, ATMYB75\_MYB75\_PAP1\_SIAA1\_production of anthocyanin pigment 1 A\_84\_P11191 0,02353903 5,88E-04 4,83706 2,27413 AT5G43620 Pre-mRNA cleavage complex II A 84 P16645 0.03490096 0,0016857 4,828329 2,271524 AT4G13480 AtMYB79, MYB79 AtMYB79\_MYB79\_myb domain protein 79 A\_84\_P21473 0,04680366 0,0034123 4,815778 2,267769 AT4G15210 ATBETA-AMY, AT- AT-BETA-AMY\_ATBETA-AMY\_BAM5\_BMY1\_RAM1\_beta-amylase 5 A 84\_P805566 0.02951533 0.0011157 4.805011 2,26454 AT5G24780 ATVSP1, VSP1 ATVSP1 VSP1 vegetative storage protein 1 A 84 P12975 0.01455063 1,47E-04 4,789286 2,25981 AT1G19550 Glutathione S-transferase family protein A\_84\_P16077 MAPKKK18\_\_\_mitogen-activated protein kinase kinase kinase 18 0,03484798 0,0016771 4,784685 2,258424 AT1G05100 MAPKKK18 A\_84\_P862058 0,01188738 8,37E-05 4,774704 2,255411 AT3G12145 FLOR1, FLR1 FLOR1\_FLR1\_FTM4\_\_Leucine-rich repeat (LRR) family protein A 84 P115562 0.01572616 1,90E-04 4,772359 2,254703 AT1G73325 Kunitz family trypsin and protease inhibitor protein A\_84\_P16802 0,03920303 0,0022235 4,756288 2,249836 AT5G14470 AtGALK2\_GALK2\_GHMP kinase family protein A\_84\_P786837 0.00903297 3.61E-05 4.748268 2.247401 AT5G13080 ATWRKY75, WRKY ATWRKY75 WRKY75 WRKY DNA-binding protein A\_84\_P87559 0,02167651 4,88E-04 4,743054 2,245816 AT1G66500 Pre-mRNA cleavage complex II A 84 P541993 0.01354508 1,16E-04 4,736571 2,243843 AT2G27310 F-box family protein A\_84\_P21703 0,01320943 1,08E-04 4,710181 2,235783 AT5G13080 ATWRKY75, WRKY ATWRKY75\_WRKY75\_WRKY DNA-binding protein 75 A 84 P15864 Curculin-like (mannose-binding) lectin family protein 0.01424899 1,40E-04 4,658196 2,219771 AT5G18470 A\_84\_P787776 0,00178308 2,89E-07 4,650138 2,217274 AT3G56260 A\_84\_P11731 0.01222481 8.89E-05 4,62808 2,210414 AT3G24500 ATMBF1C, MBF1C ATMBF1C\_MBF1C\_multiprotein bridging factor 1C A\_84\_P14710 0,0286969 0,0010111 4,622901 2,208798 AT3G56710 SIB1 SIB1\_\_sigma factor binding protein 1 A 84 P92059 0.02237896 5,24E-04 4,619537 2,207748 AT5G67480 ATBT4, BT4 ATBT4 BT4 BTB and TAZ domain protein 4 A\_84\_P12086 0,01246891 9,59E-05 4,596134 2,200421 AT5G19100 Eukaryotic aspartyl protease family protein A 84 P10148 0,01797131 2,91E-04 4,589444 2,198319 AT5G03270 LOG6 LOG6 lysine decarboxylase family proteinLOG6 lysine decarboxylase family protein A\_84\_P816079 0,0241966 6,69E-04 4,581136 2,195705 AT4G17230 SCL13 SCL13 SCARECROW-like 13SCL13 SCARECROW-like 13 A\_84\_P819821 0,03879603 DNAJ heat shock family proteinDNAJ heat shock family protein 0,0021411 4,579017 2,195038 AT2G20560 A 84 P504929 0.0373915 0.0019897 4.57067 2.192406 AT3G29000 Calcium-binding EF-hand family proteinCalcium-binding EF-hand family protein

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A_84_P301340 0,00764839			2,191456 AT1G80120		Protein of unknown function (DUF567)
A_84_P784216 0,04759051			2,191389 AT5G24780		ATVSP1_VSP1_vegetative storage protein 1
A_84_P18553 0,02376995	,		2,179587 AT4G21390	B120	B120_S-locus lectin protein kinase family protein
A_84_P15209 0,01720161			2,178997 AT1G15330		Cystathionine beta-synthase (CBS) proteinCystathionine beta-synthase (CBS) protein
A_84_P828124 0,01363577					AtERF73_ERF73_HRE1Integrase-type DNA-binding superfamily protein
A_84_P174151 0,03189378			2,159036 AT2G40330	PYL6, RCAR9	PYL6_RCAR9PYR1-like 6
A_84_P24128 0,02236889	5,19E-04	4,462134	2,157734 AT3G54150		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P205068 0,01202061	,		2,156737 AT1G17744		
A_84_P11850 0,00894727			2,153923 AT3G57760		Protein kinase superfamily protein
A_84_P13499 0,02864917	0,0010029	4,445462	2,152333 AT2G14160		RNA-binding (RRM/RBD/RNP motifs) family protein
A_84_P12726 0,01742975	2,52E-04	4,440763	2,150807 AT3G11020	DREB2, DREB2B	DREB2_DREB2BDRE/CRT-binding protein 2B
A_84_P784935 0,01756549	2,56E-04	4,433978	2,148602 AT1G15330		Cystathionine beta-synthase (CBS) proteinCystathionine beta-synthase (CBS) protein
A_84_P13568 0,01958881	3,59E-04	4,419572	2,143907 AT2G33830		AtDRM2_DRM2Dormancy/auxin associated family protein
A_84_P156125 0,03585444			2,140303 AT3G61190		BAP1_BON association protein 1
A_84_P17388 0,00894727		4,404374	2,138937 AT3G06500	A/N-InvC	A/N-InvCPlant neutral invertase family protein
A_84_P135945 0,01320943	1,07E-04	4,404077	2,13884 AT3G11840	PUB24	PUB24plant U-box 24
A_84_P20736 0,02403887	6,57E-04	4,391066	2,134571 AT5G66700	ATHB53, HB53, HB	ATHB53_HB-8_HB53homeobox 53
A_84_P145309 0,04139324	0,0025452	4,353202	2,122077 AT4G13820		Leucine-rich repeat (LRR) family protein
A_84_P268810 0,01188738	8,40E-05	4,323877	2,112326 AT4G27560		UDP-Glycosyltransferase superfamily proteinUDP-Glycosyltransferase superfamily protein
A_84_P18030 0,02160571	4,83E-04	4,274418	2,095728 AT1G30720		FAD-binding Berberine family protein
A_84_P567982 0,00640993	7,87E-06	4,252192	2,088207 AT4G15210	ATBETA-AMY, AT-	AT-BETA-AMY_ATBETA-AMY_BAM5_BMY1_RAM1beta-amylase 5
A_84_P549947 0,01771716	2,71E-04	4,248887	2,087085 AT2G18210		
A_84_P20977 0,02353903	5,90E-04	4,23665	2,082924 AT1G14480		Ankyrin repeat family protein
A_84_P139169 0,02401475	6,48E-04	4,205016	2,072111 AT1G67810	SUFE2	SUFE2_sulfur E2
A_84_P849804 0,01961223	3,68E-04	4,187997	2,06626 AT2G18210		
A_84_P15797 0,0364524	0,0018701	4,187597	2,066123 AT4G15100	scpl30	scpl30serine carboxypeptidase-like 30
A_84_P827867 0,03518269			2,065222 AT1G65880		BZO1_benzoyloxyglucosinolate 1
A_84_P12963 0,0116891	7,53E-05	4,182253	2,064281 AT4G15440	CYP74B2, HPL1	CYP74B2_HPL1hydroperoxide lyase 1
A_84_P870191 0,04017467	0,0023847	4,159566	2,056433 AT1G21320;	C	
A_84_P13173 0,02646521	8,51E-04	4,15641	2,055338 AT5G67300	ATMYB44, ATMYB	ATMYB44_ATMYBR1_MYB44_MYBR1myb domain protein r1
A_84_P12969 0,01390775	1,34E-04	4,153408	2,054296 AT4G17230	SCL13	SCL13_SCARECROW-like 13SCL13_SCARECROW-like 13
A_84_P97476 0,02951533	0,0011131	4,146306	2,051827 AT5G24780	ATVSP1, VSP1	ATVSP1_VSP1vegetative storage protein 1
A_84_P767469 0,02100477	4,54E-04	4,129149	2,045845 AT5G37270		RING/U-box superfamily protein
A_84_P269630 0,02684096	8,78E-04	4,098218	2,034997 AT2G27505		FBD-like domain family protein
A_84_P834641 0,04908544	0,0038744	4,091984	2,032801 AT3G29000		Calcium-binding EF-hand family proteinCalcium-binding EF-hand family protein
A_84_P23096 0,01686909	2,30E-04	4,089145	2,031799 AT3G26200	CYP71B22	CYP71B22cytochrome P450, family 71, subfamily B, polypeptide 22
A_84_P847323 0,01156977	6,58E-05	4,084339	2,030103 AT2G18210		
A_84_P22507 0,00772467	1,43E-05	4,082771	2,029549 AT5G27520	AtPNC2, PNC2	AtPNC2_PNC2_peroxisomal adenine nucleotide carrier 2
A_84_P279280 0,01797131	2,91E-04	4,073453	2,026252 AT5G44590		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P23455 0,02943616	0,0011009	4,058723	2,021026 AT1G68620		alpha/beta-Hydrolases superfamily protein
A_84_P10947 0,00387638	1,26E-06	4,050553	2,018119 AT4G04610	APR, APR1, ATAP	APR_APR1_ATAPR1_PRH19APS reductase 1
A_84_P14013 0,01693314	2,33E-04	4,042065	2,015092 AT5G40000		P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P755325 0,00904516	3,71E-05	4,030572	2,010985 AT2G06045		transposable element gene
A_84_P856887 0,02532245	7,79E-04	4,024943	2,008968 AT3G10985	ATWI-12, SAG20,	ATWI-12_SAG20_WI12senescence associated gene 20
A_84_P11345 0,00772467	1,46E-05	4,011036	2,003975 AT1G17750	AtPEPR2, PEPR2	AtPEPR2_PEPR2_PEP1 receptor 2
A_84_P179084 0,01504028			2,003947 AT5G35735		Auxin-responsive family protein
A_84_P22015 0,01246891	9,28E-05	3,99287	1,997426 AT1G30040	ATGA2OX2, GA2O	ATGA2OX2_GA2OX2_gibberellin 2-oxidase
A_84_P20910 0,01305407	1,05E-04	3,986538	1,995136 AT1G72360	AtERF73, ERF73, H	AtERF73_ERF73_HRE1Integrase-type DNA-binding superfamily protein
A_84_P23353 0,02507846	7,59E-04	3,985152	1,994635 AT4G38390	RHS17	RHS17_root hair specific 17
A_84_P56650 0,01305407					AtGDPD2_GDPD2_PLC-like phosphodiesterases superfamily protein
A_84_P20428 0,04788074	0,0035903	3,935472	1,976537 AT4G13310	CYP71A20	CYP71A20cytochrome P450, family 71, subfamily A, polypeptide 20
	.,	,	,	-	

A_84_P96076	0,01177365	8,02E-05	3,921685	1,971474 AT3G25790	HHO	myb-like transcription factor family protein
A_84_P564464	0,01020095	4,91E-05	3,915044	1,969029 AT4G27460		CBSX5Cystathionine beta-synthase (CBS) family protein
A_84_P820222	0,02751414	9,28E-04	3,906483	1,96587 AT1G73480		alpha/beta-Hydrolases superfamily proteinalpha/beta-Hydrolases superfamily protein
A_84_P10871	0,01747336	2,53E-04	3,882391	1,956945 AT3G50280		HXXXD-type acyl-transferase family protein
A_84_P18425	0,00178308	2,51E-07	3,86338	1,949863 AT3G47960		AtNPF2.10_GTR1_NPF2.10Major facilitator superfamily protein
A_84_P24091	0,02009357	3,97E-04	3,862192	1,94942 AT3G45960	ATEXLA3, ATEXPL	ATEXLA3_ATEXPL3_ATHEXP BETA 2.3_EXLA3_EXPL3expansin-like A3
A_84_P769675	0,029931	0,0011632	3,862049	1,949367 AT5G66210	CPK28	CPK28_calcium-dependent protein kinase 28
A_84_P811178	0,01909557	3,41E-04	3,861123	1,94902 AT4G16146		cAMP-regulated phosphoprotein 19-related protein
A_84_P17461	0,01185074	8,24E-05	3,860947	1,948955 AT3G43430		RING/U-box superfamily protein
A_84_P14693	0,009203	3,92E-05	3,859746	1,948506 AT3G59750		LecRK-V.8_Concanavalin A-like lectin protein kinase family protein
A_84_P210868	0,0110149	5,93E-05	3,855405	1,946882 AT4G16146		cAMP-regulated phosphoprotein 19-related protein
A_84_P21126	0,01961223	3,65E-04	3,853399	1,946132 AT2G32020		Acyl-CoA N-acyltransferases (NAT) superfamily protein
A_84_P790164	,	9,49E-06	,	1,94563 AT5G41080	AtGDPD2, GDPD2	AtGDPD2_GDPD2_PLC-like phosphodiesterases superfamily protein
A_84_P818318	0,01246891	9,29E-05	,	1,945327 AT4G27560		UDP-Glycosyltransferase superfamily protein
A_84_P22522	0,01386093		,	1,943737 AT5G39020		Malectin/receptor-like protein kinase family protein
A_84_P11439	0,03764553			1,941316 AT1G52560		HSP20-like chaperones superfamily protein
A_84_P169853	,			1,933858 AT3G49580	LSU1	LSU1_response to low sulfur 1
A_84_P17380	0,0184221					ATPK19_ATPK2_ATS6K2_S6K2_serine/threonine protein kinase 2
A_84_P545008	0,0308525			1,924648 AT4G31875	AII 10, AII 12, A	
A_84_P132425	0,01971263			1,921982 AT3G46110		Domain of unknown function (DUF966)Domain of unknown function (DUF966)
A_84_P21203	0,02403887	5,82E-04 6,56E-04	,	1,919073 AT1G26730		EXS (ERD1/XPR1/SYG1) family protein
A_84_P794434	0,03518269			1,907865 AT4G27460		CBSX5_Cystathionine beta-synthase (CBS) family protein
A_84_P12859	0,01439785			1,903495 AT4G12410		SAUR35SAUR-like auxin-responsive protein family
A_84_P542288		,	,	1,902994 AT4G17470		alpha/beta-Hydrolases superfamily proteinalpha/beta-Hydrolases superfamily protein
A_84_P542377	0,03915126			1,89904 AT5G03550		
A_84_P20121	0,04708578			1,898592 AT1G07860;	0	
A_84_P15366	0,01363577	,	,	1,897491 AT1G11210	1 8 9 9	Protein of unknown function (DUF761)
A_84_P16742	0,00877275	-		1,893577 AT4G14680	APS3	APS3_Pseudouridine synthase/archaeosine transglycosylase-like family protein
A_84_P72634	0,02740352	,	,	1,892277 AT4G36500		
A_84_P10209	0,02069154	,	,	1,890096 AT5G25930		Protein kinase family protein with leucine-rich repeat domain
A_84_P20724	0,01961223					ANAC102_NAC102NAC domain containing protein 102
A_84_P16285	0,03529944			1,886457 AT2G29720		CTF2BFAD/NAD(P)-binding oxidoreductase family protein
A_84_P162443	0,03988752			1,882175 AT5G64510	TIN1	TIN1
A_84_P19959	0,04340026	0,0027966	3,652484	1,868878 AT1G32970		Subtilisin-like serine endopeptidase family protein
A_84_P788885	0,02114772	4,59E-04	3,650146	1,867954 AT1G56300		Chaperone DnaJ-domain superfamily proteinChaperone DnaJ-domain superfamily protein
A_84_P14664	0,00772467	1,45E-05	3,64758	1,86694 AT3G53160	UGT73C7	UGT73C7_UDP-glucosyl transferase 73C7
A_84_P111682	0,03146277	0,0013105	3,611352	1,852539 AT5G43520		Cysteine/Histidine-rich C1 domain family protein
A_84_P23394	0,00764839	1,17E-05	3,607725	1,851089 AT5G05730	AMT1, ASA1, JDL1	AMT1_ASA1_JDL1_TRP5_WEI2anthranilate synthase alpha subunit 1
A_84_P12575	0,01630908	2,10E-04	3,599378	1,847748 AT2G23030	SNRK2.9, SNRK2-9	SNRK2-9_SNRK2.9_SNF1-related protein kinase 2.9
A_84_P567134	0,04661662	0,0033663	3,596957	1,846777 AT4G27657		
A_84_P847558	0,0266426	8,63E-04	3,586693	1,842654 AT1G55450		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P856597	0,01232764	9,08E-05	3,581019	1,84037 AT5G16190	ATCSLA11, CSLA1	ATCSLA11_CSLA11cellulose synthase like A11
A_84_P210768	0,01961223	3,79E-04	3,572125	1,836783 AT5G19230		Glycoprotein membrane precursor GPI-anchored
A_84_P18856	0,00894727			1,831739 AT5G17490	AtRGL3, RGL3	AtRGL3_RGL3RGA-like protein 3
A_84_P16133	0,02454009			1,830524 AT1G79310		AtMC7_AtMCP2a_MC7_MCP2a_metacaspase 7
A 84 P17683	0.01592639			1,826122 AT4G39580	, <b>.</b> ,,	Galactose oxidase/kelch repeat superfamily protein
A_84_P796533	0,0116891	,	,	1,824163 AT3G46650		UDP-Glycosyltransferase superfamily protein
A_84_P575245					ATCSI A11, CSI A1	ATCSLA11_CSLA11cellulose synthase like A11
A_84_P856591						ANAC102_NAC102NAC domain containing protein 102
A_84_P203438				1,818126 AT4G34138		UGT73B1UDP-glucosyl transferase 73B1
/1_0+_i 200400	0,0211-010	3,702-04	0,020220	1,010120714004100	0011001	
A_84_P603728	0,03916847	0 0022167	2 510101	1,815215 AT5G02600	NAKR1 NDCCC	NAKR1_NPCC6Heavy metal transport/detoxification superfamily protein

A_84_P23171 0,02114772	, ,	,	AT-HSFA7A, HSFA	AT-HSFA7A_HSFA7A_heat shock transcription factor A7A
A_84_P831247 0,04115299		3 1,809261 AT5G43520		Cysteine/Histidine-rich C1 domain family proteinCysteine/Histidine-rich C1 domain family protein
A_84_P537274 0,03440424	0,0016283 3,48534		ABO3, ATWRKY63	ABO3_ATWRKY63_WRKY63ABA overly sensitive mutant 3
A_84_P118712 0,01769782		3 1,799772 AT1G56300		Chaperone DnaJ-domain superfamily proteinChaperone DnaJ-domain superfamily protein
A_84_P54170 0,00992271		1,798333 AT3G57450		
A_84_P10874 0,02582126		5 1,797073 AT3G50970	LTI30, XERO2	LTI30_XERO2dehydrin family protein
A_84_P768949 0,03884352	0,0021488 3,469673			
A_84_P12431 0,01771716		1,787811 AT1G74420	ATFUT3, FUT3	ATFUT3_FUT3_fucosyltransferase 3
A_84_P20043 0,03768138		3 1,787085 AT1G54070		Dormancy/auxin associated family protein
A_84_P23164 0,00682995			ATERF#011, CEJ1,	ATERF#011_CEJ1_DEAR1cooperatively regulated by ethylene and jasmonate 1
A_84_P18141 0,01961223		1,779398 AT1G55450		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P23287 0,00461552		1,773734 AT4G22530		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P15703 0,02244603		1,772143 AT4G18340		Glycosyl hydrolase superfamily protein
A_84_P16727 0,01365065				AUR3_BRU6_GH3-2_GH3.2_YDK1Auxin-responsive GH3 family protein
A_84_P800816 0,02040927				ATWI-12_SAG20_WI12senescence associated gene 20ATWI-12
A_84_P19047 0,01063874				A ATSOT16_ATST5A_CORI-7_SOT16sulfotransferase 16
A_84_P205978 0,01742975		5 1,766564 AT4G31950	CYP82C3	CYP82C3cytochrome P450, family 82, subfamily C, polypeptide 3
A_84_P23838 0,02009357		1,766149 AT1G73480		alpha/beta-Hydrolases superfamily proteinalpha/beta-Hydrolases superfamily protein
A_84_P855665 0,01246891	9,38E-05 3,394857	1,763351 AT2G18200		
A_84_P562234 0,04669211	0,0033886 3,389558	3 1,761097 AT1G59640	BPE, BPEp, BPEub	BPE_BPEp_BPEub_ZCW32BIG PETAL
A_84_P763062 0,0151022	1,68E-04 3,389215	5 1,760951 AT4G22710	CYP706A2	CYP706A2cytochrome P450, family 706, subfamily A, polypeptide 2
A_84_P582597 0,0457815	0,0032287 3,385733	3 1,759468 AT1G09932		Phosphoglycerate mutase family protein
A_84_P22853 0,00904516	3,77E-05 3,38344	1,758491 AT1G75960		AMP-dependent synthetase and ligase family protein
A_84_P22308 0,01163687	7,27E-05 3,378833	3 1,756525 AT4G10390		Protein kinase superfamily protein
A_84_P10305 0,01246891	9,49E-05 3,373704	1,754334 AT5G59730	ATEXO70H7, EXO	ATEXO70H7_EXO70H7exocyst subunit exo70 family protein H7
A_84_P118462 0,02449875	7,01E-04 3,368924	1,752288 AT2G28400		Protein of unknown function, DUF584
A_84_P752754 0,01020095	4,92E-05 3,368518	1,752114 AT1G64195		Defensin-like (DEFL) family protein
A_84_P13988 0,03707299	0,0019508 3,366215	5 1,751127 AT5G25450		Cytochrome bd ubiquinol oxidase, 14kDa subunit
A_84_P169053 0,02894387	0,0010428 3,339133	1,739473 AT3G45730		
A_84_P818968 0,01693864	2,33E-04 3,33096	5 1,735938 AT3G15630		
A_84_P763641 0,03916847	0,002218 3,328925	5 1,735056 AT4G06529		transposable element gene
A_84_P843872 0,04254071	0,0026896 3,315494	1,729224 AT1G53430		Leucine-rich repeat transmembrane protein kinaseLeucine-rich repeat transmembrane protein kinase
A_84_P868217 0,01828971	3,00E-04 3,31116	1,727337 AT1G52030	F-ATMBP, MBP1.2,	F-ATMBP_MBP1.2_MBP2myrosinase-binding protein 2F
A_84_P18550 0,0237198	6,06E-04 3,307844	1,725891 AT4G20780	CML42	CML42_calmodulin like 42
A_84_P23721 0,00606654		1,722128 AT1G14200		RING/U-box superfamily protein
A_84_P560583 0,02054225	4,25E-04 3,288508	1,717433 AT2G18670;	0	
A_84_P183724 0,02494651	7,47E-04 3,287958	1,717192 AT2G35290		SAUR79
A_84_P19046 0,04465805	0,0030117 3,269159	1,708919 AT1G69920	ATGSTU12, GSTU	ATGSTU12_GSTU12_glutathione S-transferase TAU 12
A_84_P69204 0,0215952		1,707155 AT1G21110		IGMT3O-methyltransferase family protein
A_84_P807259 0,04812316				ATMYB44_ATMYBR1_MYB44_MYBR1myb domain protein r1
A_84_P141439 0,01911949		2 1,706337 AT5G24770		ATVSP2_VSP2_vegetative storage protein 2
A 84 P814936 0,02353903		1,705892 AT4G30210		AR2_ATR2P450 reductase 2AR2_ATR2P450 reductase 2
A_84_P172941 0,02400674		3 1,705668 AT2G18690	,	
A_84_P13022 0,02114772			ATFTSH6, FTSH6	ATFTSH6_FTSH6FTSH protease 6
A_84_P11679 0,0234887		3 1,701504 AT2G23680	,	Cold acclimation protein WCOR413 family
A 84 P611805 0,00606654		1,700999 AT5G26600		Pyridoxal phosphate (PLP)-dependent transferases superfamily protein
A_84_P11802 0,02454009	, ,	1,700742 AT3G46690		UDP-Glycosyltransferase superfamily protein
A_84_P763881 0,02353903		5 1,699652 AT4G20830		FAD-binding Berberine family proteinFAD-binding Berberine family protein
A_84_P15965 0,00900545		1,698468 AT5G56980		
A_84_P136725 0,01177365		1,690841 AT5G36920		
A_84_P133525 0,03051006				ATPSK3 (FORMER SYMBOL)_ATPSK4_PSK4_phytosulfokine 4 precursor
<u></u> 1 100020 0,00001000	0,0012010 0,213400	, 1,00007 A100+9700		

A\_84\_P12073 0.04543232 0.0031481 3.210028 1.682586 AT1G18290 A 84 P10473 0,01742975 2,49E-04 3,184558 1,671093 AT1G59870 ABCG36, ATABCG ABCG36 ATABCG36 ATPDR8 PEN3 ABC-2 and Plant PDR ABC-type transporter family protein A\_84\_P813990 0,01375994 1,24E-04 3,183021 1,670397 AT4G30530 GGP1 GGP1\_Class I glutamine amidotransferase-like superfamily protein A\_84\_P524641 0,02702 8,91E-04 3,181917 1,669896 AT4G39675 6,79E-04 3,171227 1,665041 AT2G18690 A 84 P816512 0,02430013 A\_84\_P598475 0,01842527 3,13E-04 3,161928 1,660805 AT5G26600 Pyridoxal phosphate (PLP)-dependent transferases superfamily protein A\_84\_P16642 1,25E-04 3,159096 1,659512 AT4G12720 AtNUDT7, GFG1, N AtNUDT7\_GFG1\_NUDT7\_\_MutT/nudix family protein 0.01375994 A\_84\_P808818 0,02524157 7,71E-04 3,158492 1,659236 AT5G24770; ATVSP2,VSP2,ATVSP1,VSP1 A 84 P813874 0,02269863 5,43E-04 3,155744 1,65798 AT4G12720 AtNUDT7, GFG1, N AtNUDT7\_GFG1\_NUDT7\_\_MutT/nudix family protein A\_84\_P22296 0,01114487 6,07E-05 3,147514 1,654213 AT4G05010 AtFBS3\_FBS3\_\_F-box family protein A 84 P109302 0,01526442 1,74E-04 3,144848 1,65299 AT1G19570 ATDHAR1, DHAR1 ATDHAR1 DHAR1 DHAR5 dehydroascorbate reductase A 84 P16192 0.02381684 6,25E-04 3,143213 1,65224 AT1G77450 anac032, NAC032 NAC032 anac032 NAC domain containing protein 32 A\_84\_P798326 0,01830757 3,06E-04 3,136388 1,649104 AT5G50570; SPL13,SPL13A,SPL13,SPL13B A\_84\_P21924 0,02237896 5,22E-04 3,132248 1,647199 AT1G78050 PGM PGM\_\_phosphoglycerate/bisphosphoglycerate mutase 0.01560944 A 84 P80879 1,81E-04 3,131741 1,646965 AT2G46510 AIB, ATAIB AIB ATAIB JAM1 ABA-inducible BHLH-type transcription factor A\_84\_P784658 0,01948675 3,55E-04 3,126082 1,644356 AT5G56980 A 84 P547572 0.01020095 4,94E-05 3,125545 1,644108 AT4G02360 Protein of unknown function, DUF538 A 84 P63594 0.03681569 0,0019242 3,123849 1,643325 AT4G18205 Nucleotide-sugar transporter family protein A 84 P16568 4.31E-04 3.116968 1.640143 AT3G55840 0.02066042 Hs1pro-1 protein A\_84\_P810288 0,03518269 0,0017109 3,116542 1,639946 AT3G56880 VQ motif-containing proteinVQ motif-containing protein UGT73B2 UDP-glucosyltransferase 73B2 A 84 P60830 0.0390369 0,0021757 3,109611 1,636734 AT4G34135 UGT73B2 A\_84\_P500185 0,03581283 0,0017993 3,108379 1,636162 AT1G63590 Receptor-like protein kinase-related family protein A\_84\_P118232 0,02067851 4,35E-04 3,108127 1,636046 AT5G16360 NC domain-containing protein-related A 84 P812024 0,01100802 5.89E-05 3,102263 1,633321 AT1G28330 DRM1, DYL1 AtDRM1 DRM1 DYL1 dormancy-associated protein-like 1 A\_84\_P15515 0,01707055 2,36E-04 3,095456 1,630152 AT3G19390 Granulin repeat cysteine protease family protein 1,94E-04 3,091225 1,628179 AT3G06850 BCE2, DIN3, LTA1 BCE2\_DIN3\_LTA1\_2-oxoacid dehydrogenases acyltransferase family protein A\_84\_P23064 0,01592639 A 84 P753508 0.03358237 0,0015265 3,085983 1,62573 AT1G55525 other RNA A\_84\_P10080 0,03914714 0,0022025 3,085648 1,625574 AT4G29610 Cvtidine/deoxycvtidylate deaminase family protein A\_84\_P10415 0,01771716 2,67E-04 3,073254 1,619767 AT1G52030 F-ATMBP, MBP1.2, F-ATMBP\_MBP1.2\_MBP2\_myrosinase-binding protein 2F A 84 P831354 0.01066107 5,66E-05 3,072931 1,619615 AT4G17470 alpha/beta-Hydrolases superfamily proteinalpha/beta-Hydrolases superfamily protein A\_84\_P52340 0,01322879 1,10E-04 3,070525 1,618486 AT3G26840 PES2\_Esterase/lipase/thioesterase family protein A 84\_P763462 0.01633501 2,12E-04 3,066847 1,616756 AT4G03510 ATRMA1, RMA1 ATRMA1 RMA1 RING membrane-anchor 1ATRMA1 RMA1 RING membrane-anchor 1 A 84 P13078 0.00894727 3,35E-05 3,065897 1,616309 AT5G42650 AOS, CYP74A, DD AOS CYP74A DDE2 allene oxide synthase A\_84\_P532079 5PTASE11, AT5PT 5PTASE11\_AT5PTASE11\_\_inositol polyphosphate 5-phosphatase 11 0,0151022 1,68E-04 3,063726 1,615287 AT1G47510 A\_84\_P10329 0,02452861 7,04E-04 3,063496 1,615179 AT5G65280 GCL1 GCL1\_GCR2-like 1 A 84 P19210 0.04639193 0.0033254 3.063278 1.615076 AT2G46400 ATWRKY46, WRKY ATWRKY46 WRKY46 WRKY DNA-binding protein 46 A\_84\_P604458 0,03956232 Protein of unknown function (DUF626) 0,0022778 3,059521 1,613306 AT3G57210 PROPEP4 elicitor peptide 4 precursor A 84 P110112 0.02991192 0.0011579 3.058019 1.612598 AT5G09980 PROPEP4 A\_84\_P72974 0,02100477 4,54E-04 3,056071 1,611678 AT5G50570; SPL13,SPL13A,SPL13,SPL13B A 84 P787273 0.01961223 3,68E-04 3,0525 1,609991 AT5G24600 Protein of unknown function, DUF599 A\_84\_P12143 0,02582126 8,07E-04 3,045242 1,606557 AT5G45340 CYP707A3 CYP707A3\_cytochrome P450, family 707, subfamily A, polypeptide 3CYP707A3 A 84 P509078 0.03367786 0,0015589 3,044057 1,605995 AT1G69900 Actin cross-linking proteinActin cross-linking protein A\_84\_P762419 0,03916847 0,0022178 3,039217 1,6037 AT3G15518 A\_84\_P784166 0.0265841 8.58E-04 3.03847 1.603345 AT5G45340 CYP707A3 CYP707A3\_cytochrome P450, family 707, subfamily A, polypeptide 3CYP707A3 A\_84\_P514608 0,00961027 4,30E-05 3,033142 1,600813 AT3G09032 A 84 P284990 0.03255169 0.0014193 3.023977 1.596447 AT4G15610 Uncharacterised protein family (UPF0497)Uncharacterised protein family (UPF0497) A\_84\_P21604 0,03993093 0,0023274 3,021445 1,595239 AT5G46590 anac096, NAC096 NAC096\_anac096\_NAC domain containing protein 96 A 84 P22149 0,01462891 1,55E-04 3,020397 1,594738 AT3G13310 DJC66 Chaperone DnaJ-domain superfamily protein 3,019281 1,594205 AT1G05560 A 84 P19958 0.02951533 0,001114 UGT1, UGT75B1 UGT1 UGT75B1 UDP-glucosyltransferase 75 A\_84\_P815262 3,015878 1,592578 AT3G06850 BCE2, DIN3, LTA1 BCE2\_DIN3\_LTA1\_2-oxoacid dehydrogenases acyltransferase family protein 0,03308225 0,0014838 A 84 P10136 0.03518269 0.0017144 3.014202 1.591776 AT4G36670 AtPLT6. AtPMT6. P AtPLT6 AtPMT6 PLT6 PMT6 Major facilitator superfamily protein

A\_84\_P20247 0.03051006 0,0012132 3,01163 1,590545 AT3G21780 UGT71B6 UGT71B6\_UDP-glucosyl transferase 71B6 A 84 P19074 0,04633552 0.0032945 3,008857 1,589215 AT1G18570 AtMYB51, BW51A, AtMYB51 BW51A BW51B HIG1 MYB51 myb domain protein 51 A\_84\_P22489 0,0111888 6,12E-05 3,000947 1,585418 AT5G22250 AtCAF1b, CAF1b AtCAF1b\_CAF1b\_Polynucleotidyl transferase, ribonuclease H-like superfamily protein A\_84\_P12120 PMAT1\_\_HXXXD-type acyl-transferase family protein 0,02400674 6,37E-04 2,999374 1,584662 AT5G39050 PMAT1 0,04527197 0.0031214 2.997715 1.583863 AT5G22660 FBD, F-box, Skp2-like and Leucine Rich Repeat domains containing protein A 84 P189894 A\_84\_P21591 0,02924256 0,0010816 2,992981 1,581583 AT5G43170 AZF3, ZF3 AZF3\_ZF3\_\_zinc-finger protein 3AZF3\_ZF3\_\_zinc-finger protein 3 Uncharacterised protein family (UPF0497)Uncharacterised protein family (UPF0497) A\_84\_P817440 0,02454009 7,18E-04 2,989123 1,579722 AT4G15610 A\_84\_P21537 0,00682995 9,40E-06 2,987816 1,579091 AT5G17850 Sodium/calcium exchanger family protein A 84 P16943 0.02368718 6,04E-04 2,984989 1,577725 AT1G52410 TSA1 AtTSA1 TSA1 TSK-associating protein 1 A\_84\_P204138 0,02991192 0,001155 2,983229 1,576875 AT1G66080 A 84 P828333 0,01627859 2,08E-04 2,981689 UGT71C2 UDP-glucosyl transferase 71C2 1,57613 AT2G29740 UGT71C2 A\_84\_P594466 0,04114922 0.0025111 2,978932 1,574795 AT2G33130 RALF18, RALFL18 RALF18 RALFL18 ralf-like 18 A\_84\_P13228 0.01686909 2,29E-04 2,978648 1,574657 AT4G34131 UGT73B3 UGT73B3 UDP-glucosvl transferase 73B3 A\_84\_P809682 0,03784298 0,0020342 2,974286 1,572543 AT5G54940 Translation initiation factor SUI1 family proteinTranslation initiation factor SUI1 family protein A 84 P853160 0,02963863 0.0011257 2.974284 1.572542 AT3G11820 ATSYP121, ATSYR AT-SYR1 ATSYP121 ATSYR1 PEN1 SYP121 SYR1 syntaxin of plants 121 A\_84\_P19057 0,02060719 4,29E-04 2,971792 1,571333 AT1G67970 AT-HSFA8, HSFA8 AT-HSFA8\_HSFA8\_heat shock transcription factor A8 A 84 P826434 0,04737008 0,0034909 2,971617 1,571248 AT5G43170 AZF3, ZF3 AZF3 ZF3 zinc-finger protein 3 A\_84\_P89269 0.04017467 0,002366 2,971615 1,571247 AT1G06225 CLE3 CLE3 CLAVATA3/ESR-RELATED 3 A\_84\_P14672 3.55E-04 0.01948675 2,97068 1,570793 AT1G23850 A\_84\_P23654 0,0143883 1,43E-04 2,966837 1,568926 AT1G52000 Mannose-binding lectin superfamily protein A 84 P500176 0.00894727 2,84E-05 2,95021 1,560818 AT1G61065 Protein of unknown function (DUF1218) A\_84\_P17279 0,02704264 8,93E-04 2,947885 1,55968 AT2G25090 CIPK16, SnRK3.18 AtCIPK16\_CIPK16\_SnRK3.18 \_\_CBL-interacting protein kinase 16 A 84 P550101 0,02084717 4,47E-04 2,941421 1,556513 AT3G14660 CYP72A13 CYP72A13 cytochrome P450, family 72, subfamily A, polypeptide 13 A 84 P13560 0.02820842 9,75E-04 2,941267 1,556438 AT2G32150 Haloacid dehalogenase-like hydrolase (HAD) superfamily protein A\_84\_P137739 0,01305407 1,04E-04 2,938295 1,554979 AT3G63060 EDL3 EDL3\_EID1-like 3 A\_84\_P824985 0,04465805 0,0030205 2,93521 1,553464 AT2G18670; 0 A 84 P16120 0.01686909 2,31E-04 2,928472 1,550148 AT1G10140 Uncharacterised conserved protein UCP031279 A\_84\_P23396 0,03782426 0,0020323 2,918577 1,545265 AT5G06320 NHL3 NHL3 NDR1/HIN1-like 3NHL3 NDR1/HIN1-like 3 A\_84\_P11606 0,01474425 1,57E-04 2,913899 1,542951 AT2G29480 ATGSTU2, GST20, ATGSTU2\_GST20\_GSTU2\_glutathione S-transferase tau 2 A 84 P572262 0.0467632 0,0034082 2,912071 1,542046 AT3G10815 RING/U-box superfamily protein A\_84\_P829722 0,04856481 0,0037753 2,903335 1,537711 AT4G23190 AT-RLK3, CRK11 AT-RLK3\_CRK11\_cysteine-rich RLK (RECEPTOR-like protein kinase) 11 A\_84\_P130216 0,03518269 0,0017123 2,899507 1,535808 AT2G37750 A 84 P84909 0.03558141 0.0017731 2.899047 1.535579 AT3G17110 0,0013912 2,898405 1,535259 AT4G18197 ATPUP7, PEX17, P ATPUP7\_PEX17\_PUP7\_purine permease 7 A\_84\_P764548 0,03220695 A\_84\_P182564 0,01382326 1,28E-04 2,895632 1,533878 AT4G21323 Subtilase family protein A 84 P81409 0.02069154 4,37E-04 2,894878 1,533503 AT3G15630 A\_84\_P803216 0,02609744 NHL3\_\_NDR1/HIN1-like 3NHL3\_\_NDR1/HIN1-like 3 8,26E-04 2,893577 1,532854 AT5G06320 NHL3 2.88175 1.526945 AT2G40140 ATSZF2, CZF1, SZ ATSZF2, CZF1, SZF2, ZFAR1 zinc finger (CCCH-type) family protein A 84 P857301 0.03330867 0.0014993 A\_84\_P199584 0,01539895 1,77E-04 2,861063 1,516551 AT3G14200 Chaperone DnaJ-domain superfamily protein A 84 P14864 0.03782426 0,0020321 2,859129 1,515575 AT4G37260 ATMYB73, MYB73 ATMYB73 MYB73 myb domain protein 73 A\_84\_P12030 0,04036312 0,0024062 2,858766 1,515392 AT5G01100 FRB1\_O-fucosyltransferase family protein A 84 P772475 0,00574999 5,73E-06 2,853494 1,51273 AT1G66760 MATE efflux family proteinMATE efflux family protein A\_84\_P552447 0,02376995 6,12E-04 2,852966 1,512463 AT1G44414 A\_84\_P19431 0.0246688 7.27E-04 2.852798 1.512378 AT3G62150 ABCB21. PGP21 ABCB21\_PGP21\_P-glycoprotein 21 A\_84\_P18081 0,04809133 0,0036197 2,851654 1,511799 AT1G76410 ATL8 ATL8\_\_RING/U-box superfamily protein 0.02023179 4,06E-04 2,85125 1,511595 AT5G61590 Integrase-type DNA-binding superfamily protein A 84 P21661 A\_84\_P185784 0,04759051 0,0035302 2,850656 1,511294 AT2G39920 HAD superfamily, subfamily IIIB acid phosphatase A 84 P167173 0.04887474 2.85026 1,511094 AT4G25480 ATCBF3, CBF3, DR ATCBF3 CBF3 DREB1A dehydration response element B1A 0.0038209 A 84 P559517 0,01163687 6.99E-05 2,846862 1,509373 AT1G28330 DRM1, DYL1 AtDRM1\_DRM1\_DYL1\_\_dormancy-associated protein-like 1 1,50782 AT2G40140 ATSZF2, CZF1, SZ ATSZF2\_CZF1\_SZF2\_ZFAR1\_\_zinc finger (CCCH-type) family protein A\_84\_P17256 0,0409498 0,0024853 2,8438 A 84 P197444 0.03173508 0.0013273 2.843176 1.507504 AT3G22160 JAV1\_VQ motif-containing protein

A 84 P10799 0.04473491 0.0030371 2.841544 1.506675 AT3G29190 Terpenoid cyclases/Protein prenyltransferases superfamily protein A 84 P116712 0,01830757 3,08E-04 2,840573 1,506182 AT1G29180 Cysteine/Histidine-rich C1 domain family protein A 84 P245685 0,0293697 0,0010918 2,837193 1,504464 AT3G44300 AtNIT2, NIT2 AtNIT2 NIT2 nitrilase 2 A\_84\_P16624 GRAS family transcription factor 0,03032501 0,0011939 2,832481 1,502066 AT4G08250 A 84 P545146 0,01255292 9,74E-05 2,825738 1,498628 AT5G40180 A\_84\_P55660 0,03220695 0,0013931 2,822829 1,497142 AT3G56880 VQ motif-containing proteinVQ motif-containing protein Fes1A\_Fes1AFes1A\_Fes1A A\_84\_P271340 0,04464122 0,0030034 2,811616 1,4914 AT3G09350 Fes1A A\_84\_P821119 0,04927701 0,0039103 2,809513 1,49032 AT3G09350 Fes1A\_Fes1AFes1A\_Fes1A Fes1A A 84 P819884 0.03864233 2,801693 1,486299 AT4G38550 Arabidopsis phospholipase-like protein (PEARLI 4) family 0.0021264 A\_84\_P609600 1,43E-04 2,799154 1,484991 AT2G40200 basic helix-loop-helix (bHLH) DNA-binding superfamily protein 0,0143883 A 84 P10284 0,02430013 6,76E-04 2,798562 1,484686 AT5G54170 Polyketide cyclase/dehydrase and lipid transport superfamily protein A\_84\_P123322 0,01253431 9,70E-05 2,796931 1,483845 AT1G58200 MSL3 MSL3 MSCS-like 3 0,0011431 2,796564 1,483655 AT5G22570 ATWRKY38, WRKY ATWRKY38\_WRKY38\_WRKY DNA-binding protein 38 A\_84\_P845967 0.02987884 A\_84\_P71564 0.02894719 0,0010504 2,790486 1,480516 AT2G19800 MIOX2 MIOX2\_myo-inositol oxygenase 2 A 84 P15041 0.02248672 5,32E-04 2,787846 1,479151 AT5G61890 Integrase-type DNA-binding superfamily protein A\_84\_P16057 0,04195379 0,0026195 2,786024 1,478208 AT1G45145 ATH5, ATTRX5, LIV ATH5\_ATTRX5\_LIV1\_TRX-h5\_TRX5\_thioredoxin H-type 5 A 84 P809927 0.0209969 4,51E-04 2,784638 1,47749 AT5G07440 GDH2 GDH2 glutamate dehydrogenase 2 A 84 P21022 0.03202478 0.0013684 2.782358 1.476308 AT2G15480 UGT73B5 UGT73B5 UDP-glucosyl transferase 73B5 A 84 P859107 0.04899432 ERD7 Senescence/dehvdration-associated protein-related 0.0038609 2.777443 1.473757 AT2G17840 ERD7 A\_84\_P785967 0,02130474 4,68E-04 2,777187 1,473624 AT5G54940 Translation initiation factor SUI1 family protein A 84 P828523 0.00894727 2,60E-05 2,774368 1,472159 AT3G46110 Domain of unknown function (DUF966) A\_84\_P23419 0,01156977 6,66E-05 2,767869 1,468776 AT5G13200 GRAM domain family protein A 84 P17231 0.04661662 0,003371 2,76387 1,46669 AT2G29710 UDP-Glycosyltransferase superfamily protein G6PD3 glucose-6-phosphate dehydrogenase 3 A 84 P17042 0.04195379 0.0026186 2,757106 1,463155 AT1G24280 G6PD3 A\_84\_P169313 0,01710836 2,38E-04 2,750229 1,459552 AT2G36590 ATPROT3, ProT3 ATPROT3\_ProT3\_proline transporter 3 A\_84\_P12066 0,03211456 0,0013797 2,742419 1,455449 AT5G11410 Protein kinase superfamily protein A 84 P13072 0.01305407 1,04E-04 2,738441 1,453355 AT5G40850 UPM1 UPM1 urophorphyrin methylase 1 1,45E-04 2,734765 1,451417 AT3G14050 ATRSH2, AT-RSH2 AT-RSH2\_ATRSH2\_RSH2 RELA/SPOT homolog 2 A\_84\_P17400 0,01451385 A\_84\_P17802 0,01375994 1,27E-04 2,729744 1,448766 AT5G41610 ATCHX18, CHX18 ATCHX18\_CHX18\_cation/H+ exchanger 18 UGT1 UGT75B1 UDP-glucosyltransferase 75 A 84 P818633 0,04733288 0,0034826 2,723729 1,445583 AT1G05560 UGT1, UGT75B1 A\_84\_P19570 0,03580366 0,0017977 2,722464 1,444913 AT4G38550 Arabidopsis phospholipase-like protein (PEARLI 4) family A 84 P19170 1,28E-04 2,718617 1,442873 AT2G29420 ATGSTU7, GST25, ATGSTU7\_GST25\_GSTU7\_glutathione S-transferase tau 7 0.0138317 A 84 P15485 0.02581204 8,05E-04 2,716761 1,441888 AT3G11820 ATSYP121, ATSYR AT-SYR1 ATSYP121 ATSYR1 PEN1 SYP121 SYR1 syntaxin of plants 121 A\_84\_P211628 0,04417133 0,0029155 2,716733 1,441873 AT2G40080 ELF4 ELF4\_Protein of unknown function (DUF1313) A\_84\_P767650 0,01679116 2,23E-04 2,716406 1,441699 AT5G23510 A 84 P15467 0,03822042 0,0020775 2,715343 1,441135 AT3G01290 AtHIR2, HIR2 AtHIR2 HIR2 SPFH/Band 7/PHB domain-containing membrane-associated protein family A\_84\_P233859 0,03437764 0,0016186 2,715088 1,440999 AT2G28570 A 84 P119812 0.01372322 1.19E-04 2.712464 1.439604 AT1G50740 Transmembrane proteins 14C A\_84\_P11193 0,02750804 9,25E-04 2,705641 1,43597 AT5G44210 ATERF9, ATERF-9 ATERF-9\_ATERF9\_ERF9\_erf domain protein 9 A 84 P813469 0,01177365 7,89E-05 2,705083 1,435673 AT2G15890 MEE14 MEE14 maternal effect embryo arrest 14 A\_84\_P22839 0,03518269 0,0017111 2,694863 1,430212 AT1G73260 ATKTI1, KTI1 ATKTI1\_KTI1\_kunitz trypsin inhibitor 1 A 84 P14602 0.04572882 0,0032069 2,690116 1,427669 AT1G59590 ZCF37 ZCF37 ZCF37 A\_84\_P556737 0,00894727 2,83E-05 2,687887 1,426473 AT5G65207 A 84 P858400 0.02548116 7.89E-04 2.687429 1.426227 AT1G76790 IGMT5 IGMT5 O-methyltransferase family proteinIGMT5 O-methyltransferase family protein A\_84\_P758905 0,02894387 0,0010451 2,686678 1,425823 AT3G30415 A 84 P233559 0,02355661 5,95E-04 2,685708 1,425303 AT1G10340 Ankyrin repeat family protein A\_84\_P601310 0,02342488 5,84E-04 2,68549 1,425185 AT1G11070 A 84 P16332 0.03187662 0,0013499 2,683619 1,42418 AT2G29450 AT103-1A, ATGST AT103-1A ATGSTU1 ATGSTU5 GSTU5 glutathione S-transferase tau 5 A 84 P813465 0,01375994 1,26E-04 2,682411 1,423531 AT2G15890 MEE14 MEE14 maternal effect embryo arrest 14 A\_84\_P786976 0,04030779 0,0023982 2,677043 1,42064 AT2G37750 A 84 P858464 0.00903297 3.64E-05 2.669566 1.416605 AT3G10020

A 04 D47000	0 000 44 50	0 0040050	0 050570			
A_84_P17963	0,0364153			1,411194 AT1G13300	HRS1	HRS1myb-like transcription factor family protein
A_84_P823802	0,03001025		-	1,411065 AT3G09830		Protein kinase superfamily protein
A_84_P19214	0,02484235	,	,	1,409522 AT2G32510		MAPKKK17mitogen-activated protein kinase kinase kinase 17
A_84_P19413	0,02020679		2,650528		ATCPK32, CDPK32	2 ATCPK32_CDPK32_CPK32_calcium-dependent protein kinase 32
A_84_P21381	0,01592639		2,647591	1,40468 AT4G18950	000	Integrin-linked protein kinase familyIntegrin-linked protein kinase family
A_84_P16702	0,01386093			1,403317 AT4G30530	GGP1	GGP1_Class I glutamine amidotransferase-like superfamily protein
A_84_P761881	0,04831925			1,400905 AT3G04640		glycine-rich protein
A_84_P13338	0,01189048			1,398532 AT1G18710	AtMYB47, MYB47	AtMYB47_MYB47myb domain protein 47
A_84_P786702	0,01782063			1,398088 AT3G09830		Protein kinase superfamily protein
A_84_P12088	0,01961223			1,393287 AT5G22630	ADT5	ADT5_arogenate dehydratase 5
A_84_P21836	0,02732394			1,393035 AT1G72940		Toll-Interleukin-Resistance (TIR) domain-containing protein
A_84_P12888	0,01375994			1,393002 AT4G23600		CORI3_JR2Tyrosine transaminase family protein
A_84_P17107	0,01433996			1,390866 AT1G73080	ATPEPR1, PEPR1	ATPEPR1_PEPR1_PEP1 receptor 1
A_84_P816048 0	,02364189	,	2,618699	1,38885 AT1G79245		
A_84_P598498 0		3,10E-05	2,617607	1,388248 AT5G41120		Esterase/lipase/thioesterase family protein
A_84_P289354 0	,01769782	2,65E-04	2,613421	1,38594 AT2G15890	MEE14	MEE14maternal effect embryo arrest 14
A_84_P816929	0,03049443	0,0012048	2,611863	1,385079 AT5G54170		Polyketide cyclase/dehydrase and lipid transport superfamily protein
A_84_P15250	0,02449762	6,97E-04	2,611232	1,38473 AT1G66760		MATE efflux family proteinMATE efflux family protein
A_84_P548152	0,04811797	0,0036328	2,607072	1,38243 AT2G05400		Ubiquitin-specific protease family C19-related protein
A_84_P16040	0,01742975	2,49E-04	2,606768	1,382262 AT2G17840	ERD7	ERD7Senescence/dehydration-associated protein-related
A_84_P12642	0,0254004	7,82E-04	2,604687	1,38111 AT3G10020		
A_84_P840707	0,0398743	0,0023188	2,602607	1,379957 AT1G07500		SMR5SMR5
A_84_P11917	0,01961223	3,71E-04	2,599207	1,378072 AT4G13180		NAD(P)-binding Rossmann-fold superfamily protein
A_84_P20050	0,03076559	0,0012329	2,595113	1,375797 AT1G14370	APK2A, Kin1, PBL2	2 APK2A_Kin1_PBL2protein kinase 2A
A_84_P764555	0,02765526	9,40E-04	2,594751	1,375596 AT4G18195	ATPUP8, PUP8	ATPUP8_PUP8_purine permease 8
A_84_P20057	0,04437989			1,371507 AT1G63580		Receptor-like protein kinase-related family protein
A_84_P20399	0,00894727			1,370729 AT4G03400	DFL2. GH3-10	DFL2_GH3-10_Auxin-responsive GH3 family protein
A_84_P825287	0,01961223			1,370154 AT2G15480;		
A_84_P17278	0,04040338					ATGSTU6_GST24_GSTU6glutathione S-transferase tau 6
A 84 P13740	0,04260025			1,368324 AT3G57740	,	Protein kinase superfamily protein
A_84_P509290 0	,			1,368141 AT3G11385		Cysteine/Histidine-rich C1 domain family protein
A_84_P149248	0,03764553			1,366087 AT1G21120	IGMT2	IGMT2O-methyltransferase family protein
A_84_P22843	0,03916847			1,365538 AT1G76790		IGMT5_O-methyltransferase family proteinIGMT5
A 84 P816425 0	,			1,365308 AT3G30415;		
A_84_P790545 0	,			1,365157 AT1G49520		SWIB complex BAF60b domain-containing protein
A_84_P293774	0,01020095			1,362685 AT5G55120	VTC5	VTC5_galactose-1-phosphate guanylyltransferase (GDP)s;GDP-D-glucose phosphorylases
A_84_P20176	0,03731508			1,361881 AT2G44790		UCC2_uclacyanin 2
A_84_P12990	0,02312453					AtREC8_DIF1_REC8_SYN1Rad21/Rec8-like family protein
A 84 P11918	0,0147321					ATCSLA15_CSLA15Nucleotide-diphospho-sugar transferases superfamily protein
A_84_P556854	0,01163687			1,359944 AT1G25275		
A_84_P18342	0,02400674					D A37_ATPDX1.2_PDX1.2_pyridoxine biosynthesis 1.2
A_84_P11961	0,04624212			1,358398 AT4G27410		ANAC072_RD26NAC (No Apical Meristem) domain transcriptional regulator superfamily protein
A_84_P759616 0	,			1,357723 AT3G30405	711070072, 11020	transposable element gene
A_84_P715787 0		3,33E-04		1,357625 AT1G32928		analopoouble element gene
A 84 P61100	0,0161733		2,558041	1,35504 AT5G12010		
A_84_P206968 0	,	,	,	,		ATPUP18_PUP18purine permease 18
A_84_P824268 0				1,353535 AT1G56660	ATF UF 10, FUF 10	ATT OF TO_F OF TO_PUTITIE PETITIEASE TO
A_84_P544677 0				1,348222 AT2G30400		ATOFP2 OFP2 ovate family protein 2
A_84_P803184 0		5,62E-04 4,02E-04		1,346111 AT3G10020	ATOFEZ, UFFZ	A + O + 2 - O + 2 - O + a + a + a + a + a + a + a + a + a +
					DDND6	RRN26
A_84_P770411	0,0286969		-	1,34474 ATMG00020		
A_84_P819429	0,01163687	7,40E-05	2,539609	1,344000 ATTG59640	оге, вгер, вгеи	BPE_BPEp_BPEub_ZCW32BIG PETAL

A\_84\_P14539 0.01020095 4.94E-05 2.538698 1.344088 AT1G53440 Leucine-rich repeat transmembrane protein kinase A 84 P21943 0.04769728 0.0035538 2,53539 1,342208 AT1G32170 XTH30, XTR4 XTH30 XTR4 xyloglucan endotransglucosylase/hydrolase 30 A\_84\_P85009 0,03380537 0,0015721 2,532451 1,340534 AT3G44190 FAD/NAD(P)-binding oxidoreductase family protein A\_84\_P227239 0,03184637 0,0013409 2,531027 1,339723 AT5G13210 Uncharacterised conserved protein UCP015417 0,0012091 2,522825 CRK10 RLK4 cysteine-rich RLK (RECEPTOR-like protein kinase) 10 A 84 P14776 0,03051006 1,33504 AT4G23180 CRK10, RLK4 A\_84\_P22452 0,00857212 1,81E-05 2,515416 1,330797 AT5G06860 ATPGIP1, PGIP1 ATPGIP1\_PGIP1\_polygalacturonase inhibiting protein 1 AT-RLK3\_CRK11\_\_cysteine-rich RLK (RECEPTOR-like protein kinase) 11 A\_84\_P528136 0,02353903 5,93E-04 2,509955 1,327661 AT4G23190 AT-RLK3, CRK11 A\_84\_P790566 0,02524157 7,70E-04 2,507474 1,326235 AT1G69900 Actin cross-linking proteinActin cross-linking protein A 84 P18180 0.03837948 0,002103 2,505292 1,324979 AT2G31880 EVR, SOBIR1 EVR SOBIR1 Leucine-rich repeat protein kinase family protein A\_84\_P14392 0,02612251 8,31E-04 2,503665 1,324041 AT2G43550 Scorpion toxin-like knottin superfamily protein A 84 P575955 0,04552429 0,003164 2,501719 1,32292 AT3G62990 A 84 P12402 0.01719517 2,42E-04 2,501221 1,322633 AT1G69370 CM3, cm-3 CM3 cm-3 chorismate mutase 3 A\_84\_P197554 0,02732394 9,10E-04 2,494302 1,318636 AT2G28650 ATEXO70H8, EXO ATEXO70H8\_EXO70H8\_exocyst subunit exo70 family protein H8 A\_84\_P513046 0.03454188 0.0016501 2,491851 1,317218 AT4G25110 AtMC2. AtMCP1c. AtMC2\_AtMCP1c\_MC2\_MCP1c\_metacaspase 2 A 84 P10026 0.04830764 0.0036958 2.485292 1.313415 AT4G13110 BSD domain-containing proteinBSD domain-containing protein A\_84\_P581339 0,01828971 3,00E-04 2,48407 1,312706 AT4G12005 A 84 P12218 0.02242965 5,28E-04 2,477961 1,309154 AT5G64870 SPFH/Band 7/PHB domain-containing membrane-associated protein family A 84 P824914 0.04972693 0.003995 2,477426 1,308842 AT4G18197; ATPUP7, PEX17, PUP7 A 84 P532052 0.03238549 0.0014086 2.472546 1.305997 AT1G30757 A\_84\_P21550 0,01185074 8,29E-05 2,470846 1,305005 AT5G24270 ATSOS3, CBL4, SO ATSOS3\_CBL4\_SOS3\_Calcium-binding EF-hand family protein A 84 P16138 0,029571 0.0011218 2,470398 1,304744 AT1G30700 FAD-binding Berberine family protein A\_84\_P141219 0,01774115 2,76E-04 2,469971 1,304494 AT5G35320 A 84 P579587 0,01375994 1,21E-04 2,468474 1,303619 AT4G20830 FAD-binding Berberine family proteinFAD-binding Berberine family protein A 84 P10613 0.04831925 0.0037168 2,463903 1,300945 AT2G42530 COR15B COR15B cold regulated 15b A\_84\_P844671 0,02684096 8,78E-04 2,460304 1,298837 AT4G38470 STY46 STY46\_ACT-like protein tyrosine kinase family protein A\_84\_P13549 0,00764839 1,12E-05 2,458024 1,297499 AT2G39420 alpha/beta-Hydrolases superfamily protein A 84 P848384 0,00877275 2,32E-05 2,457736 1,29733 AT5G23510 A\_84\_P23347 0,01861906 3,20E-04 2,453821 1,29503 AT4G36010 Pathogenesis-related thaumatin superfamily protein A\_84\_P168223 0,02894387 0,001045 2,449246 1,292337 AT1G19770 ATPUP14, PUP14 ATPUP14\_PUP14\_\_purine permease 14 A 84 P12053 0.03086358 0,0012502 2,448501 1,291899 AT5G07440 GDH2 GDH2 glutamate dehydrogenase 2 A\_84\_P548542 0,02400674 6,41E-04 2,447158 1,291107 AT4G30090 A 84 P19196 0.02924256 0,001077 2,445617 1,290198 AT1G75490 Integrase-type DNA-binding superfamily protein A 84 P786801 0,03006957 0,0011748 2,444663 1,289636 AT5G13210 Uncharacterised conserved protein UCP015417 A\_84\_P82539 AtPCR2 PCR2 PLANT CADMIUM RESISTANCE 2 0,03681569 0,0019186 2,444237 1,289384 AT1G14870 AtPCR2, PCR2 A\_84\_P20574 0,02500151 7,52E-04 2,442544 1,288384 AT5G10830 S-adenosyl-L-methionine-dependent methyltransferases superfamily protein A 84 P868891 0.0012108 2,442013 1.288071 AT1G52400 ATBG1, BGL1, BGL ATBG1 BGL1 BGLU18 beta glucosidase 18 0.03051006 A\_84\_P193364 0,03529676 0,0017265 2,439675 1,286689 AT1G56660 A 84 P16319 0.02381684 6.25E-04 2.439413 1.286534 AT2G20350 Integrase-type DNA-binding superfamily protein A\_84\_P10142 0,03308225 0,001484 2,43681 1,284994 AT5G01760 ENTH/VHS/GAT family protein A 84 P174131 0.02725007 9,04E-04 2,435835 1,284416 AT5G14730 A\_84\_P114372 0,01613558 2,02E-04 2,433768 1,283192 AT5G53050 alpha/beta-Hydrolases superfamily protein A 84 P13322 0.01564355 1,85E-04 2,4328 1,282618 AT1G21680 DPP6 N-terminal domain-like protein systemic acquired resistance (SAR) regulator protein NIMIN-1-related A\_84\_P175914 0,04669211 0,0033885 2,423554 1,277124 AT4G01895 A 84 P816258 0.03204315 0.0013707 2.414566 1.271764 AT2G36220 A\_84\_P820724 0,02045072 4,15E-04 2,414354 1,271637 AT4G23600 CORI3, JR2 CORI3\_JR2\_\_Tyrosine transaminase family protein A 84 P11544 0.01911949 3,44E-04 2,403924 1,265391 AT1G33590 Leucine-rich repeat (LRR) family protein A\_84\_P811890 0,04760732 0,0035348 2,402274 1,264401 AT4G08950 EXO EXO Phosphate-responsive 1 family protein A 84 P814710 0,0019756 2,400459 1,26331 AT1G14870 AtPCR2, PCR2 AtPCR2 PCR2 PLANT CADMIUM RESISTANCE 2 0.03731508 AtGH3.11\_FIN219\_JAR1\_\_Auxin-responsive GH3 family protein A 84 P18264 0.01785415 2,85E-04 2,392477 1,258505 AT2G46370 FIN219, JAR1 A\_84\_P20835 0,03450806 0,0016363 2,391751 1,258067 AT1G80820 ATCCR2, CCR2 ATCCR2\_CCR2\_cinnamoyl coa reductase A 84 P23343 0.02009357 4.00E-04 2.390708 1.257438 AT4G35110 Arabidopsis phospholipase-like protein (PEARLI 4) family

A 84 P75664	0,04418246	0 0020183	2 389869	1,256931 AT2G36220		
A_84_P16180	0,03206007	,	,	1,256636 AT1G11050		Protein kinase superfamily protein
A 84 P824135	0,01386738		-			ATPUP14_PUP14_purine permease 14
A_84_P10306	0,04096361	,	,	1,254717 AT5G59930	ATF 0F 14, F 0F 14	Cysteine/Histidine-rich C1 domain family protein
A_84_P96976	0,00904516				AT\N/L12 SAG20	ATWI-12_SAG20_WI12senescence associated gene 20
A_84_P805135				1,253961 AT3G15450	ATWI-12, 3AG20,	Aluminium induced protein with YGL and LRDR motifs
A_84_P823929					ATCOVE12 COVE	•
					AIGRASIS, GRAS	ATGRXS13_GRXS13_Glutaredoxin family protein
A_84_P843621	0,04831925		-	1,247097 AT2G31010		Protein kinase superfamily proteinProtein kinase superfamily protein
A_84_P21192	0,02998462	,	,	1,244903 AT3G19930	,	ATSTP4_STP4_sugar transporter 4
A_84_P19368	0,03452952	0,0016402			ASNI, AT-ASNI, D	ASN1_AT-ASN1_DIN6glutamine-dependent asparagine synthase 1
A_84_P18271	0,03910996			1,243445 AT1G07500		SMR5SMR5
A_84_P576707	0,03367786	0,0015574	,	,		alpha 1,4-glycosyltransferase family protein
A_84_P20555	0,01790467	-	-	1,236988 AT5G05400		LRR and NB-ARC domains-containing disease resistance protein
A_84_P10893	0,01226299		2,356899			ABCG19_ATWBC19_WBC19white-brown complex homolog 19
A_84_P20389	0,01622046	,	,	1,235999 AT4G01026	,	PYL7_RCAR2PYR1-like 7PYL7_RCAR2PYR1-like 7
A_84_P23258	0,04137047			1,235898 AT4G11370	RHA1A	RHA1ARING-H2 finger A1A
A_84_P11710	0,04975333			1,234645 AT1G53080		Legume lectin family protein
A_84_P786905	0,02948737			1,23364 AT5G41120		Esterase/lipase/thioesterase family protein
A_84_P11250	0,02991192	0,0011573	2,349519	1,232366 AT5G59570	BOA	BOAHomeodomain-like superfamily protein
A_84_P845323	0,01396545	1,36E-04	2,348723	1,231876 AT4G08170		Inositol 1,3,4-trisphosphate 5/6-kinase family protein
A_84_P120892	0,01961223	3,76E-04	2,343634	1,228747 AT4G08170		Inositol 1,3,4-trisphosphate 5/6-kinase family protein
A_84_P20495	0,01771716	2,70E-04	2,340631	1,226897 AT4G32800		Integrase-type DNA-binding superfamily protein
A_84_P19755	0,04989897	0,0040283	2,335947	1,224008 AT5G58750		NAD(P)-binding Rossmann-fold superfamily protein
A_84_P103496	0,02364212	6,02E-04	2,332659	1,221975 AT5G05140		Transcription elongation factor (TFIIS) family protein
A_84_P18946	0,02403887	6,54E-04	2,332654	1,221973 AT1G09970	LRR XI-23, RLK7	LRR XI-23_RLK7_Leucine-rich receptor-like protein kinase family protein
A_84_P545592	0,04555509	0,0031725	2,331709	1,221388 AT2G39650		Protein of unknown function (DUF506)
A_84_P10597	0,01785415	2,82E-04	2,327028	1,218489 AT1G27770	ACA1, PEA1	ACA1_PEA1autoinhibited Ca2+-ATPase 1ACA1_PEA1autoinhibited Ca2+-ATPase 1
A_84_P830058	0,04899432	0.0038659	2,323218	1,216125 AT1G63860		Disease resistance protein (TIR-NBS-LRR class) family
A 84 P15109	0,01719517		-		ERD10, LTI29, LTI4	ERD10_LTI29_LTI45Dehydrin family protein
A_84_P569157	,			1,215229 AT5G67410	-, -,	
A_84_P857480					ERD10, LTI29, LTI4	ERD10_LTI29_LTI45Dehydrin family protein
A_84_P702605			-	1,212496 AT3G14362		DVL19_RTFL10_ROTUNDIFOLIA like 10
A_84_P752539	0,00877048			1,210278 AT1G08920		ESL1_ERD (early response to dehydration) six-like 1
A_84_P12321	0.04616675	0.0032749				ATSYP124_SYP124_syntaxin of plants 124
A_84_P167943	0,03103756	,				ATERF-4_ATERF4_ERF4_RAP2.5ethylene responsive element binding factor 4
A_84_P23850	0,04379391			1,205387 AT2G43520		ATTI2_TI2_trypsin inhibitor protein 2
A_84_P820727	0,03305386			1,205185 AT4G24160	ATTIZ, TIZ	alpha/beta-Hydrolases superfamily protein
A_84_P17618	0,02267799			1,204991 AT4G24160		alpha/beta-Hydrolases superfamily protein
A_84_P750801	0,04465805			1,203772 AT1G42490		
					OTV/40	CTV/4C ACT like protein turneine kingen femily protein
A_84_P79769	0,03172537	,	,	1,203758 AT4G38470	51140	STY46ACT-like protein tyrosine kinase family protein
A_84_P565337	0,0286969	0,0010109		1,203057 AT4G21865		ATROL ROLLING hats shows iters to
A_84_P21673	0,04475471					ATBG1_BGL1_BGLU18beta glucosidase 18
A_84_P21603	0,01176281			1,194228 AT5G46330		FLS2_Leucine-rich receptor-like protein kinase family protein
A_84_P181994						AtGDPD1_GDPD1_SRG3_senescence-related gene 3
A_84_P108412	0,01686909		-	1,191798 AT4G22690	CYP706A1	CYP706A1cytochrome P450, family 706, subfamily A, polypeptide 1
A_84_P20775	0,00464882			1,191684 AT5G58400		Peroxidase superfamily protein
A_84_P212918					EDS5, SCORD3, S	EDS5_SCORD3_SID1MATE efflux family protein
A_84_P277930	0,02184876		-	1,190896 AT2G02680		Cysteine/Histidine-rich C1 domain family protein
A_84_P16068	0,01785415			1,190496 AT1G33790		jacalin lectin family protein
A_84_P816095	0,01774115	2,74E-04	2,282243	1,190453 AT4G01026	PYL7, RCAR2	PYL7_RCAR2PYR1-like 7
A_84_P182114	0,02226756	5,14E-04	2,281288	1,189849 AT5G02950		Tudor/PWWP/MBT superfamily protein

A 84 P519221 0.01961223 3,78E-04 2,280608 1,189418 AT4G14860 At0FP11, OFP11 At0FP11\_OFP11\_ovate family protein 11 A 84 P869434 0.03184718 0,0013427 2,277785 1,187632 AT4G23600 CORI3, JR2 CORI3 JR2 Tyrosine transaminase family protein A 84 P189784 0,02069154 4,37E-04 2,276053 1,186534 AT3G18690 MKS1 MKS1 MAP kinase substrate 1 A\_84\_P18737 Methylenetetrahydrofolate reductase family protein 0,04234786 0,0026625 2,274057 1,185268 AT5G38710 0.0325659 0,001427 2,272636 1,184366 AT4G18950 Integrin-linked protein kinase familyIntegrin-linked protein kinase family A 84 P784441 A\_84\_P805118 0,029931 0,0011617 2,269276 1,182232 AT3G15450 Aluminium induced protein with YGL and LRDR motifs 6,36E-04 2,266853 1,180691 AT1G27130 ATGSTU13, GST12 ATGSTU13\_GST12\_GSTU13\_ glutathione S-transferase tau 13 A\_84\_P815327 0,02400674 A\_84\_P537920 0,01686909 2,30E-04 2,264265 1,179043 AT4G33985 Protein of unknown function (DUF1685) A 84 P21728 0,01462891 1,55E-04 2,263773 1,178729 AT1G20490 AMP-dependent synthetase and ligase family protein A\_84\_P814684 0,01769782 2,63E-04 2,263524 1,178571 AT1G21110; IGMT3,IGMT4 A 84 P12206 0,01830757 3,05E-04 2,26074 1,176795 AT5G61810 APC1 APC1 Mitochondrial substrate carrier family protein A 84 P810995 0,04831925 0,0037049 2,260141 1,176413 AT2G41430 CID1, ERD15, LSR CID1 ERD15 LSR1 dehydration-induced protein (ERD15) A\_84\_P15182 0,03364271 0,0015513 2,258459 1,175339 AT1G60260 BGLU5 BGLU5 beta glucosidase 5 A\_84\_P852295 0.03624299 0.001841 2,255902 1,173704 AT3G47340 ASN1, AT-ASN1, D ASN1\_AT-ASN1\_DIN6\_\_glutamine-dependent asparagine synthase 1 AtRDUF2 RDUF2 zinc finger (C3HC4-type RING finger) family protein A 84 P22597 0.03884352 0.0021489 2.255885 1.173694 AT5G59550 A\_84\_P17674 0,02493108 7,45E-04 2,253785 1,17235 AT1G27130 ATGSTU13, GST12 ATGSTU13\_GST12\_GSTU13\_glutathione S-transferase tau 13 A 84 P17031 0.00894727 3,11E-05 2,253709 1,172301 AT1G03850 ATGRXS13, GRXS ATGRXS13\_GRXS13\_Glutaredoxin family protein A\_84\_P259140 0,01798792 2,92E-04 2,249105 1,169351 AT1G28050 BBX13 B-box type zinc finger protein with CCT domain ATRBOHD, RBOH ATRBOHD RBOHD respiratory burst oxidase homologue D A 84 P12153 0.01386093 1,30E-04 2,247369 1,168237 AT5G47910 A\_84\_P248365 0,04831925 0,0037045 2,246696 1,167805 AT4G37240 A 84 P805073 0.02695982 8,87E-04 2,239347 1,163078 AT3G15450 Aluminium induced protein with YGL and LRDR motifs A\_84\_P846967 0,02075475 4,41E-04 2,237025 1,161581 AT5G53050 alpha/beta-Hydrolases superfamily proteinalpha/beta-Hydrolases superfamily protein A 84 P15156 0.04008859 0.0023517 2.236603 1.161309 AT1G61470 Polynucleotidyl transferase, ribonuclease H-like superfamily protein A 84 P22445 0.02577352 8,02E-04 2,235833 1,160812 AT5G04720 ADR1-L2, PHX21 ADR1-L2 PHX21 ADR1-like 2 A\_84\_P859574 0,03053011 0,0012168 2,235738 1,160751 AT5G04720 ADR1-L2, PHX21 ADR1-L2\_PHX21\_\_ADR1-like 2 A\_84\_P755699 0,03359263 0,0015343 2,233412 1,15925 AT2G34580 A 84 P144119 0.03926466 0.002229 2,23331 1,159184 AT4G17250 A 84 P849068 0.04469125 0,0030285 2,233185 1,159102 AT3G06760 Drought-responsive family proteinDrought-responsive family protein A\_84\_P510552 0,03916847 0,0022138 2,229786 1,156905 AT5G41100 A 84 P17955 0.03025034 0,0011882 2,229298 1,15659 AT1G22380 AtUGT85A3, UGT8 AtUGT85A3 UGT85A3 UDP-glucosyl transferase 85A3 A\_84\_P21886 0,00857212 1,87E-05 2,229261 1,156566 AT1G55920 ATSERAT2;1, SAT ATSERAT2;1\_SAT1\_SAT5\_SERAT2;1\_serine acetyltransferase 2;1 A\_84\_P15536 0.01871661 3,22E-04 2,228236 1,155902 AT3G16470 JAL35, JR1 JAL35\_JR1\_Mannose-binding lectin superfamily protein A 84 P12293 0.00882356 2,35E-05 2,219093 1,14997 AT1G11330 S-locus lectin protein kinase family protein A\_84\_P197484 PYL4 RCAR10 PYR1-like 4 0,02951533 0,0011166 2,217134 1,148696 AT2G38310 PYL4, RCAR10 A\_84\_P15219 0,04942583 0,0039277 2,212806 1,145877 AT1G02860 BAH1. NLA BAH1\_NLA\_\_SPX (SYG1/Pho81/XPR1) domain-containing protein A 84 P22019 0.03899062 0,0021661 2,211227 1,144847 AT2G44390 Cysteine/Histidine-rich C1 domain family protein A\_84\_P273770 0,01163687 6,94E-05 2,210556 1,144409 AT1G28380 NSL1 NSL1\_MAC/Perforin domain-containing protein A 84 P844839 0.04086231 0.0024725 2.209603 1.143787 AT4G39030 EDS5. SCORD3. S EDS5 SCORD3 SID1 MATE efflux family protein A\_84\_P11755 0,02430013 6,76E-04 2,208952 1,143362 AT3G14990 AtDJ1A, DJ1A AtDJ1A\_DJ-1a\_DJ1A\_\_Class I glutamine amidotransferase-like superfamily protein A 84 P589129 0.03133682 0,001296 2,207034 1,142109 AT2G40095 Alpha/beta hydrolase related protein A\_84\_P131945 0,00894727 3,36E-05 2,206865 1,141998 AT5G53060 RCF3\_SHI1\_\_RNA-binding KH domain-containing protein A 84 P14143 0,02359227 5,98E-04 2,203991 1,140119 AT5G13550 SULTR4;1 SULTR4;1 sulfate transporter 4.1 A\_84\_P89489 0,02429591 6,74E-04 2,203869 1,140039 AT3G16330 A\_84\_P565178 0.01830757 3.04E-04 2.197584 1.135919 AT3G25882 NIMIN-2 NIMIN-2 NIM1-interacting 2 A\_84\_P21875 0.03308225 0,0014772 2,196975 1,135518 AT1G63180 UGE3 UGE3\_UDP-D-glucose/UDP-D-galactose 4-epimerase 3 A 84 P272750 0.01386093 1,30E-04 2,196947 1,1355 AT2G22300 CAMTA3, SR1 CAMTA3 SR1 signal responsive 1CAMTA3 SR1 signal responsive 1 A\_84\_P254190 0,00971693 4,37E-05 2,196283 1,135064 AT1G75230 DNA glycosylase superfamily protein A 84 P14972 0.00178308 2,87E-07 2,191738 1,132075 AT5G44070 ARA8, ATPCS1, CA ARA8 ATPCS1 CAD1 PCS1 phytochelatin synthase 1 (PCS1) A 84 P10688 0.04362199 0.0028285 2,191377 1,131838 AT1G30070 SGS domain-containing protein A\_84\_P24037 1,51E-04 2,188676 1,130058 AT3G18830 ATPLT5, ATPMT5, ATPMT5\_PMT5\_polyol/monosaccharide transporter 5 0,0145606 A 84 P109922 0.01634308 2.13E-04 2.188611 1.130015 AT4G17900 PLATZ transcription factor family protein

A\_84\_P13942 0.02449762 6,93E-04 2,187364 1,129193 AT1G06520 ATGPAT1, GPAT1 ATGPAT1\_GPAT1\_sn-2-GPAT1\_glycerol-3-phosphate acyltransferase 1 A 84 P15324 0,01560944 1,81E-04 2,18504 1,12766 AT1G33560 ADR1 ADR1 Disease resistance protein (CC-NBS-LRR class) family A\_84\_P78279 0,0320088 0,0013662 2,184396 1,127235 AT1G67920 A\_84\_P813323 0,03913938 0,0021956 2,17921 1,123805 AT1G70780; CPuORF28 A 84 P785683 0,03959857 0,0022951 2,177729 1,122825 AT1G27770 ACA1, PEA1 ACA1 PEA1 autoinhibited Ca2+-ATPase 1 A\_84\_P532349 0,00466105 3,16E-06 2,177452 1,122641 AT3G09520 ATEXO70H4, EXO ATEXO70H4\_EXO70H4\_exocyst subunit exo70 family protein H4 A\_84\_P16128 BGLU6\_\_beta glucosidase 6BGLU6\_\_beta glucosidase 6 0,01020095 4,85E-05 2,175282 1,121203 AT1G60270 BGLU6 A\_84\_P97266 0,03800573 0,0020552 2,175144 1,121111 AT4G18510 CLE2 CLE2 CLAVATA3/ESR-related 2 A 84 P21159 0.03636191 0.0018572 2.173139 1.119781 AT3G04010 O-Glycosyl hydrolases family 17 protein A\_84\_P249125 0,02095783 4,50E-04 2,171825 1,118908 AT1G68500 A 84 P21889 0.02038725 4,11E-04 2,171567 1,118736 AT1G74360 Leucine-rich repeat protein kinase family protein A 84 P22932 0,01771716 2,68E-04 2,169214 1,117172 AT2G22770 NAI1 NAI1 basic helix-loop-helix (bHLH) DNA-binding superfamily protein FAD/NAD(P)-binding oxidoreductase family protein A\_84\_P821329 0,04348974 0,0028056 2,167369 1,115945 AT3G44190 A\_84\_P190534 0,04806924 0,0036159 2,167053 1,115734 AT1G68450 PDE337 PDE337\_\_VQ motif-containing protein AKN2 APK2 APS-kinase 2 A 84 P19576 0.02237896 5,24E-04 2,164362 1,113942 AT4G39940 AKN2, APK2 A\_84\_P836958 0,03225273 0,0013985 2,162703 1,112835 AT1G61470 Polynucleotidyl transferase, ribonuclease H-like superfamily protein A 84 P13248 0.01830757 3,05E-04 2,161595 1,112096 AT1G03800 ATERF10, ERF10 ATERF10\_ERF10\_ERF domain protein 10 A\_84\_P750244 0,04598934 0,0032541 2,161464 1,112009 AT1G69140 A\_84\_P20445 5,40E-05 2,160613 1,111441 AT4G21410 CRK29 0.01065349 CRK29 cvsteine-rich RLK (RECEPTOR-like protein kinase) 29 A\_84\_P212328 0,0184221 3,11E-04 2,159282 1,110551 AT3G50010 Cysteine/Histidine-rich C1 domain family protein A 84 P859177 0.03051006 0,0012111 2,158004 1,109697 AT4G22780 ACR7 ACR7 ACT domain repeat 7ACR7 ACT domain repeat 7 A\_84\_P22084 0,04465805 0,0030177 2,157833 1,109583 AT3G11580 AP2/B3-like transcriptional factor family protein A 84 P312543 0,02524157 7.67E-04 IQ calmodulin-binding motif family protein 2,15743 1,109314 AT3G52870 A 84 P89769 0.02612251 8,35E-04 2,151389 1,105269 AT2G35930 PUB23 AtPUB23 PUB23 plant U-box 23 A\_84\_P505223 0,01686909 2,30E-04 2,15087 1,10492 AT5G35732 A\_84\_P22039 0,01156977 6,70E-05 2,149966 1,104314 AT2G31010 Protein kinase superfamily protein A 84 P23152 0,03178847 0,0013312 2,14981 1,104209 AT3G47380 Plant invertase/pectin methylesterase inhibitor superfamily protein A\_84\_P596065 0,00894727 2,47E-05 2,149805 1,104206 AT1G49520 SWIB complex BAF60b domain-containing protein A\_84\_P816283 0,03818448 0,0020738 2,148263 1,10317 AT1G53280 AtDJ1B, DJ1B AtDJ1B\_DJ-1b\_DJ1B\_\_Class I glutamine amidotransferase-like superfamily protein A 84 P230289 0.03518269 0,0017091 2,145975 1,101633 AT2G44080 ARL ARL ARGOS-like A\_84\_P119322 0,02430013 6,79E-04 2,142498 1,099294 AT3G14060 A\_84\_P24136 0.02114772 4,60E-04 2,139821 1,09749 AT3G55940 Phosphoinositide-specific phospholipase C family protein A 84 P808847 0,01504028 1,64E-04 2,139649 1,097374 AT2G23320 WRKY15 AtWRKY15 WRKY15 WRKY DNA-binding protein 15 A\_84\_P13943 1,74E-04 2,138164 1,096373 AT5G07460 ATMSRA2, PMSR2 ATMSRA2\_PMSR2 \_\_peptidemethionine sulfoxide reductase 2 0,01526442 A\_84\_P597426 0,02894719 0,0010505 2,137677 1,096044 AT4G21320 HSA32 HSA32\_\_Aldolase-type TIM barrel family protein A 84 P23998 0.01320943 1,08E-04 2,136488 1,095242 AT1G53430 Leucine-rich repeat transmembrane protein kinase A\_84\_P14116 3,20E-04 2,134052 1,093596 AT5G66790 0,01861906 Protein kinase superfamily protein A\_84\_P192974 0,01066107 5.47E-05 2.133877 1.093477 AT5G65660 hydroxyproline-rich glycoprotein family protein A\_84\_P203128 0,02300682 5,62E-04 2,132088 1,092267 AT3G29575 AFP3 AFP3\_\_ABI five binding protein 3 0.03399409 Major facilitator superfamily protein A\_84\_P23923 0,0015858 2,131501 1,091869 AT2G37900 A\_84\_P784302 0,03939561 0,0022518 2,129073 1,090225 AT1G01720 ANAC002, ATAF1 ANAC002\_ATAF1\_\_NAC (No Apical Meristem) domain transcriptional regulator superfamily protein A 84 P14449 0,0242297 6,71E-04 2,125369 1,087713 AT2G34810 FAD-binding Berberine family protein A\_84\_P824439 0,00894727 3,29E-05 2,125032 1,087484 AT2G22300 CAMTA3, SR1 CAMTA3\_SR1\_\_signal responsive 1 A\_84\_P571306 0.02991192 0.0011546 2.123456 1.086414 AT2G38500 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein A 84 P21994 0.01592639 1,95E-04 2,122978 1,086089 AT2G26440 PME12\_Plant invertase/pectin methylesterase inhibitor superfamily A\_84\_P286620 0,02497593 7,51E-04 2,120676 1,084524 AT4G33940 RING/U-box superfamily protein 0,0020177 2,118493 1,083038 AT5G44070 ARA8, ATPCS1, CA ARA8\_ATPCS1\_CAD1\_PCS1\_phytochelatin synthase 1 (PCS1) A\_84\_P816321 0,03768138 A 84 P796756 0,04234786 0,0026626 2,1174 1,082293 AT1G79245 A\_84\_P11807 0.04025845 0.0023943 2.116581 1.081736 AT3G47800 Galactose mutarotase-like superfamily protein A\_84\_P22614 NPX1\_\_nuclear protein X1 0,01958881 3,59E-04 2,110794 1,077786 AT5G63320 NPX1 A 84 P68194 0.04538051 0,003142 2,107592 1,075596 AT5G46710 PLATZ transcription factor family protein

A\_84\_P294564 0.02324895 5.75E-04 2.106822 1.075069 AT3G11420 Protein of unknown function (DUF604)Protein of unknown function (DUF604) A 84 P17200 0,04418246 0,0029286 2,105115 1,073899 AT1G16110 WAKL6 WAKL6 wall associated kinase-like 6 A\_84\_P588557 0,00894727 2,98E-05 2,104569 1,073525 AT4G36830 HOS3-1 HOS3-1 GNS1/SUR4 membrane protein family A\_84\_P828208 7,30E-04 2,104473 1,073459 AT5G53060 RCF3\_SHI1\_\_RNA-binding KH domain-containing protein 0,02469361 0.04811797 0.0036293 2.103174 1.072568 AT5G65500 U-box domain-containing protein kinase family protein A 84 P19785 A\_84\_P163223 0,03636191 0,0018604 2,102561 1,072147 AT3G13760 Cysteine/Histidine-rich C1 domain family protein A\_84\_P13481 AtWRKY15\_WRKY15\_WRKY DNA-binding protein 15 0,01320943 1,10E-04 2,102203 1,071902 AT2G23320 WRKY15 A\_84\_P832916 0,02430118 6,80E-04 2,098414 1,0693 AT2G44380 Cysteine/Histidine-rich C1 domain family protein A 84 P21166 3,06E-04 2,096378 1,067899 AT3G09830 0,01830757 Protein kinase superfamily protein A\_84\_P54290 0,03681569 0,0019221 2,095145 alpha/beta-Hydrolases superfamily protein 1,06705 AT3G19970 A 84 P608949 0,04788074 0,0035934 2,091447 1,064502 AT4G15450 Senescence/dehydration-associated protein-related A\_84\_P803506 0.00387638 1,09E-06 2,088249 1,062294 AT3G30775 ATPDH, ATPOX, A AT-POX ATPDH ERD5 PDH1 PRO1 PRO1 Methylenetetrahydrofolate reductase family protein A\_84\_P53650 0,03359263 0,001546 2,087328 1,061657 AT5G57910 A\_84\_P18467 0,03827128 0,0020864 2,086823 1,061308 AT3G57520 AtSIP2, RS2, SIP2 AtSIP2\_RS2\_SIP2\_seed imbibition 2 A 84 P88579 0.02449762 6,98E-04 2,085612 1,060471 AT1G15430 Protein of unknown function (DUF1644) A\_84\_P563858 0,0497107 0,0039875 2,084348 1,059596 AT4G38340 NLP3\_Plant regulator RWP-RK family protein A 84 P813213 0.02009357 3,96E-04 2,081168 1,057393 AT4G22690 CYP706A1 CYP706A1 cytochrome P450, family 706, subfamily A, polypeptide 1 A\_84\_P22967 0.03446271 0.0016322 2,080063 1,056627 AT2G22500 DIC1, ATPUMP5, U ATPUMP5 DIC1 UCP5 uncoupling protein 5 ZAR1 HOPZ-ACTIVATED RESISTANCE 1 A\_84\_P23167 0.02449762 6.99E-04 2.077801 1.055058 AT3G50950 ZAR1 A\_84\_P11601 0.01872867 3,24E-04 2,076943 1,054461 AT2G39660 BIK1 BIK1\_\_botrytis-induced kinase1 A 84 P823526 0.02178353 4,93E-04 2,076692 1,054287 AT5G04250 Cysteine proteinases superfamily protein A\_84\_P52100 0.0227833 5,52E-04 2,075827 1,053686 AT4G21910 MATE efflux family protein Integral membrane HPP family protein A 84 P274520 0.04954357 0,0039493 2,075162 1,053224 AT3G47980 AtWRKY15 WRKY15 WRKY DNA-binding protein 15 A 84 P808837 0.01185074 8,24E-05 2,074917 1,053054 AT2G23320 WRKY15 A\_84\_P827290 0,04017467 0,002382 2,071514 1,050686 AT4G13110 BSD domain-containing protein A\_84\_P522242 0,02409643 6,61E-04 2,071476 1,050659 AT1G07985 Expressed protein A 84 P854328 0.04970967 0,0039798 2,070135 1,049725 AT5G02020 SIS SIS SIS SIS A\_84\_P13841 0,01592639 1,96E-04 2,069877 1,049545 AT4G25380 AtSAP10, SAP10 AtSAP10 SAP10 stress-associated protein 10 A\_84\_P754645 0,0340082 0,0015885 2,06863 1,048676 AT1G31540 Disease resistance protein (TIR-NBS-LRR class) family A 84 P127291 0.04321904 0,0027746 2,066848 1,047432 AT5G62865 A\_84\_P19986 0,03944673 0,0022656 2,063735 1,045258 AT1G01720 ANAC002, ATAF1 ANAC002\_ATAF1\_NAC (No Apical Meristem) domain transcriptional regulator superfamily protein A 84\_P92089 0.0336938 0.001565 2.063111 1.044822 AT1G19310 RING/U-box superfamily protein A 84 P21831 0.04989897 0,0040285 2,062548 1,044428 AT1G15380 GLYI4 GLYI4 Lactoylglutathione lyase / glyoxalase I family protein 0,00272 2,061704 1,043837 AT4G21200 ATGA2OX8, GA2O ATGA2OX8\_GA2OX8 gibberellin 2-oxidase 8 A\_84\_P21390 0.04277083 A\_84\_P571830 0,04413573 0,0029101 2,059866 1,04255 AT5G65925 A 84 P14321 0,03533791 0.0017409 2.057364 1.040797 AT1G74440 Protein of unknown function (DUF962) A\_84\_P20148 6,50E-04 2,056926 1,04049 AT2G30870 ATGSTF10, ATGST ATGSTF10\_ATGSTF4\_ERD13\_GSTF10\_\_glutathione S-transferase PHI 10 0,02403026 A 84 P870134 0.03117012 0.0012714 2.056679 1.040317 AT1G60270 BGLU6 BGLU6 beta alucosidase 6BGLU6 beta alucosidase 6 A\_84\_P852060 0,04811797 0,0036294 2,056495 1,040188 AT1G19770 ATPUP14, PUP14 ATPUP14\_PUP14\_purine permease 14 A 84 P845406 0.04387216 0.0028764 2.049024 1.034937 AT3G47340 ASN1, AT-ASN1, D ASN1 AT-ASN1 DIN6 glutamine-dependent asparagine synthase 1 A\_84\_P750962 0,01971263 3,85E-04 2,04538 1,032369 AT1G18200 AtRABA6b, RABA6 AtRABA6b\_RABA6b\_RABA6b\_RAB GTPase homolog A6B A 84 P15150 0.03359263 0,0015412 2,044461 1,03172 AT1G24100 UGT74B1 UGT74B1 UDP-glucosyl transferase 74B1 0,0020486 2,040776 1,029118 AT3G30775 ATPDH, ATPOX, A AT-POX\_ATPDH\_ERD5\_PDH1\_PRO1\_PRODH\_\_Methylenetetrahydrofolate reductase family protein A\_84\_P22153 0,03789915 A\_84\_P848526 0.02018128 4.02E-04 2.039846 1.028461 AT3G17120 Protein kinase superfamily proteinProtein kinase superfamily protein A\_84\_P13013 0,01921593 3,46E-04 2,039038 1,027888 AT1G18390 A 84 P58430 0.02728939 9,07E-04 2,038989 1,027854 AT3G17120 A\_84\_P158205 0.0403545 0,0024019 2,038049 1,027189 AT1G22470 A 84 P750311 0,01872867 3,26E-04 2,037992 1,027148 AT1G72120 AtNPF5.14 NPF5.14 Major facilitator superfamily protein A 84 P218298 0.03490096 0,0016866 2,037426 1,026748 AT2G42760 A\_84\_P186194 Homeodomain-like superfamily protein 0,04813688 0,0036466 2,035825 1,025614 AT2G35640 A 84 P11922 0.02511608 7.61E-04 2.028394 1.020338 AT4G18280 alvcine-rich cell wall protein-related

A_84_P12018	0,00387638			1,019729 AT4G15420		Ubiquitin fusion degradation UFD1 family protein
A_84_P502958	0,01989875			1,018489 AT2G21500		RING/U-box superfamily protein
A_84_P22649	0,00894727			1,018225 AT5G13110	G6PD2	G6PD2glucose-6-phosphate dehydrogenase 2
A_84_P157325	0,02189142	4,99E-04		1,017181 AT1G69360		Plant protein of unknown function (DUF863)Plant protein of unknown function (DUF863)
A_84_P19763	0,01663725	2,20E-04		1,016977 AT5G60300		DORN1_LecRK-I.9Concanavalin A-like lectin protein kinase family protein
A_84_P20789	0,00918073	3,89E-05	2,02262	1,016225 AT1G71400	AtRLP12, RLP12	AtRLP12_RLP12receptor like protein 12
A_84_P11083	0,02454013	7,19E-04	2,021431	1,015377 AT4G36900	DEAR4, RAP2.10	DEAR4_RAP2.10related to AP2 10
A_84_P822179	0,02342488			1,015242 AT5G02020		SIS_SIS_SIS_
A_84_P23020	0,03270588	0,0014445	2,020978	1,015054 AT2G22850	AtbZIP6, bZIP6	AtbZIP6_bZIP6basic leucine-zipper 6
A_84_P13306	0,04527197	0,0031223	2,018107	1,013002 AT1G03090	MCCA	MCCA_methylcrotonyl-CoA carboxylase alpha chain, mitochondrial 3-methylcrotonyl-CoA carboxylase 1
A_84_P13455	0,03187662	0,0013483	2,01475	1,010601 AT2G30360	CIPK11, PKS5, SIF	P CIPK11_PKS5_SIP4_SNRK3.22SOS3-interacting protein 4
A_84_P304080	0,01163687	7,15E-05	2,00996	1,007167 AT4G36930	SPT	SPTbasic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P12054	0,01830757	3,05E-04	2,009852	1,00709 AT5G07580		Integrase-type DNA-binding superfamily protein
A_84_P11868	0,01673145	2,22E-04	2,009152	1,006587 AT3G62260		Protein phosphatase 2C family protein
A_84_P97076	0,01560944	1,81E-04	2,008572	1,00617 AT1G80380		P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P510878	0,04831925	0,0037152	2,008487	1,006109 AT1G75000		GNS1/SUR4 membrane protein family
A_84_P12385	0,02745852	9,21E-04	2,008202	1,005904 AT1G80110	ATPP2-B11, PP2-E	3 ATPP2-B11_PP2-B11phloem protein 2-B11
A_84_P87289	0,02069154	4,37E-04	2,00592	1,004264 AT1G24807;	ASB1,TRP4,WEI7	
A_84_P18377	0,04695471	0,0034309	2,003492	1,002517 AT3G24420		alpha/beta-Hydrolases superfamily protein
A_84_P833963	0,03822881	0,0020797	1,995415	0,996689 AT3G11850		Protein of unknown function, DUF593
A_84_P92929	0,03612883	0,0018272	1,99399	0,995658 AT5G19240		Glycoprotein membrane precursor GPI-anchored
A_84_P812231	0,03187662	0,0013484	1,993965	0,99564 AT1G03220;	C	
A_84_P868911				0,993786 AT5G05590	PAI2	PAI2_phosphoribosylanthranilate isomerase 2
A_84_P849451	0,03490096	0,0016869	1,990562	0,993176 AT3G26910		hydroxyproline-rich glycoprotein family protein
A_84_P17672	0,03025034			0,992535 AT4G36150		Disease resistance protein (TIR-NBS-LRR class) family
A 84 P23992	0,02100477	4,54E-04	1,988682	0.991813 AT3G05640		Protein phosphatase 2C family protein
A_84_P787112	0,02353903	5,89E-04	1,987547	0,990989 AT5G56490		AtGulLO4_GulLO4_D-arabinono-1,4-lactone oxidase family protein
A 84 P17641	0,01386093			0,990803 AT4G29050		LecRK-V.9Concanavalin A-like lectin protein kinase family protein
A_84_P832631	0,04972693	0,0039936	1,987284	0,990798 AT1G11330		S-locus lectin protein kinase family proteinS-locus lectin protein kinase family protein
A 84 P845699	0,03258821				ATPLT5. ATPMT5.	ATPLT5_ATPMT5_PMT5_polyol/monosaccharide transporter 5
A_84_P22952	0,03197693					ATCB5-C_B5 #1_CB5-C_CYTB5-C_cytochrome B5 isoform C
A 84 P17213	0,02728939	9,06E-04		0,985726 AT1G58410	, - , -	Disease resistance protein (CC-NBS-LRR class) family
A_84_P15244	0,04180804	,	,	0,985124 AT1G75030	ATLP-3. TLP-3	ATLP-3_TLP-3_thaumatin-like protein 3
A_84_P848857	,			0,983795 AT3G07090		PPPDE putative thiol peptidase family protein
A_84_P89509	0,0373915			0,983748 AT5G18490		Plant protein of unknown function (DUF946)
A_84_P95119	0,03571476			0,982921 AT3G14840		Leucine-rich repeat transmembrane protein kinase
A_84_P10568	0,01786949	2,88E-04			ATEXO70B2, EXO7	ATEXO70B2_EXO70B2_exocyst subunit exo70 family protein B2
A_84_P195774	0,00900545			0,978019 AT1G30320	, -	Remorin family protein
A_84_P75444	0,02353903			0,975697 AT2G24100	ASG1	ASG1
A 84 P18359	0,03975394					AGC2 AGC2-1 AtOXI1 AGC (cAMP-dependent, cGMP-dependent and protein kinase C) kinase protein
A_84_P161083	0,01771716	,	,	0,97281 AT5G52410		
A 84 P14345	0,01323236			0,972659 AT1G76040	CPK29	CPK29_calcium-dependent protein kinase 29
A_84_P107092	,			0,972562 AT1G69050	011120	
A_84_P538274				0,969509 AT1G30755		Protein of unknown function (DUF668)Protein of unknown function (DUF668)
A_84_P503493	0,0237224			0,969122 AT5G49015		Expressed protein
A 84 P788959	0,0296875			0,968207 AT5G57910		
A_84_P308213	,	,	,	0,966776 AT3G03020		
A_84_P500277				0,966218 AT2G17705		
A_84_P250505				0,963881 AT5G46780		VQ motif-containing protein
A_84_P823769				0,960375 AT3G58980		F-box family protein
A 84 P819063	0.02582126			0,960103 AT3G46640		LUX_PCL1_Homeodomain-like superfamily protein
//_0+_i 013003	0,02002120	0,102-04	1,040440	0,000100710040		

A\_84\_P19788 0.01719517 2,42E-04 1,944603 0,959476 AT5G66210 CPK28 CPK28\_calcium-dependent protein kinase 28 A 84 P21825 0.03014083 0.0011808 1,942328 0,957787 AT1G13210 ACA.I ACA.I autoinhibited Ca2+/ATPase II A\_84\_P23186 0,01246891 9,60E-05 1,939654 0,955799 AT3G55110 ABCG18 ABCG18\_\_ABC-2 type transporter family protein A\_84\_P13692 NB-ARC domain-containing disease resistance protein 0,0241966 6,69E-04 1,938157 0,954686 AT3G46710 A 84 P15558 0.02883385 0.001027 1.937318 0.954061 AT3G20660 OCT4, AtOCT4 AtOCT4 OCT4 organic cation/carnitine transporter4 A\_84\_P10820 0,02047121 4,16E-04 1,935538 0,952735 AT3G13430 RING/U-box superfamily protein A\_84\_P23180 0,0037566 Fes1B Fes1B 0,04844125 1,928637 0,947581 AT3G53800 Fes1B A\_84\_P11232 0,00903297 3,64E-05 1,928045 0,947139 AT5G54810 ATTSB1, TRP2, TR ATTSB1\_TRP2\_TRPB\_TSB1\_tryptophan synthase beta-subunit 1 A 84 P123632 0.02454009 7,10E-04 1,927539 0,94676 AT1G70780; CPuORF28 A\_84\_P561086 0,03102973 0,0012626 1,925307 0,945088 AT5G44050 MATE efflux family protein UGT71C5 UDP-glucosyl transferase 71C5 A 84 P18131 0,01066107 5.60E-05 1,92508 0,944919 AT1G07240 UGT71C5 A 84 P156895 0,04262674 0.0027039 1.924733 0.944658 AT3G47210 Plant protein of unknown function (DUF247) DNA-binding bromodomain-containing protein A\_84\_P20006 0.01582691 1,92E-04 1,923072 0,943413 AT1G76380 AtRDUF1\_RDUF1\_\_zinc finger (C3HC4-type RING finger) family protein A\_84\_P168493 0,02850911 9.96E-04 1,922251 0,942797 AT3G46620 A 84 P259710 0.03831658 0,0020976 1,91909 0,940422 AT3G18930 RING/U-box superfamily protein A\_84\_P814679 0,01771716 2,71E-04 1,918487 0,939969 AT3G62420; ATBZIP53,BZIP53,CPuORF3 A 84 P23294 0.03152039 0,0013147 1,917984 0,93959 AT4G24230 ACBP3 ACBP3 acyl-CoA-binding domain 3 A 84 P18591 0,02991192 0,0011583 1,917885 0,939516 AT4G29950 Ypt/Rab-GAP domain of gyp1p superfamily protein A\_84\_P550411 Tetratricopeptide repeat (TPR)-like superfamily protein 0.00857965 1.89E-05 1,916685 0,938613 AT5G20190 A\_84\_P24127 0.03234271 0,0014059 1,916481 0,938459 AT3G53990 Adenine nucleotide alpha hydrolases-like superfamily protein HSA32 Aldolase-type TIM barrel family proteinHSA32 Aldolase-type TIM barrel family protein A 84 P825746 0,0281065 9,67E-04 1,913627 0,93631 AT4G21320 HSA32 A\_84\_P560703 0,02430013 6,75E-04 1,913023 0,935854 AT3G07150 A 84 P21822 7,35E-04 1,912916 0,935774 AT1G21000 PLATZ transcription factor family protein 0,02477091 A 84 P19551 0.03999281 0.0023373 1,912536 0,935487 AT4G33300 ADR1-L1 ADR1-L1 ADR1-like 1 A\_84\_P123362 0,03211456 0,0013791 1,912072 0,935137 AT1G13740 AFP2 AFP2\_\_ABI five binding protein 2 A\_84\_P256200 0,03120647 0,0012799 1,909599 0,93327 AT3G06760 Drought-responsive family proteinDrought-responsive family protein A 84 P15341 VHA-E3\_vacuolar H+-ATPase subunit E isoform 3 0.02285099 5,57E-04 1,909018 0,932831 AT1G64200 VHA-E3 AtGSH2 GSH2 GSHB glutathione synthetase 2 A\_84\_P12104 0,01785633 2,87E-04 1,907605 0,931763 AT5G27380 GSH2, GSHB A\_84\_P22312 0,04234786 0,002668 1,907347 0,931567 AT4G11360 RHA1B RHA1B\_\_\_RING-H2 finger A1B A 84 P11862 0,0409187 0,0024804 1,906296 0,930772 AT3G60690 SAUR59 SAUR-like auxin-responsive protein family A\_84\_P22159 0,02859729 0,0010001 1,906065 0,930597 AT3G13100 ABCC7, ATMRP7, ABCC7\_ATMRP7\_MRP7\_multidrug resistance-associated protein 7 ABF1 AtABF1 abscisic acid responsive element-binding factor 1 A 84 P164953 0.02449762 6.93E-04 1.904194 0,92918 AT1G49720 ABF1 A 84 P785537 0.03524169 0.0017216 1.904132 0.929133 AT5G40460 A\_84\_P16258 0.0336938 0,0015653 1,899902 0,925925 AT1G49230 ATL78\_AtATL78\_\_RING/U-box superfamily protein A\_84\_P281300 0,04465805 0,0030115 1,898538 0,924889 AT3G46640 LUX, PCL1 LUX\_PCL1\_\_Homeodomain-like superfamily protein A 84 P750263 0,02376995 6,16E-04 1,898402 0,924786 AT1G17230 Leucine-rich receptor-like protein kinase family protein A\_84\_P538776 0,03478254 RING/U-box superfamily protein 0,0016715 1,898356 0,92475 AT4G24015 AtDRM1 DRM1 DYL1 dormancy-associated protein-like 1 A 84 P812038 0.02403887 6.58E-04 1.897513 0.924109 AT1G28330 DRM1. DYL1 A\_84\_P12747 0,03529676 0,0017258 1,896174 0,923091 AT3G46700 UDP-Glycosyltransferase superfamily protein EXS (ERD1/XPR1/SYG1) family protein A 84 P121232 0.0241966 6.69E-04 1.893508 0.921061 AT2G03240 A\_84\_P531393 0,04181817 0,0026031 1,892646 0,920404 AT2G37210 LOG3 LOG3\_lysine decarboxylase family protein A 84 P17993 0.01961223 3,77E-04 1,892353 0,920181 AT1G61370 S-locus lectin protein kinase family protein 0,0018719 1,891678 0,919666 AT5G50200 ATNRT3.1, NRT3.1 ATNRT3.1\_NRT3.1\_WR3\_\_nitrate transmembrane transporters A\_84\_P856262 0,03645688 A\_84\_P298164 0,01163597 6.78E-05 1.890017 0.918399 AT5G65870 ATPSK5, PSK5 ATPSK5 PSK5 phytosulfokine 5 precursor A\_84\_P815150 0.04348974 0,002806 1,88933 0,917874 AT2G36320 A20/AN1-like zinc finger family protein A 84 P13039 0.03831641 0,002096 1,888914 0,917557 AT5G24420 PGL5 PGL5 6-phosphogluconolactonase 5 A\_84\_P818026 0,0151022 1,68E-04 1,888015 0,91687 AT4G26090 RPS2 RPS2\_\_NB-ARC domain-containing disease resistance protein A 84 P15176 0.01375994 1,23E-04 1,886798 ERD (early-responsive to dehydration stress) family protein 0,91594 AT1G11960 A\_84\_P218898 0,01961223 3,68E-04 1,886701 0,915866 AT5G27760 Hypoxia-responsive family protein A\_84\_P15820 ATMDAR2\_Pyridine nucleotide-disulphide oxidoreductase family protein 0,03988752 0,0023213 1,886149 0,915443 AT5G03630 ATMDAR2 A 84 P141359 0.01872867 3.25E-04 1.885462 0.914918 AT2G24550

A\_84\_P13968 0,02195661 5,02E-04 1,885338 0,914824 AT5G16410 HXXXD-type acyl-transferase family protein A 84 P19237 0,03051006 0,0012131 1,885 0,914564 AT2G27510 ATFD3, FD3 ATFD3 FD3 ferredoxin 3 A\_84\_P16662 0.02060719 4,29E-04 1,88297 0,91301 AT4G21570 Protein of unknown function (DUF300) A\_84\_P810987 1,881091 0,91157 AT2G41430 CID1, ERD15, LSR CID1\_ERD15\_LSR1\_\_dehydration-induced protein (ERD15) 0,00606654 6,78E-06 1,51E-04 1,880572 0,911172 AT4G26090 RPS2 RPS2 NB-ARC domain-containing disease resistance protein A 84 P20465 0.0145606 A\_84\_P17577 0,02910758 0,0010678 1,877991 0,90919 AT1G06180 ATMYB13, ATMYB ATMYB13\_ATMYBLFGN\_MYB13\_myb domain protein 13 5,02E-04 1,875028 0,906912 AT4G39890 AtRABH1c, RABH1 AtRABH1c\_RABH1c\_ RAB GTPase homolog H1C A\_84\_P16738 0.02195661 A\_84\_P123242 0,02646521 8,51E-04 1,874396 0,906426 AT5G40460 A 84 P834403 0.02057283 4,27E-04 1,873945 0,906078 AT4G09150 T-complex protein 11T-complex protein 11 A\_84\_P848389 0,02896398 0,0010525 1,873577 0,905796 AT1G07890 APX1, ATAPX01, A APX1\_ATAPX01\_ATAPX1\_CS1\_MEE6\_ ascorbate peroxidase 1 A 84 P14746 0,00961027 4.28E-05 1.873166 0.905478 AT4G11660 AT-HSFB2B, HSFB AT-HSFB2B HSF7 HSFB2B winged-helix DNA-binding transcription factor family protein A 84 P826004 0.01785415 2,84E-04 1,868904 0,902192 AT1G07000 ATEXO70B2, EXO7 ATEXO70B2 EXO70B2 exocyst subunit exo70 family protein B2 A\_84\_P535752 0,01320943 1,08E-04 1,868594 0,901953 AT2G13790 ATSERK4, BAK7, B ATSERK4\_BAK7\_BKK1\_SERK4\_somatic embryogenesis receptor-like kinase 4 A\_84\_P19740 0.03537542 0,0017467 1,867599 0,901185 AT5G54300 Protein of unknown function (DUF761) A 84 P604788 0.0491156 0,0038788 1,866795 0,900563 AT5G59510 DVL18, RTFL5 DVL18 RTFL5 ROTUNDIFOLIA like 5 A\_84\_P18315 0,04636379 0,0033158 1,864798 0,899019 AT3G09940 ATMDAR3, MDAR2 ATMDAR3\_MDAR2\_MDAR3\_MDHAR\_monodehydroascorbate reductase A 84 P14868 0.02376995 6,18E-04 1,864795 0,899017 AT5G01830 ARM repeat superfamily protein A\_84\_P10152 0.04639193 0,0033274 1,862771 0,89745 AT5G04760 Duplicated homeodomain-like superfamily protein 1.27E-04 A 84 P550081 0.01375994 1.862285 0.897074 AT3G09405 Pectinacetylesterase family protein A\_84\_P798820 0,01375994 1,25E-04 1,861436 0,896416 AT2G41430 CID1, ERD15, LSR CID1\_ERD15\_LSR1\_dehydration-induced protein (ERD15) A 84 P832013 0.04488314 0,0030705 1,860947 0,896037 AT5G07890 myosin heavy chain-related A\_84\_P251265 0,01872867 3,26E-04 1,859908 0,895231 AT1G63720 0,0017411 1,858849 0,894409 AT1G43886 transposable element gene A 84 P784997 0.03533791 A 84 P127601 0,03827128 0.0020859 1,857877 0,893655 AT5G02940 Protein of unknown function (DUF1012) A\_84\_P814940 0,04358541 0,0028142 1,854072 0,890697 AT4G30210 AR2, ATR2 AR2\_ATR2\_\_P450 reductase 2AR2\_ATR2\_\_P450 reductase 2 CLC3\_Clathrin light chain proteinCLC3\_Clathrin light chain protein A\_84\_P103796 0,0373915 0,001985 1,851026 0,888325 AT3G51890 A 84 P22051 0,04614215 0,00327 1.848729 0.886534 AT2G19450 ABX45, AS11, ATD ABX45 AS11 ATDGAT AtDGAT1 RDS1 TAG1 membrane bound O-acyl transferase (MBOAT) protein A\_84\_P555310 0,03330867 0,0014996 1,848614 0,886444 AT2G36650 A\_84\_P222519 0,02544358 7,85E-04 1,847575 0,885633 AT1G21130 IGMT4 IGMT4\_O-methyltransferase family protein A 84 P19220 0.03688685 0.0019341 1,845863 0,884295 AT2G39210 Major facilitator superfamily protein A\_84\_P13391 0,01492891 1,60E-04 1,84541 0,883942 AT1G34420 leucine-rich repeat transmembrane protein kinase family protein A 84\_P16698 3,66E-04 1,845251 0,883817 AT4G29700 0.01961223 Alkaline-phosphatase-like family protein A 84 P16520 0.03181502 0.0013381 1,844985 0,883609 AT1G02640 ATBXL2, BXL2 ATBXL2 BXL2 beta-xylosidase 2 A\_84\_P20605 0,04784888 DMR6\_2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein 0,0035782 1,840491 0,880091 AT5G24530 DMR6 A\_84\_P16153 0,02894719 0,0010502 1,837108 0,877437 AT1G14130 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein 0,0010614 1,836306 0,876806 AT4G09150 A 84 P552884 0.02906186 T-complex protein 11T-complex protein 11 A\_84\_P13990 0,00398 Protein phosphatase 2C family protein 0,04970967 1,83612 0,87666 AT5G26010 A\_84\_P814037 0,01830216 3.01E-04 1.835812 0.876418 AT1G80380 P-loop containing nucleoside triphosphate hydrolases superfamily protein A\_84\_P280930 0,02384917 6,27E-04 1,83508 0,875843 AT1G21670 A 84 P15987 0.02237896 5,24E-04 1,834488 0,875378 AT5G61900; BON,BON1,CPN1 A\_84\_P15238 0,04826047 0,0036805 1,834137 0,875101 AT1G80320 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein A 84 P23251 0.02943616 0,001103 1,833095 0,874282 AT4G09570 ATCPK4, CPK4 ATCPK4 CPK4 calcium-dependent protein kinase 4 A\_84\_P137359 0,02820842 9,76E-04 1,83274 0,874002 AT5G27070 AGL53 AGL53 AGAMOUS-like 53 A\_84\_P15928 0.04197278 0.0026226 1.832618 0.873906 AT5G46520 VICTR\_Disease resistance protein (TIR-NBS-LRR class) family A\_84\_P816799 0,02675365 8,69E-04 1,830923 0,872571 AT4G19880 Glutathione S-transferase family protein A 84 P12695 0,04661662 0,0033746 1,827652 0,869991 AT3G25730 EDF3 EDF3 ethylene response DNA binding factor 3 A\_84\_P281740 0,03454188 0,0016456 1,827293 0,869708 AT1G78070 Transducin/WD40 repeat-like superfamily protein A 84 P20025 0,02084717 4,46E-04 1,826485 0.86907 AT1G22360 AtUGT85A2, UGT8 AtUGT85A2 UGT85A2 UDP-glucosyl transferase 85A2 A\_84\_P812152 0,04838306 0,0037403 1,824638 0,86761 AT4G21850 ATMSRB9, MSRB9 ATMSRB9\_MSRB9\_methionine sulfoxide reductase B9 A\_84\_P236133 2,26E-04 1,823634 0,866817 AT1G70420 Protein of unknown function (DUF1645) 0,01686909 A 84 P22342 0.03270588 0.0014467 1.82351 0.866718 AT4G22780 ACR7 ACR7 ACT domain repeat 7

A\_84\_P118682 0,02943616 0,0011018 1,823327 0,866573 AT3G52430 ATPAD4, PAD4 ATPAD4\_PAD4\_alpha/beta-Hydrolases superfamily protein A 84 P121882 0.04490265 0.0030802 1.822925 0.866255 AT4G30240 Syntaxin/t-SNARE family protein A 84 P173901 0.04461956 0,0029913 1,820325 0,864196 AT5G22355 Cysteine/Histidine-rich C1 domain family protein 0,01323236 1,816063 0,860814 AT4G36900 DEAR4, RAP2.10 DEAR4\_RAP2.10\_related to AP2 10DEAR4\_RAP2.10\_related to AP2 10 A\_84\_P819283 1,11E-04 0.03746976 0.0019967 1.815829 0.860628 AT2G28080 UDP-Glycosyltransferase superfamily protein A 84 P16349 A\_84\_P832358 0,01926563 3,47E-04 1,81559 0,860438 AT5G52410 A 84\_P10660 0,02208143 5,07E-04 1,814782 0,859797 AT2G29490 ATGSTU1, GST19, ATGSTU1\_GST19\_GSTU1\_glutathione S-transferase TAU 1 A\_84\_P556490 0,02237896 5,24E-04 1,813488 0,858767 AT4G23493 A 84 P23354 0.04371693 0.0028488 1,812682 0,858126 AT4G38580 ATFP6, FP6, HIPP2 ATFP6 FP6 HIPP26 farnesylated protein 6 A\_84\_P562392 0,04372359 0,0028556 1,810314 0,85624 AT2G34355 Major facilitator superfamily protein A 84 P77129 0.04657911 0,0033603 1,809002 0,855194 AT2G44370 Cysteine/Histidine-rich C1 domain family proteinCysteine/Histidine-rich C1 domain family protein A 84 P505670 0.02038725 4,11E-04 1,807134 0,853704 AT2G36080 ABS2 NGAL1 AP2/B3-like transcriptional factor family protein A\_84\_P554085 0,01564355 1,84E-04 1,806646 0,853313 AT1G02880 TPK1 TPK1 thiamin pyrophosphokinase1TPK1 thiamin pyrophosphokinase1 A\_84\_P513731 0,02369543 6,05E-04 1,805439 0,852349 AT3G12955 SAUR74 SAUR-like auxin-responsive protein family glycosyl hydrolase family 10 protein / carbohydrate-binding domain-containing protein 0,0246688 7.26E-04 1.804504 0.851603 AT1G10050 A 84 P13281 A\_84\_P816633 0,04517826 0,0031085 1,804334 0,851466 AT5G27380 GSH2, GSHB AtGSH2\_GSH2\_GSHB\_\_glutathione synthetase 2 A 84 P13439 0.03541943 0,0017505 1,800746 0,848595 AT2G39980 HXXXD-type acyl-transferase family protein A 84 P76134 0,01797131 2,91E-04 1,799786 0,847825 AT4G14270 A 84 P21026 0.04308559 1,798422 0,846731 AT2G33380 AtCLO3, CLO3, CL AtCLO3\_CLO-3\_CLO3\_RD20\_Caleosin-related family protein 0,002758 A\_84\_P820912 0,02559591 7,95E-04 1,797811 0,846241 AT3G02875 ILR1 ILR1\_Peptidase M20/M25/M40 family protein 0.0015347 1.796727 0.845371 AT2G02710 PLP, PLPA, PLPB, PLP PLPA PLPB PLPC PAS/LOV protein B A 84 P182664 0.03359263 A\_84\_P12017 0.04103823 0,0024958 1,794466 0,843555 AT4G15270 glucosyltransferase-related A 84 P15989 0.0373915 0,0019874 1,792985 0,842363 AT5G62350 Plant invertase/pectin methylesterase inhibitor superfamily protein A 84 P81259 0.03665218 0.0019062 1,79277 0,842191 AT4G21510 AtFBS2 FBS2 F-box family protein A\_84\_P16249 0,03255169 0,0014189 1,791898 0,841489 AT1G55510 BCDH BETA1 BCDH BETA1\_\_branched-chain alpha-keto acid decarboxylase E1 beta subunit A\_84\_P21190 0,02342488 5,82E-04 1,791671 0,841306 AT3G16350 Homeodomain-like superfamily protein A 84 P186874 0.03021324 0,0011853 1,787399 0,837862 AT3G54020 AtIPCS1 AtIPCS1 Arabidopsis Inositol phosphorylceramide synthase 1 A\_84\_P534040 0,01320943 1,08E-04 1,785922 0,836669 AT2G31810 ACT domain-containing small subunit of acetolactate synthase protein A\_84\_P246145 0,01647879 2,16E-04 1,784906 0,835848 AT4G32120 Galactosyltransferase family protein A 84 P585178 0.03997699 0,002335 1,784847 0,8358 AT5G53895 A\_84\_P22571 0,02400674 6,42E-04 1,781863 0,833386 AT5G52310 COR78, LTI140, LT COR78\_LTI140\_LTI78\_RD29A\_low-temperature-responsive protein 78 /desiccation-responsive protein 29A A 84\_P840181 RPM1-interacting protein 4 (RIN4) family proteinRPM1-interacting protein 4 (RIN4) family protein 0.01874703 3,28E-04 1,781358 0,832977 AT3G48450 A 84 P20571 0.00894727 2,84E-05 1,781244 0,832885 AT1G68690 AtPERK9, PERK9 AtPERK9\_PERK9\_Protein kinase superfamily protein A\_84\_P22517 Chaperone DnaJ-domain superfamily protein 0,03307353 0,0014714 1,781183 0,832836 AT5G37750 A\_84\_P11735 0,03993093 0,0023297 1,780875 0,832586 AT3G28930 AIG2 AIG2\_\_AIG2-like (avirulence induced gene) family protein A 84 P809109 0.02072643 4,40E-04 1,780716 0,832458 AT4G14270 A\_84\_P21324 0,0015431 1,77915 0,831188 AT3G62420; ATBZIP53,BZIP53,CPuORF3 0,03359263 0.0027239 A 84 P188574 0.0427998 1.7788 0.830904 AT5G02020 SIS SIS SIS SIS A\_84\_P14670 0,03331262 0,0015006 1,777766 0,830066 AT3G54200 Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family 0.003021 1.777406 0.829773 AT2G25140 CLPB4, CLPB-M, H CLPB-M CLPB4 HSP98.7 casein lytic proteinase B4 A 84 P22953 0.04465805 A\_84\_P814035 0,0265841 8,59E-04 1,776002 0,828633 AT1G80380 P-loop containing nucleoside triphosphate hydrolases superfamily protein A 84 P99616 0,04176612 0.0025886 1.775381 0.828129 AT5G08360 Protein of unknown function (DUF789) 0,00172 1,775061 0,827868 AT5G13700 APAO, ATPAO1, P APAO\_ATPAO1\_PAO1\_polyamine oxidase 1 A\_84\_P14907 0,03523168 A\_84\_P245045 0,02067666 4.33E-04 1.774782 0.827642 AT5G64660 ATCMPG2 CMPG2 ATCMPG2 CMPG2 CYS. MET. PRO. and GLY protein 2 A\_84\_P18219 0,02390528 6,31E-04 1,774007 0,827012 AT1G04770 Tetratricopeptide repeat (TPR)-like superfamily protein A 84 P17267 0,02740352 9,17E-04 1,771169 0,824702 AT2G41410 Calcium-binding EF-hand family protein 3,48E-04 1,770824 0,824421 AT4G18880 AT-HSFA4A, HSF A AT-HSFA4A\_HSF A4A\_heat shock transcription factor A4A A\_84\_P17597 0.01926882 A 84 P854510 0,03120647 0,0012808 1,770653 0,824282 AT5G02290 NAK NAK Protein kinase superfamily protein A 84 P162193 0,04398862 0.0028882 1,766062 0,820536 AT5G62070 IQD23 IQD23 IQ-domain 23 A\_84\_P16834 0,03466453 GSDA\_Cytidine/deoxycytidylate deaminase family protein 0,0016626 1,764552 0,819302 AT5G28050 A 84 P23105 0.01331538 1,12E-04 1,761843 0,817085 AT3G13110 ATSERAT2;2, SAT- ATSERAT2;2\_SAT-1\_SAT-A\_SAT-M\_SAT3\_SERAT2;2\_serine acetyltransferase 2;2

A 84 P183994 0.04883185 0.0038135 1.760907 0.816318 AT3G14870 Plant protein of unknown function (DUF641) A 84 P13308 0,04899432 0.0038572 1,759846 0,815449 AT1G79410 OCT5, AtOCT5 AtOCT5 OCT5 organic cation/carnitine transporter5 A 84 P817774 0,02676006 8,71E-04 1,758715 0,814522 AT5G61900; BON,BON1,CPN1 A\_84\_P579822 0,03172537 0,0013254 1,758058 0,813983 AT5G57880; ATPRD2,MPS1,PRD2 2,45E-04 A 84 P830975 0.01731088 1,757592 0,813601 AT1G30755 Protein of unknown function (DUF668) A\_84\_P19628 0,02454009 7,13E-04 1,75705 0,813155 AT5G10820 Major facilitator superfamily protein 5,49E-04 1,755484 0,811869 AT2G36320 A\_84\_P110642 0,02277204 A20/AN1-like zinc finger family protein A\_84\_P21264 0,04062048 0,0024375 1,75476 0,811274 AT3G48360 ATBT2, BT2 ATBT2\_BT2\_BTB and TAZ domain protein 2 A 84 P14621 0.02532245 7,78E-04 SULTR4;2 sulfate transporter 4;2 1,754397 0,810975 AT3G12520 SULTR4:2 A\_84\_P15984 0,03916847 0,0022098 1,75253 0,809439 AT5G61380 APRR1, AtTOC1, P APRR1\_AtTOC1\_PRR1\_TOC1\_\_CCT motif -containing response regulator protein A 84 P10998 0,04418246 0,0029216 1,752452 0,809375 AT4G23570 SGT1A SGT1A phosphatase-related A 84 P806857 0,02524157 7,72E-04 1,752452 0,809375 AT5G28050 GSDA Cytidine/deoxycytidylate deaminase family protein A\_84\_P18708 0.0457815 0,0032323 1,752219 0,809183 AT5G23050 AAE17 AAE17\_acyl-activating enzyme 17 ATSTP1\_STP1\_sugar transporter 1 A\_84\_P10638 0.02146564 4,74E-04 1,751707 0,808762 AT1G11260 ATSTP1, STP1 A 84 P830088 0,04017467 0.0023865 1,749064 0,806583 AT2G03240 EXS (ERD1/XPR1/SYG1) family protein A\_84\_P97176 0,04856481 0,0037757 1,745036 0,803257 AT5G43190 Galactose oxidase/kelch repeat superfamily protein A 84 P21538 0,03697815 0,0019423 1,744943 0,80318 AT5G18170 GDH1 GDH1\_\_glutamate dehydrogenase 1 A\_84\_P23528 0.03317259 0.0014912 1.744134 0.802511 AT5G55700 BAM4, BMY6 BAM4 BMY6 beta-amylase 4 1.86E-04 A\_84\_P20907 0.01564355 1.738913 0.798185 AT1G49050 Eukaryotic aspartyl protease family protein A\_84\_P571270 0,0282571 9.80E-04 1,737593 0,797091 AT2G28056 MIR172\_MIR172A\_\_MIR172/MIR172A; miRNA MIR172, MIR172A A 84 P93839 0,00877048 2,06E-05 1,737379 0,796912 AT1G78460 SOUL heme-binding family protein A\_84\_P806863 0,01710836 2,38E-04 1,734841 0,794804 AT5G28050 GSDA\_Cytidine/deoxycytidylate deaminase family protein A 84 P22063 2,85E-05 1,734817 0,794784 AT2G48010 RKF3 RKF3 receptor-like kinase in in flowers 3 0,00894727 A 84 P13470 0,0313028 0,0012919 1,73436 0,794403 AT2G47890 B-box type zinc finger protein with CCT domain A\_84\_P85169 0,01176281 7,78E-05 ARO4\_armadillo repeat only 4 1,732777 0,793086 AT3G26600 ARO4 A\_84\_P241903 0,03438171 0,0016196 1,732716 0,793035 AT5G50200 ATNRT3.1, NRT3.1 ATNRT3.1\_NRT3.1\_WR3\_\_nitrate transmembrane transporters A 84 P18314 0.01065925 5,43E-05 1,732669 0,792996 AT3G10640 VPS60.1 VPS60.1 SNF7 family protein A\_84\_P157795 0,01803394 2,94E-04 1,732505 0,792859 AT3G21070 ATNADK-1, NADK1ATNADK-1\_NADK1\_\_NAD kinase 1 A\_84\_P15732 0,02532245 7,79E-04 1,730452 0,791149 AT4G25390 Protein kinase superfamily protein A 84 P21970 0.04811797 0.0036322 1,729536 0,790385 AT2G22200 Integrase-type DNA-binding superfamily protein A\_84\_P816956 0,01769782 2,64E-04 1,729453 0,790316 AT1G64190 6-phosphogluconate dehydrogenase family protein A\_84\_P19863 0.02403887 6,54E-04 1,729295 0,790184 AT1G23830 A 84 P816646 0.03954039 0,0022747 1,72713 0,788377 AT5G27380 GSH2, GSHB AtGSH2 GSH2 GSHB glutathione synthetase 2 A\_84\_P254390 0,04661662 Protein of unknown function (DUF688) 0,0033712 1,726698 0,788015 AT2G30990 A\_84\_P202508 0,03308225 0,0014848 1,725519 Protein of unknown function (DUF789) 0,78703 AT1G73210 A 84 P16794 0,04899432 0.0038536 1,725002 0,786598 AT1G18390 Protein kinase superfamily protein A\_84\_P824802 0,03051006 0,0012073 1,724344 0,786048 AT2G44370 Cysteine/Histidine-rich C1 domain family protein A 84 P88159 0.04538051 0.0031435 1.722204 0.784256 AT2G45360 Protein of unknown function (DUF1442) A\_84\_P170233 0,01246891 9,27E-05 1,720364 0,782714 AT2G38000 chaperone protein dnaJ-related A 84 P503984 0.0161733 2,03E-04 1,719128 0,781677 AT3G12510 MADS-box family protein 8,37E-05 1,718365 0,781036 AT3G22890 APS1 APS1\_\_ATP sulfurylase 1 A\_84\_P862614 0,01188738 A 84 P82909 0.0467632 0.0034077 1.718289 0.780973 AT1G17830 Protein of unknown function (DUF789) A\_84\_P273060 0,03308225 0,0014743 1,716116 0,779147 AT3G51090 Protein of unknown function (DUF1640) A 84 P599034 0.01246891 9.46E-05 1,715161 0,778344 AT3G16860 COBL8 COBL8 COBRA-like protein 8 precursor A\_84\_P854693 0,02612945 8,36E-04 1,713683 0,777101 AT5G63970 RGLG3\_Copine (Calcium-dependent phospholipid-binding protein) family A 84 P135625 0,03542483 0,0017563 1,711251 0,775051 AT4G25670; CPuORF12 A\_84\_P224559 0,04234786 0,0026691 1,706787 0,771283 AT5G13760 Plasma-membrane choline transporter family protein A 84 P849522 2,28E-04 1,706371 0,770931 AT3G63010 ATGID1B, GID1B ATGID1B GID1B alpha/beta-Hydrolases superfamily protein 0.01686909 A 84 P22207 0.03492962 0,0016899 1,705816 0,770462 AT3G47550 RING/FYVE/PHD zinc finger superfamily protein 1,705339 0,770059 AT4G11850 MEE54, PLDGAMM MEE54\_PLDGAMMA1\_phospholipase D gamma 1 A\_84\_P288524 0,03217042 0,001385 A 84 P868111 0.02353903 5.91E-04 1.704156 0.769057 AT3G54020 AtIPCS1 AtIPCS1\_Arabidopsis Inositol phosphorylceramide synthase 1

A 84 P21333 0.04572882 0,0032067 1,702465 0,767625 AT1G12200 FMO FMO\_\_Flavin-binding monooxygenase family protein A 84 P257990 0,03178847 0,0013318 1,701722 0,766995 AT3G48350 CEP3 CEP3 Cysteine proteinases superfamily protein A 84 P867087 0.03416876 0,0016007 1,699788 0,765355 AT5G54930 AT hook motif-containing protein A\_84\_P561436 0,03916847 0,0022206 1,698545 0,764299 AT2G07749; ORF251 5,99E-04 1,697497 0,763409 AT5G15960 KIN1 A 84 P810688 0.02362118 KIN1 stress-responsive protein (KIN1) / stress-induced protein (KIN1) A\_84\_P16701 0,02267799 5,40E-04 1,696147 0,762261 AT4G30350 SMXL2 \_\_Double Clp-N motif-containing P-loop nucleoside triphosphate hydrolases superfamily protein A\_84\_P137419 0,03051635 0,0012156 1,695076 0,76135 AT1G21010 A\_84\_P753864 0,00668285 8,51E-06 1,694665 0,761 AT1G67365 other RNA 1,694385 0,760761 AT4G25020 D111/G-patch domain-containing protein A 84 P301020 0,03146277 0,0013111 A\_84\_P762519 0,04078834 1,690676 other RNA 0,0024636 0,7576 AT3G21781 A 84 P90109 0,0482928 0,0036917 1,68979 0,756844 AT3G05800 AIF1 AIF1 AtBS1(activation-tagged BRI1 suppressor 1)-interacting factor 1 A 84 P856001 0.03359263 0.0015367 1.686956 0.754422 AT5G17460 A\_84\_P809392 0,01451456 1,46E-04 1,686218 0,753791 AT1G02880 TPK1 TPK1 thiamin pyrophosphokinase1TPK1 thiamin pyrophosphokinase1 A\_84\_P167973 0,02376995 6,19E-04 1,685983 0,75359 AT3G48450 RPM1-interacting protein 4 (RIN4) family proteinRPM1-interacting protein 4 (RIN4) family protein 0.03134441 0,0012987 1,685746 0,753387 AT2G38750 ANNAT4 ANNAT4 AtANN4 annexin 4 A 84 P15372 A\_84\_P852163 0,04387216 0,0028761 1,685675 0,753326 AT5G20250 DIN10, RS6 DIN10\_RS6\_\_Raffinose synthase family proteinDIN10\_RS6\_\_Raffinose synthase family protein A 84 P761363 0.04311589 0,002762 1,685613 0,753274 AT3G13550 CIN4, COP10, EMB CIN4\_COP10\_EMB144\_FUS9\_\_Ubiquitin-conjugating enzyme family protein A 84 P159095 0,02445181 6.89E-04 1,68142 0,749681 AT1G33700 Beta-glucosidase, GBA2 type family protein A 84 P21624 0.01156977 6.63E-05 1,681355 0,749624 AT1G64280 ATNPR1, NIM1, NP ATNPR1\_NIM1\_NPR1\_SAI1\_\_regulatory protein (NPR1) A\_84\_P12389 0.02067666 4,32E-04 1,681123 0,749425 AT1G14860 atnudt18, NUDT18 NUDT18\_atnudt18\_nudix hydrolase homolog 18 A 84 P821021 0,04927701 0,0039099 1,680366 0,748775 AT1G75020 LPAT4 LPAT4 lysophosphatidyl acyltransferase 4 A\_84\_P177674 0,0184221 3,11E-04 1,679499 0,748031 AT5G25210 A 84 P765657 0.01504028 1,64E-04 1,677917 0,746672 AT4G40065 other RNA A 84 P18514 0.03722991 0,0019627 1,677671 0,74646 AT1G06000 UDP-Glycosyltransferase superfamily protein A\_84\_P13957 0,04141799 0,0025496 alpha/beta-Hydrolases superfamily protein 1,677585 0,746386 AT5G11650 A\_84\_P19477 0,02430013 6,79E-04 1,676546 0,745492 AT4G12080 AHL1, ATAHL1 AHL1\_ATAHL1\_\_AT-hook motif nuclear-localized protein 1 A 84 P754799 0.04603513 0.0032603 1,676209 0,745202 AT1G07780; PAI1,TRP6,PAI2 A\_84\_P785697 0.04368648 0,0028423 1,67508 0,74423 AT3G10260 Reticulon family protein A\_84\_P19130 0,04387216 0,0028727 1,674623 0,743836 AT2G46270 GBF3 GBF3\_\_G-box binding factor 3 A 84 P20210 0,02312453 5,69E-04 1,673013 0,742449 AT3G03190 ATGSTF11, ATGST ATGSTF11\_ATGSTF6\_GSTF11\_glutathione S-transferase F11 A\_84\_P833361 0,03681569 0,0019269 1,672622 0,742111 AT2G30100 pentatricopeptide (PPR) repeat-containing protein A\_84\_P21398 0.02877405 0.001019 1,6693 0,739244 AT4G23270 CRK19 CRK19\_cysteine-rich RLK (RECEPTOR-like protein kinase) 19 A 84 P19945 0.04572882 0.0032093 1.667122 0.73736 AT1G72850 Disease resistance protein (TIR-NBS class) A\_84\_P529509 0,01971263 3,84E-04 1,66661 0,736917 AT1G77360 APPR6\_\_Tetratricopeptide repeat (TPR)-like superfamily protein A\_84\_P184384 0,01156977 6,65E-05 1,666043 0,736425 AT1G68440 A 84 P861072 0.04254559 0.0026909 1,664049 0,734698 AT3G26740 CCL CCL CCR-likeCCL CCR-like A\_84\_P18041 1,56E-04 1,663158 0,733925 AT1G32690 0,01469936 A\_84\_P715804 0.02681149 8.74E-04 1.662493 0.733348 AT5G18540 A\_84\_P21584 0,02376995 6,13E-04 1,662449 0,73331 AT1G72210 bHLH096\_bHLH96\_basic helix-loop-helix (bHLH) DNA-binding superfamily protein A 84 P761889 0.0145606 1,51E-04 1,65973 0,730948 AT3G16565 alanine-tRNA ligases;nucleic acid binding;ligases, forming aminoacyl-tRNA and related compounds A\_84\_P852438 0,02454009 7,14E-04 1,659251 0,730532 AT5G24590 ANAC091, TIP ANAC091\_TIP\_TCV-interacting proteinANAC091\_TIP\_TCV-interacting protein A 84 P17654 0,03681569 0.0019256 1,659207 0,730494 AT4G31980 A\_84\_P11048 0,02047567 4,18E-04 1,656537 0,72817 AT4G34810 SAUR5\_SAUR-like auxin-responsive protein family A\_84\_P82139 0.03959271 0.0022835 1.656263 0.727932 AT5G65920 ARM repeat superfamily protein A\_84\_P67324 0,02883385 0,0010241 1,65555 0,72731 AT2G25930 ELF3, PYK20 ELF3\_PYK20\_\_hydroxyproline-rich glycoprotein family protein A 84 P95179 0.03359263 0.0015326 1,654151 0,726091 AT1G44050 Cysteine/Histidine-rich C1 domain family protein A\_84\_P817161 0.02684096 8,77E-04 1,653485 0,72551 AT4G30470 NAD(P)-binding Rossmann-fold superfamily proteinNAD(P)-binding Rossmann-fold superfamily protein A 84 P17499 0,03474192 0.0016679 A20/AN1-like zinc finger family protein 1,651308 0,723609 AT3G52800 A 84 P766219 0,00877275 2,29E-05 1,650766 0,723136 AT5G26600 Pyridoxal phosphate (PLP)-dependent transferases superfamily protein A\_84\_P20393 Cysteine/Histidine-rich C1 domain family protein 0,04473491 0,0030464 1,649941 0,722414 AT4G01910 A 84 P233289 0.03612883 0.0018274 1.64993 0.722405 AT1G80610

A\_84\_P10413 0,01305407 1.05E-04 1.649713 0.722215 AT1G03905 ABCI19 ABCI19\_P-loop containing nucleoside triphosphate hydrolases superfamily protein A 84 P79549 0.00877275 2,21E-05 1,647751 0,720498 AT3G26090 ATRGS1, RGS1 ATRGS1 RGS1 G-protein coupled receptors:GTPase activators A\_84\_P809384 0,04406328 0,0029002 1,646058 0,719015 AT1G76180 ERD14 ERD14\_\_Dehydrin family proteinERD14\_\_Dehydrin family protein A\_84\_P833006 0,04418246 0,0029253 1,645951 0,718922 AT5G07730 0.02951533 0.0011169 A 84 P851226 1,645702 0,718703 AT3G46640 LUX, PCL1 LUX PCL1 Homeodomain-like superfamily protein A\_84\_P79235 0,01498409 1,63E-04 1,643919 0,717139 AT3G11850 Protein of unknown function, DUF593Protein of unknown function, DUF593 0,001826 A\_84\_P801314 0,03612883 1,64258 0,715963 AT5G26600 Pyridoxal phosphate (PLP)-dependent transferases superfamily protein A\_84\_P14271 0,04465805 0,0030097 1,642277 0,715697 AT1G66350 RGL, RGL1 RGL RGL1 RGA-like 1 A 84 P19565 0.02676006 8,71E-04 CYP81F1, CYP91A CYP81F1 CYP91A2 cytochrome P450, family 91, subfamily A, polypeptide 2 1,640146 0,713824 AT4G37430 A\_84\_P525365 0,0015556 1,638989 0,712806 AT3G57500 0,03367786 A 84 P258390 0.04066901 0,0024491 1,638342 0,712236 AT4G25170 Uncharacterised conserved protein (UCP012943) A 84 P789465 0.02955248 0,0011204 1,637404 0,711411 AT1G49720 ABF1 ABF1 AtABF1 abscisic acid responsive element-binding factor 1 A\_84\_P805290 0,04324035 0,0027809 1,63669 0,710781 AT1G75800 Pathogenesis-related thaumatin superfamily protein A\_84\_P224479 0,02053168 4,20E-04 1,63641 0,710534 AT5G16650 Chaperone DnaJ-domain superfamily protein A 84 P10858 0.01771716 2,71E-04 1,635349 0,709599 AT3G47160 RING/U-box superfamily protein A\_84\_P816640 0,0243154 6,81E-04 1,634456 0,708811 AT5G27380 GSH2, GSHB AtGSH2\_GSH2\_GSHB\_\_glutathione synthetase 2 A 84 P11453 0,02445181 6,90E-04 1,63373 0,70817 AT1G53320 AtTLP7, TLP7 AtTLP7\_TLP7\_tubby like protein 7 A\_84\_P55070 0.02682222 8,75E-04 1,632891 0,707428 AT4G13030 P-loop containing nucleoside triphosphate hydrolases superfamily protein A\_84\_P100746 0,01785415 2.81E-04 1,63274 0,707295 AT3G28220 TRAF-like family protein A\_84\_P784699 0.04760732 0,0035326 1,63013 0,704987 AT5G41600 BTI3, RTNLB4 BTI3\_RTNLB4\_\_VIRB2-interacting protein A 84 P13014 0,0364947 0.0018807 1.629889 0.704774 AT5G12110 Glutathione S-transferase, C-terminal-like; Translation elongation factor EF1B/ribosomal protein S6 A\_84\_P129666 0,03884352 0,0021491 1,629578 0,704498 AT5G07730 A 84 P815258 6,70E-05 1,629353 0,704299 AT4G25690; CPuORF13 0,01156977 A 84 P13043 0.02507846 7,56E-04 1,629177 0,704144 AT5G25440 Protein kinase superfamily protein A\_84\_P18693 0,03405143 0,0015914 1,628954 0,703946 AT1G18270 ketose-bisphosphate aldolase class-II family protein D-mannose binding lectin protein with Apple-like carbohydrate-binding domain A\_84\_P785373 0,02991192 0,0011546 1,628449 0,703499 AT1G78850 A 84 P183754 0,04181817 0,0026041 1,628319 0,703384 AT3G27420 A\_84\_P11150 0,0308525 0,0012461 1,627381 0,702552 AT5G24590 ANAC091, TIP ANAC091 TIP TCV-interacting proteinANAC091 TIP TCV-interacting protein A\_84\_P107602 0,03875106 0,0021359 1,627369 0,702541 AT3G17860 JAI3, JAZ3, TIFY6B JAI3\_JAZ3\_TIFY6B \_\_jasmonate-zim-domain protein 3 A 84 P12615 0.02238792 5,26E-04 1,626652 0,701905 AT2G32235; 0 A\_84\_P812535 0,01830757 3,03E-04 1,626203 0,701508 AT4G11650 ATOSM34, OSM34 ATOSM34\_OSM34\_osmotin 34ATOSM34\_OSM34\_osmotin 34 0,0036061 A\_84\_P11473 0.04799153 1,626075 0,701394 AT1G49650 alpha/beta-Hydrolases superfamily protein A 84 P12764 0,04714022 0.0034525 1,625593 0,700966 AT3G50760 GATL2 GATL2 galacturonosyltransferase-like 2 A\_84\_P18117 Pathogenesis-related thaumatin superfamily protein 0,02400674 6,46E-04 1,624354 0,699866 AT1G75800 A\_84\_P765062 0,02943565 0,0010996 1,623314 0,698942 AT4G24415 MIR824A MIR824A\_MIR824a; miRNA A 84 P17036 0,0145606 1,50E-04 1,622934 0,698605 AT1G77000 ATSKP2;2, SKP2B ATSKP2;2\_SKP2B\_\_RNI-like superfamily protein A\_84\_P22863 0,01565107 1,88E-04 1,621051 0,696929 AT1G22280 PAPP2C PAPP2C\_\_phytochrome-associated protein phosphatase type 2C A\_84\_P205698 0.04572418 0.0031885 1.620705 0.696622 AT2G27260 Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family A\_84\_P811421 0,03707299 0,0019498 1,620353 0,696308 AT5G20250 DIN10, RS6 DIN10\_RS6\_\_Raffinose synthase family proteinDIN10\_RS6\_\_Raffinose synthase family protein A 84 P803838 0.03544915 0,0017626 1,618159 0,694353 AT4G25670; CPuORF12CPuORF13 A\_84\_P824252 0,03850592 0,0021143 1,614481 0,691071 AT3G08710 ATH9, TH9, TRX H ATH9\_TH9\_TRX H9\_thioredoxin H-type 9 A 84 P238313 0,04288919 0.0027335 1,61416 0,690784 AT4G26060 Ribosomal protein L18ae family A\_84\_P23843 0,04261217 0,0026991 1,612606 0,689394 AT2G03200 Eukaryotic aspartyl protease family protein A\_84\_P86519 0.04552032 0.0031627 1.61199 0.688843 AT5G04040 SDP1 SDP1\_Patatin-like phospholipase family protein A\_84\_P18500 0,03217042 0,0013846 1,611855 0,688722 AT4G01700 Chitinase family protein A 84 P13049 0.01622046 2,05E-04 1,610694 0,687682 AT1G55850 ATCSLE1, CSLE1 ATCSLE1 CSLE1 cellulose synthase like E1 A\_84\_P562730 0,02039214 4,12E-04 1,609726 0,686815 AT4G38060 CCI2 A 84 P10724 0.00230433 4,27E-07 1,60929 0,686424 AT2G30020 Protein phosphatase 2C family protein A 84 P19752 0.02054225 4,24E-04 1,607769 0,68506 AT5G57900 SKIP1 SKIP1\_SKP1 interacting partner 1 A\_84\_P16007 1,607305 0,684644 AT5G66880 SNRK2.3, SNRK2-3 SNRK2-3\_SNRK2.3\_SRK2I\_sucrose nonfermenting 1(SNF1)-related protein kinase 2.3 0,01958881 3,60E-04 A 84 P198394 0.04461956 0.0029922 1.606945 0.68432 AT4G16670 Plant protein of unknown function (DUF828) with plant pleckstrin homology-like region

A 84 P848088	0,04556191	0,003174	1 60694	0,684226 AT1G63830		PLAC8 family proteinPLAC8 family protein
A_84_P764462	0,04550191	0,0020249	,	0,683988 AT4G03510		ATRMA1_RMA1_RING membrane-anchor 1
A 84 P786348	0,03432886	0,0020249		0,683464 AT1G69360		Plant protein of unknown function (DUF863)
A_84_P23887	0,03432880	,	,	0,683384 AT2G22080		Fiant protein of unknown function (DOF603)
A_84_P15331	0,03518269			0,682013 AT2G43620		Chitinase family protein
A_84_P18852	0,02400674			0,681831 AT5G15870		glycosyl hydrolase family 81 protein
	0,02400074			0,681418 AT3G26740	CCI	CCL_CCR-likeCCL_CCR-like
A_84_P16468						
A_84_P13801	0,02100365	-			A1031034, 031034	ATOSM34_OSM34_osmotin 34
A_84_P848912	0,01775137			0,680077 AT5G42050		DCD (Development and Cell Death) domain protein
A_84_P857541	0,04813688	0,003646		0,678658 AT4G26750	EXT-like	EXT-like_LIP5hydroxyproline-rich glycoprotein family protein
A_84_P11479	0,02115263			0,678472 AT1G01260		JAM2_basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P10767	0,01320943				ATHSFATE, HSFAT	ATHSFA1E_HSFA1E_heat shock transcription factor A1E
A_84_P858372	0,03529944			0,674731 AT1G75800		Pathogenesis-related thaumatin superfamily protein
A_84_P514578	0,04042752	,	,	0,674687 AT2G48060		
A_84_P515753	0,03258517			0,674644 AT4G31510		
A_84_P12278	0,03359263	0,0015442		0,674027 AT1G12320		Protein of unknown function (DUF1442)
A_84_P830480	0,0364947				ATFIP1[V], ATFIPS	ATFIP1[V]_ATFIPS5_FIP1[V]_FIPS5homolog of yeast FIP1 [V]
A_84_P19320	0,03949743			0,672394 AT3G16510		Calcium-dependent lipid-binding (CaLB domain) family protein
A_84_P567376	0,02452861			0,671153 AT5G66050		Wound-responsive family proteinWound-responsive family protein
A_84_P17510	0,04152048	0,0025578	1,592232	0,67105 AT1G23870	ATTPS9, TPS9	ATTPS9_TPS9trehalose-phosphatase/synthase 9
A_84_P761677	0,03507878	0,0017004	1,592227	0,671046 AT3G45638		other RNA
A_84_P22255	0,03051006	0,001209	1,591696	0,670565 AT3G58710	ATWRKY69, WRKY	ATWRKY69_WRKY69WRKY DNA-binding protein 69
A_84_P17345	0,02354743	5,93E-04	1,591464	0,670355 AT1G44790		ChaC-like family protein
A_84_P846313	0,0457815	0,0032268	1,591035	0,669966 AT3G11420		Protein of unknown function (DUF604)
A_84_P23665	0,03076559	0,0012383	1,589834	0,668876 AT1G03610		Protein of unknown function (DUF789)
A_84_P23239	0,0227833	5,52E-04	1,589633	0,668694 AT4G04220	AtRLP46, RLP46	AtRLP46_RLP46receptor like protein 46
A_84_P21167	0,04444991	0,002972	1,588555	0,667715 AT3G02875	ILR1	ILR1_Peptidase M20/M25/M40 family protein
A_84_P573251	0,03206014	0,0013751	1,588419	0,667591 AT3G47200		Plant protein of unknown function (DUF247)
A_84_P796327	0,048729	0,0037986		0,6674 AT5G20900	JAZ12, TIFY3B	JAZ12_TIFY3Biasmonate-zim-domain protein 12
A_84_P609756	0.01386738	1,33E-04	1,586999	0,666301 AT3G50030		ARM-repeat/Tetratricopeptide repeat (TPR)-like protein
A_84_P826661	0,0364947			0,664471 AT3G51890		CLC3_Clathrin light chain proteinCLC3_Clathrin light chain protein
A_84_P22526	0,04922931			0,664459 AT5G40100		Disease resistance protein (TIR-NBS-LRR class) family
A_84_P12986	0,00772467			0,664071 AT5G04020		calmodulin binding
A_84_P86089	0,01163687			0,662608 AT4G25030		g
A 84 P790810	0,02454009	-		0,662493 AT5G02200	FHI	FHLfar-red-elongated hypocotyl1-like
A_84_P724318	0,02551475	7,91E-04	,	0,661804 AT5G06865		other RNA
A_84_P76794	0,02390528			0,659668 AT3G26680	ATSNM1_SNM1	ATSNM1_SNM1_DNA repair metallo-beta-lactamase family protein
A_84_P533074	0,04994319			0,659073 AT1G78895		Reticulon family protein
A_84_P75484	0,04521387	0,0031124	,	,	NTMC2T6.2_NTMC	NTMC2T6.2_NTMC2TYPE6.2Calcium-dependent lipid-binding (CaLB domain) family protein
A_84_P13407	0,01592639		1,577642			CATSOT17_ATST5C_SOT17sulfotransferase 17
A_84_P868662	0,03187662	0,0013471		0,657027 AT4G30470	A130117, A13130	NAD(P)-binding Rossmann-fold superfamily protein
A_84_P845383	0,01947035	,	,	0,655734 AT3G51090		Protein of unknown function (DUF1640)
A_84_P11179	0,03076559	0,0012368		0,655284 AT5G40170		AtRLP54_RLP54_receptor like protein 54
					AINLE 34, NLF 34	
A_84_P511815	0,04103823 0,03134441	0,0024953 0,0012999		0,654747 AT2G18193 0,653233 AT5G51480	eken	P-loop containing nucleoside triphosphate hydrolases superfamily protein SKS2_SKU5 similar 2
A_84_P21622					01/02	
A_84_P824199	0,04418246	0,0029374	,	0,652991 AT5G66050	כוחח	Wound-responsive family protein DD2 protein pump interactor 2
A_84_P582979	0,03916847			0,651629 AT3G15340		PPI2_proton pump interactor 2
A_84_P11244	0,01331538	-				ATEXO70B1_EXO70B1exocyst subunit exo70 family protein B1
A_84_P219858	0,04669211	,	,	0,647718 AT5G13190	,	AtGILP_GILP
A_84_P23085	0,0362616	0,0018465				AFH1_AHF1_ATFH1_FH1formin homology 1
A_84_P809423	0,0487809	0,003805	1,566463	0,647511 AT1G76180	ERD14	ERD14Dehydrin family protein

A_84_P571809 0,030758	· · · ·	,	
A_84_P10689 0,04418246		0,646726 AT2G18750	
A_84_P826018 0,01989875	, , ,	0,646652 AT4G30240	, ,,
A_84_P16892 0,02376995		3 0,646521 AT1G56140	· · ·
A_84_P16856 0,03044238		0,645931 AT5G41600	
A_84_P171153 0,04917615		6 0,644665 AT2G37940	
A_84_P15422 0,02230205	5,17E-04 1,563223	3 0,644524 AT2G23450	D Protein kinase superfamily protein
A_84_P785057 0,02023179			0 ATSERK4, BAK7, B ATSERK4_BAK7_BKK1_SERK4somatic embryogenesis receptor-like kinase 4
A_84_P24061 0,04633552	0,0032936 1,562764	1 0,6441 AT3G19010	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
A_84_P509060 0,04176612	0,0025841 1,561248	3 0,642699 AT1G63830	D PLAC8 family proteinPLAC8 family protein
A_84_P857361 0,02026665	4,07E-04 1,560733	3 0,642223 AT4G28400	D Protein phosphatase 2C family protein
A_84_P121962 0,04769728	0,0035547 1,560642	2 0,642139 AT3G27880	Protein of unknown function (DUF1645)
A_84_P14902 0,03850592	0,0021144 1,560615	5 0,642115 AT5G11670	) ATNADP-ME2, NA ATNADP-ME2_NADP-ME2NADP-malic enzyme 2
A_84_P16129 0,04017467	0,002379 1,56003	3 0,641574 AT1G60140	0 ATTPS10, TPS10 ATTPS10_TPS10_trehalose phosphate synthase
A_84_P825682 0,04641205	0,0033372 1,558116	0,639803 AT5G66070	D RING/U-box superfamily protein
A_84_P562314 0,0453766	0,0031366 1,557658	3 0,639379 AT2G04080	D MATE efflux family protein
A_84_P515273 0,02091737	4,49E-04 1,55716	5 0,638922 AT1G61255	
A_84_P826495 0,04633552	0,0032966 1,555966	6 0,637811 AT3G55450	) PBL1 PBL1_PBS1-like 1PBL1_PBS1-like 1
A_84_P716286 0,01276785		2 0,637334 AT5G48530	
A_84_P286360 0,03533791			) ATLCAT3, LCAT3 ATLCAT3_LCAT3_lecithin:cholesterol acyltransferase 3
A_84_P858684 0,01961223		0,635305 AT4G39090	
A_84_P23854 0,00772467		1 0,634606 AT2G31810	
A_84_P20252 0,01592639			) ATNUDT13, ATNU ATNUDT13_ATNUDX13_NUDX13nudix hydrolase homolog 13
A_84_P295744 0,01961223	, ,	0,631772 AT2G36310	
A_84_P846636 0,01652713			
A 84 P23679 0,04521387			) AtJ8, AtToc12, J8, AtJ8_AtToc12_DJC22_J8_Toc12_Chaperone DnaJ-domain superfamily protein
A_84_P23188 0,02740352		0,629969 AT3G55450	
A_84_P824693 0,02511608		0,626987 AT5G58787	
A_84_P23929 0,02449762			ATTPS11, ATTPSB ATTPS11_ATTPSB_TPS11trehalose phosphatase/synthase 11
A_84_P852053 0,03529944		0,625298 AT4G25030	
A_84_P787591 0,03533791		0,623578 AT4G25050	
		0,622792 AT1G03740	· ·
A_84_P19881 0,02020968	, , ,	,	
A_84_P16434 0,04421492			) ATH9, TH9, TRX H ATH9_TH9_TRX H9thioredoxin H-type 9
A_84_P18545 0,04991401			ATWRKY65, WRKY ATWRKY65_WRKY65_WRKY DNA-binding protein 65
A_84_P23535 0,02477556			0 CIPK21, SnRK3.4 CIPK21_SnRK3.4_CBL-interacting protein kinase 21
A_84_P20352 0,00894727		5 0,617209 AT3G55890	
A_84_P17180 0,02342488		2 0,615606 AT1G18210	<b>3 7</b>
A_84_P818289 0,03367786		3 0,615391 AT3G47550	<b>v</b> , <i>y</i> ,
A_84_P823703 0,0288939	, , ,	7 0,615011 AT5G52882	
A_84_P592683 0,02497593		7 0,614632 AT2G30720	
A_84_P816721 0,03076559			3; CPuORF49,ATHSF4,AT-HSFB1,HSF4,HSFB1
A_84_P271010 0,03006957		3 0,613449 AT4G02550	
A_84_P23695 0,02991109	0,0011466 1,529087	7 0,61267 AT1G09250	
A_84_P600173 0,03454188	· · · ·	3 0,612294 AT5G03490	, , , , , , , , , , , , , , , , , , , ,
A_84_P11377 0,04181817		0,611334 AT1G22890	
A_84_P848098 0,02381684	6,25E-04 1,522123	3 0,606085 AT3G08710	) ATH9, TH9, TRX H ATH9_TH9_TRX H9thioredoxin H-type 9
A_84_P102836 0,02140161	4,71E-04 1,520263	3 0,604321 AT3G54620	) ATBZIP25, BZIP25, ATBZIP25_BZIP25_BZO2H4basic leucine zipper 25
A_84_P532550 0,02100365	4,52E-04 1,519573	3 0,603666 AT4G18140	O SSP4b SSP4b_SCP1-like small phosphatase 4b
A_84_P16726 0,03585444	0,0018047 1,519003	3 0,603125 AT4G36140	disease resistance protein (TIR-NBS-LRR class), putative
A_84_P247395 0,01386093	1,32E-04 1,518802	2 0,602934 AT3G26470	D Powdery mildew resistance protein, RPW8 domain
A_84_P13876 0,03438211	0,0016204 1,517646	6 0,601835 AT4G33160	D F-box family protein
	.,		

A\_84\_P11368 0.01658668 2.19E-04 1.516155 0.600417 AT1G61260 Protein of unknown function (DUF761) A 84 P104016 0.03075464 0.0012272 1,515679 0,599964 AT1G05805 AKS2 basic helix-loop-helix (bHLH) DNA-binding superfamily protein A\_84\_P563023 0.0362616 0,0018465 1,514719 0,599051 AT1G17145 RING/U-box superfamily protein A\_84\_P601724 RIN4\_\_RPM1 interacting protein 4 0,01565107 1,87E-04 1,513274 0,597673 AT3G25070 RIN4 ATCNGC20, CNBT ATCNGC20 CNBT1 CNGC20 cyclic nucleotide-binding transporter 1 A 84 P24015 0,02991192 0.0011549 1,511968 0,596428 AT3G17700 A\_84\_P560796 0,02546071 7,87E-04 1,510989 0,595493 AT3G49550 8,82E-04 A\_84\_P190014 0,02693361 1,51036 0,594892 AT4G36030 ARO3 ARO3 armadillo repeat only 3 A\_84\_P527116 0,02167651 4,88E-04 1,508931 0,593527 AT3G48180 A 84 P817922 0.0407948 0.0024649 1,508367 0,592987 AT5G39410 Saccharopine dehydrogenase A\_84\_P14067 0,04896518 0,0038439 1,50828 0,592904 AT5G54860 Major facilitator superfamily protein A 84 P210718 0.04533307 0,0031308 1,508174 0,592803 AT3G60200 A\_84\_P268440 0.03991389 0.0023248 1,508117 0,592749 AT2G15270 A\_84\_P195794 0,04176612 0,0025827 1,505281 0,590033 AT4G19390 Uncharacterised protein family (UPF0114) A\_84\_P306090 0.019105 3,41E-04 1,505148 0,589905 AT2G05260 alpha/beta-Hydrolases superfamily protein PARG2 poly(ADP-ribose) glycohydrolase 2 A 84 P145439 0.04473491 0.0030442 1,50365 0,588469 AT2G31865 PARG2 A\_84\_P15196 0,03134441 0,0013002 1,503363 0,588193 AT1G03080 NET1D\_kinase interacting (KIP1-like) family protein A 84 P822818 0.04975333 0.0040029 1,502816 0,587669 AT3G03310 ATLCAT3, LCAT3 ATLCAT3\_LCAT3\_lecithin:cholesterol acyltransferase 3 A\_84\_P17328 0.02189142 4,99E-04 1,502294 0,587167 AT2G39350 ABCG1 ABCG1 ABC-2 type transporter family protein A\_84\_P826468 0.03359263 0.0015334 1.501221 0.586136 AT1G21010 A\_84\_P22022 0.04917615 0,0038908 1,501039 0,585961 AT2G22330 CYP79B3 CYP79B3\_\_cytochrome P450, family 79, subfamily B, polypeptide 3 A 84 P66154 0,0145606 1,51E-04 1,500872 0,585801 AT5G63880 VPS20.1 VPS20.1 SNF7 family protein A\_84\_P10582 0,02277204 5,48E-04 1,50043 0,585376 AT1G78600 BBX22, DBB3, LZF BBX22\_DBB3\_LZF1\_STH3\_\_light-regulated zinc finger protein 1 A 84 P171853 0,01163687 7,08E-05 1,500097 0,585055 AT1G13390 A 84 P103326 0,02449767 7,01E-04 -1,50047 -0,58541 AT1G61990 Mitochondrial transcription termination factor family protein A\_84\_P597006 0,03355331 0,0015169 -1,50197 O-Glycosyl hydrolases family 17 protein -0,58686 AT1G66250 A\_84\_P20046 0,0322775 0,0014009 -1,50441 -0,5892 AT1G14280 PKS2 PKS2\_phytochrome kinase substrate 2 A 84 P219518 0,04473491 0,003047 -1,50485 -0,58962 AT1G65295 -1,50514 -0,5899 AT1G66730 AtLIG6, LIG6 A\_84\_P833726 0,03014083 0,0011813 AtLIG6 LIG6 DNA LIGASE 6 A\_84\_P249845 0,03960313 0,0022977 -1,50591 -0,59063 AT3G46870 Pentatricopeptide repeat (PPR) superfamily protein A 84 P820158 0,04418246 0.0029296 -1,50729 -0,59195 AT2G18050 HIS1-3 HIS1-3 histone H1-3 A\_84\_P135105 0,02612251 8,32E-04 -1,50775 -0,59239 AT3G17640 Leucine-rich repeat (LRR) family protein A\_84\_P21908 0.0451422 0.0031029 -1.50784 -0.59249 AT1G51830 Leucine-rich repeat protein kinase family protein A 84 P597910 0.04633552 0.0032962 -1,50811 ATSOFL2, SOFL2 ATSOFL2 SOFL2 SOB five-like 2 -0,59274 AT1G68870 ATCFM3B, CFM3B ATCFM3B CFM3B CRM family member 3B A\_84\_P274710 0,04173238 0,0025776 -1.50832 -0,59294 AT4G14510 A\_84\_P16440 0,03628755 0,0018514 -1,5093 -0,59388 AT3G04140 Ankyrin repeat family protein A 84 P814976 4,88E-04 -1,50984 -0,5944 AT2G21970 SEP2 SEP2 stress enhanced protein 2 0,02167651 A\_84\_P16685 0,0033204 -1,51131 GDSL-like Lipase/Acylhydrolase superfamily protein 0,04636379 -0,5958 AT4G26790 A 84 P199044 0.03927432 0.0022339 -1.51182 -0.59629 AT1G63150 Tetratricopeptide repeat (TPR)-like superfamily protein A\_84\_P848439 0,04416341 0,002914 -1,51262 -0,59705 AT1G11860 Glycine cleavage T-protein family A 84 P254330 0,03739099 0.0019835 -1,51277 -0,59719 AT4G17600 LIL3:1 LIL3:1 Chlorophyll A-B binding family protein A\_84\_P15056 0,038253 0,0020837 -1,51368 HSL2\_HAESA-like 2 -0,59806 AT5G65710 HSL2 A 84 P502211 0,04006887 0,002348 -1,51496 -0,59928 AT3G18970 MEF20 MEF20 mitochondrial editing factor 20 0,04017467 A\_84\_P852804 0,0023723 -1,51576 -0,60004 AT5G21430 CRRL, NdhU CRRL\_NdhU\_\_Chaperone DnaJ-domain superfamily protein A\_84\_P792605 0.0342136 0.0016069 -1.5158 -0.60008 AT3G61310 AHL11 AT hook motif DNA-binding family protein A\_84\_P520864 0,02991192 0,0011505 -1,51653 -0,60078 AT3G23370 RNA-binding (RRM/RBD/RNP motifs) family protein A 84 P22024 0.01902633 3,38E-04 -1,51675 -0,60098 AT1G07640 OBP2 OBP2 URP3 Dof-type zinc finger DNA-binding family protein ATOFP7\_OFP7\_ovate family protein 7 A\_84\_P269540 0,04636379 0,0033192 -1,51692 -0,60114 AT2G18500 ATOFP7, OFP7 A 84 P187824 0,04521387 0,0031152 -1,51754 -0,60173 AT2G31625; ATDPB2,CYL2,DPB2 A 84 P19654 0,01504028 1,66E-04 -1,51765 -0,60184 AT5G23070 AtTK1b\_TK1b\_\_Thymidine kinase A\_84\_P10143 AtMAN6\_MAN6\_Glycosyl hydrolase superfamily protein 0,04006887 0,0023487 -1,51774 -0,60192 AT5G01930 AtMAN6, MAN6 A 84 P21919 0,03910996 0,002191 -1,51794 -0,60212 AT1G25440 BBX15 B-box type zinc finger protein with CCT domain

A_84_P242453	0,0362341	0,0018344 -1,51816	-0,60232 AT5G22020		Calcium-dependent phosphotriesterase superfamily protein
A_84_P784376	0,01633501	2,12E-04 -1,5189	-0,60302 AT3G61550		RING/U-box superfamily protein
A_84_P22494	0,03884352	0,0021488 -1,52094		EFO2, RUP2	EFO2_RUP2Transducin/WD40 repeat-like superfamily protein
A_84_P823506	0,04418246	0,0029406 -1,52108	-0,6051 AT1G06690		NAD(P)-linked oxidoreductase superfamily protein
A_84_P18709	0,04461956	0,0029978 -1,52111			PYRDpyrimidine d
A_84_P19909	0,03764553	0,0020148 -1,52128	-0,60529 AT1G04180	YUC9	YUC9_YUCCA 9
A_84_P863005	0,04317679	0,0027689 -1,52141	-0,60541 AT1G80030		DJA7Molecular chaperone Hsp40/DnaJ family protein
A_84_P814482	0,03999281	0,0023384 -1,52358	-0,60747 AT2G43710	FAB2, SSI2	AtSSI2_FAB2_LDW1_SSI2Plant stearoyl-acyl-carrier-protein desaturase family protein
A_84_P16824	0,00884543	2,41E-05 -1,52381	-0,60768 AT5G25280		serine-rich protein-related
A_84_P841750	0,04636379	0,003312 -1,52436	-0,6082 AT5G15980		Pentatricopeptide repeat (PPR) superfamily protein
A_84_P231009	0,02894387	0,0010463 -1,52491	-0,60873 AT1G01070		UMAMIT28nodulin MtN21 /EamA-like transporter family protein
A_84_P17580	0,03359263	0,0015368 -1,52718			ATOPT7_OPT7oligopeptide transporter 7
A_84_P810803	0,02951533	0,0011147 -1,52933	-0,6129 AT3G55360	ATTSC13, CER10,	ATTSC13_CER10_ECR_GLH6_TSC133-oxo-5-alpha-steroid 4-dehydrogenase family protein
A_84_P229999	0,04455438	0,002981 -1,52992	-0,61346 AT4G31330		Protein of unknown function, DUF599
A_84_P832601	0,04691907	0,0034228 -1,53085	-0,61433 ATCG01010	NDHF	NDHFNADH-Ubiquinone oxidoreductase (complex I), chain 5 protein
A_84_P798859	0,04831925	0,0037132 -1,53094	-0,61442 AT1G73970		
A_84_P814104	0,02991192	0,001159 -1,53114	-0,6146 AT5G23060	CaS	CaScalcium sensing receptor
A_84_P176234	0,03090113	0,001254 -1,53174	-0,61517 AT2G36070	ATTIM44-2, TIM44-	ATTIM44-2_TIM44-2translocase inner membrane subunit 44-2
A_84_P832189	0,04811797	0,0036324 -1,53269	-0,61607 AT5G67030	ABA1, ATABA1, AT	ABA1_ATABA1_ATZEP_IBS3_LOS6_NPQ2_ZEPzeaxanthin epoxidase (ZEP) (ABA1)
A_84_P796549	0,03959642	0,0022876 -1,53299	-0,61634 AT3G06868		
A_84_P551820	0,03454188	0,0016479 -1,53385	-0,61716 AT3G04770	RPSAb	RPSAb40s ribosomal protein SA B
A_84_P244465	0,03358237	0,001526 -1,53398	-0,61728 AT3G28920	AtHB34, HB34, ZH	AtHB34_HB34_ZHD9homeobox protein 34
A_84_P13830	0,04669211	0,0033867 -1,53413	-0,61742 AT4G22910	CCS52A1, FZR2	CCS52A1_FZR2FIZZY-related 2
A_84_P751425	0,04831925	0,0037181 -1,5344	-0,61768 AT1G64770	NDF2, NDH45, Pns	NDF2_NDH45_PnsB2NDH-dependent cyclic electron flow 1
A_84_P17816	0,03026108	0,0011907 -1,53508	-0,61831 AT5G45428;	CPuORF24	
A_84_P52410	0,04418246	0,002932 -1,53508	-0,61831 AT4G37080		Protein of unknown function, DUF547
A_84_P22890	0,0312771	0,0012901 -1,53527	-0,61849 AT1G14410	ATWHY1, PTAC1,	ATWHY1_PTAC1_WHY1ssDNA-binding transcriptional regulator
A_84_P21158	0,02943565	0,0010994 -1,53594	-0,61912 AT3G04550		
A_84_P20800	0,02342488	5,81E-04 -1,53617	-0,61934 AT1G17700	PRA1.F1	PRA1.F1_prenylated RAB acceptor 1.F1
A_84_P786032	0,04991401	0,0040314 -1,53671	-0,61985 AT4G26850	VTC2	VTC2mannose-1-phosphate guanylyltransferase (GDP)s;
A_84_P200084	0,01198826	8,55E-05 -1,53701	-0,62012 AT5G22090		Protein of unknown function (DUF3049)
A_84_P15878	0,0320905	0,0013771 -1,53746	-0,62055 AT5G25265		
A_84_P18535	0,04368648	0,0028415 -1,53781	-0,62088 AT4G13020	MHK	MHK_Protein kinase superfamily protein
A_84_P13453	0,01610925	2,00E-04 -1,53835	-0,62139 AT2G34060		Peroxidase superfamily protein
A_84_P835993	0,02780123	9,50E-04 -1,539	-0,62199 AT3G07610	IBM1	IBM1Transcription factor jumonji (jmjC) domain-containing protein
A_84_P826941	0,04488314	0,0030738 -1,53919	-0,62217 AT1G03400		2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
A_84_P13380	0,03010546	0,0011771 -1,5398	-0,62275 AT1G79890		RAD3-like DNA-binding helicase protein
A_84_P13476	0,02009357	3,99E-04 -1,53997	-0,62291 AT2G18230	AtPPa2, PPa2	AtPPa2_PPa2pyrophosphorylase 2
A_84_P16395	0,04292951	0,0027411 -1,54004	-0,62297 AT2G03480	QUL2	QUL2QUASIMODO2 LIKE 2
A_84_P558547	0,04838306	0,0037401 -1,5408	-0,62368 AT5G65850		F-box and associated interaction domains-containing protein
A_84_P23671	0,02069154	4,36E-04 -1,54309	-0,62582 AT1G22850		SNARE associated Golgi protein family
A_84_P21554	0,02892086	0,0010409 -1,54343	-0,62614 AT5G25380	CYCA2;1	CYCA2;1cyclin a2;1
A_84_P173411	0,03927432	0,0022348 -1,54364	-0,62633 AT3G22210		
A_84_P17664	0,04482194	0,0030622 -1,54375		KCS17	KCS173-ketoacyl-CoA synthase 17
A_84_P829818	0,02695145	8,86E-04 -1,54404	-0,62671 AT4G39470		Tetratricopeptide repeat (TPR)-like superfamily protein
A_84_P533478	0,02436664	6,85E-04 -1,54422			
A_84_P816178	0,03003507			ATCDSP32, CDSP	ATCDSP32_CDSP32chloroplastic drought-induced stress protein of 32 kD
A_84_P12263	0,04636379	0,0033053 -1,54481			ALG6, ALG8 glycosyltransferase family
A_84_P525958	0,03557096	0,0017704 -1,54536		LYM3	LYM3_LYP3Peptidoglycan-binding LysM domain-containing protein
A_84_P792532	0,04639575	0,0033295 -1,54585	-0,6284 AT1G16489		other RNA
A 84 P758952	0,03219107				Polyketide cyclase/dehydrase and lipid transport superfamily protein
		.,	.,		· · · · · · · · · · · · · · · · · · ·

A_84_P15712	0,03189056	0,001356 -1,54895		3, ATSCAR3_SCAR3_WAVE2SCAR family protein
A_84_P15048	0,04465805	0,0030141 -1,54912	-0,63145 AT5G63710	Leucine-rich repeat protein kinase family protein
A_84_P512133	0,04648656	0,0033515 -1,54949	-0,6318 AT4G15810	P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P14035	0,03542483	0,0017535 -1,5505	-0,63274 AT5G45960	GDSL-like Lipase/Acylhydrolase superfamily protein
A_84_P519451	0,03091118	0,0012557 -1,55126	-0,63344 AT5G52950	
A_84_P846263	0,03173109	0,0013264 -1,55146	-0,63362 AT1G11860	Glycine cleavage T-protein familyGlycine cleavage T-protein family
A_84_P17110	0,04829036	0,0036839 -1,55179	-0,63393 AT1G77850 ARF17	ARF17_auxin response factor 17
A_84_P814302	0,03959271	0,0022859 -1,55325	-0,63529 AT5G11420	Protein of unknown function, DUF642
A_84_P819943	0,0443487	0,0029617 -1,55391	-0,63591 AT3G14400 UBP25	UBP25_ubiquitin-specific protease 25
A_84_P837815	0,00904516	3,77E-05 -1,55438	-0,63634 AT5G44410	FAD-binding Berberine family protein
A_84_P200834	0,04972693	0,0039951 -1,55494	-0,63686 AT1G58070	
A_84_P283520	0,04478252	0,0030585 -1,55502	-0,63694 AT4G28740	
A_84_P10351	0,03001025	0,0011687 -1,55533	-0,63722 AT5G20380 PHT4;5	PHT4;5_phosphate transporter 4;5
A_84_P820541	0,04831884	0,0037018 -1,55577	-0,63763 AT3G02640	
A_84_P15584	0,04327241	0,002785 -1,55611	-0,63794 AT3G47250	Plant protein of unknown function (DUF247)
A_84_P21608	0,02584636	8,12E-04 -1,55631	-0.63813 AT5G47630 mtACP3	mtACP3 mitochondrial acyl carrier protein 3
A_84_P127121	0,03569937	0,0017858 -1,55639	-0,63821 AT5G44650 AtCEST, CEST, Y	
A_84_P15095	0,03014083	0,00118 -1,55737		I, FD-GOGAT_GLS1_GLU1_GLUSglutamate synthase 1
A_84_P166003	0,03681569	0,001924 -1,55868	-0,64032 AT3G13510	Protein of Unknown Function (DUF239)
A 84 P18937	0,0317688	0,0013295 -1,55925	-0,64085 AT1G08640 CJD1	CJD1Chloroplast J-like domain 1
A_84_P787659	0,03120647	0,0012794 -1,56015	-0,64169 AT4G09970	
A_84_P12807	0,04780289	0,0035726 -1,56119	-0,64264 AT3G60720 PDLP8	PDLP8_plasmodesmata-located protein 8
A_84_P16721	0,0475861	0,0035275 -1,56141	-0,64285 AT4G35030	Protein kinase superfamily protein
A_84_P547377	0,0340082	0,0015886 -1,56178	-0,64319 AT2G43110	
A_84_P11745	0,03907514	0,0021864 -1,56196	-0,64336 AT3G29200 ATCM1, CM1	ATCM1_CM1chorismate mutase 1
A_84_P11371	0,02417585	6,66E-04 -1,56309	-0,6444 AT1G64680	
A_84_P764999	0,03916847	0,0022195 -1,56309	-0,6444 AT4G26795	other RNA
A_84_P17948	0,02924256	0,001081 -1,56385	-0,6451 AT1G01860 PFC1	PFC1Ribosomal RNA adenine dimethylase family protein
A_84_P19075	0,02924250	6,79E-05 -1,56482	-0,64599 AT1G19920 APS2, ASA1	APS2_ASA1Pseudouridine synthase/archaeosine transglycosylase-like family protein
A_84_P819016	0,02826573	9,81E-04 -1,56575	-0,64685 AT2G17695	AF32_ASA1FSeudoundine synthase/archaeosine transgiycosylase-like family protein
A_84_P764875	0,01961223	3,72E-04 -1,56583	-0,64693 AT4G37925 NdhM, NDH-M	NDH-M_NdhMsubunit NDH-M of NAD(P)H:plastoquinone dehydrogenase complex
A_84_P22819	0,01301223	0,0019683 -1,5661	-0,64717 AT1G35910 TPPD	TPPD_Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
A_84_P763808	0,03727133	1,26E-04 -1,56614	-0,64722 AT4G08940	Ubiquitin carboxyl-terminal hydrolase family protein
A_84_P56690	0,01375994	1,27E-04 -1,56659	-0,64763 AT4G32770 ATSDX1, VTE1	ATSDX1_VTE1tocopherol cyclase, chloroplast/vitamin E deficient 1 (VTE1) / sucrose export defective 1
A_84_P824668	0,01742975	2,51E-04 -1,56706		0 AtMYB106_MYB106_NOKmyb domain protein 106
		6,86E-05 -1,56727		
A_84_P750079 A_84_P813537	0,01163687	0,0010464 -1,56758	-0,64825 AT1G75150	SBPASE_sedoheptulose-bisphosphatase
	0,02894387		-0,64854 AT3G55800 SBPASE	
A_84_P835427	0,04066901	0,0024472 -1,56852	-0,64941 AT1G66250	O-Glycosyl hydrolases family 17 protein
A_84_P223799	0,02948737	0,0011068 -1,56903	-0,64987 AT5G22100	RNA cyclase family protein
A_84_P858457	0,02381684	6,23E-04 -1,57074	-0,65144 AT4G28450	nucleotide binding;protein binding
A_84_P268710	0,0390369	0,0021804 -1,57166	-0,65229 AT2G47910 CRR6	CRR6chlororespiratory reduction 6
A_84_P238393	0,04521387	0,0031149 -1,57188	-0,65249 AT4G22900	Protein of unknown function (DUF1191)
A_84_P762707	0,03264978	0,0014382 -1,57431	-0,65472 AT3G06868	
A_84_P13335	0,03963988	0,0023015 -1,57497	-0,65533 AT1G08280	GALT29A_Glycosyltransferase family 29 (sialyltransferase) family protein
A_84_P568326	0,03010546	0,0011776 -1,57511	-0,65546 AT1G09812	
A_84_P20506	0,04730906	0,0034714 -1,57524		J ATGLR3.2_ATGLUR2_GLR3.2_GLUR2glutamate receptor 2
A_84_P13126	0,03333252	0,001503 -1,57548	-0,65579 AT5G56040	SKM2_Leucine-rich receptor-like protein kinase family protein
A_84_P819331	0,04830764	0,0036964 -1,57601	-0,65628 AT5G48790	Domain of unknown function (DUF1995)
A_84_P763996	0,04555509	0,0031723 -1,57611		D B1_BCH1_BETA-OHASE 1_chy1beta-hydroxylase 1
A_84_P23556	0,03891045	0,0021537 -1,57627	-0,65652 AT5G62230 ERL1	ERL1ERECTA-like 1
A_84_P11751	0,01132469	6,26E-05 -1,57793	-0,65804 AT3G13810 AtIDD11, IDD11	AtIDD11_IDD11indeterminate(ID)-domain 11

A_84_P13730	0,03411908	0,0015954 -1,57798	-0,65808 AT3G55340	PHIP1	PHIP1phragmoplastin interacting protein 1
A_84_P841956	0,01634308	2,13E-04 -1,57875	-0,65878 AT5G48790		Domain of unknown function (DUF1995)
A_84_P18482	0,00857212	1,84E-05 -1,5801	-0,66002 AT3G61460	BRH1	BRH1_brassinosteroid-responsive RING-H2
A_84_P11357	0,03895907	0,0021582 -1,58027	-0,66017 AT1G62500		Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
A_84_P786301	0,02915839	0,001071 -1,581	-0,66084 AT3G13940		DNA binding;DNA-directed RNA polymerases
A_84_P756542	0,01004329	4,58E-05 -1,58139	-0,66119 AT2G20724		
A_84_P817921	0,01774115	2,76E-04 -1,58141	-0,66121 AT1G68830	STN7	STN7STT7 homolog STN7
A_84_P772575	0,01742975	2,51E-04 -1,58149	-0,66128 AT1G62250		
A_84_P10436	0,02929986	0,0010857 -1,58347	-0,66309 AT1G09890		Rhamnogalacturonate lyase family protein
A_84_P20048	0,03785144	0,0020358 -1,58377	-0,66337 AT1G14345		NAD(P)-linked oxidoreductase superfamily protein
A_84_P811308	0,03914714	0,0022024 -1,58441	-0,66395 AT5G54770	THI1, THI4, TZ	THI1_THI4_TZthiazole biosynthetic enzyme, chloroplast (ARA6) (THI1) (THI4)
A_84_P847474	0,04092497	0,0024823 -1,58683	-0,66615 AT1G54385		ARM repeat superfamily proteinARM repeat superfamily protein
A_84_P849653	0,0362616	0,0018463 -1,58695	-0,66626 AT2G01680		Ankyrin repeat family protein
A_84_P788379	0,01768366	2,61E-04 -1,58755	-0,6668 AT3G14740		RING/FYVE/PHD zinc finger superfamily protein
A_84_P823140	0,02820842		-0,66682 AT5G51110		Transcriptional coactivator/pterin dehydratase
A_84_P602807	0,0145606	1,48E-04 -1,58761	-0,66686 AT4G31400	CTF7	AtCTF7_CTF7_ECO1damaged DNA binding;DNA-directed DNA polymerases
A_84_P186644	0,04418246	0,0029325 -1,5877	-0,66694 AT2G37240	••••	Thioredoxin superfamily protein
A 84 P22703	0,0145606	1,50E-04 -1,58822	-0,66741 AT1G31050		basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P294634	0,01961223	3,75E-04 -1,58869	-0,66784 AT5G53500		Transducin/WD40 repeat-like superfamily protein
A 84 P16435	0.04403443	0,0028972 -1,58924	-0.66834 AT3G07820		Pectin lyase-like superfamily protein
A_84_P23174	0,03258517	0,0014323 -1,59031		ΔΟΔ1 ΔΤΔΟΔ1 Ο	A ACA1_ATACA1_CAH1alpha carbonic anhydrase 1
A_84_P186514	0,02454009	7,16E-04 -1,59061	-0,66958 AT5G45100		BRG1_SBP (S-ribonuclease binding protein) family protein
A_84_P18751	0,0390369	0,0021773 -1,59147	-0,67036 AT1G68570	DIGT	AtNPF3.1_NPF3.1Major facilitator superfamily protein
A_84_P10327	0,04234786	0,0026648 -1,5923			ABCF5_ATGCN5_GCN5_general control non-repressible 5
	,	, ,	-0,67151 AT2G22920		
A_84_P11672	0,04799153	, ,	,		SCPL12_serine carboxypeptidase-like 12
A_84_P856078	0,02084717	4,45E-04 -1,59305		AIBCAI, AISABP	3 ATBCA1_ATSABP3_CA1_SABP3carbonic anhydrase 1
A_84_P15425	0,03913154	0,0021932 -1,59305	-0,67179 AT1G67750		Pectate lyase family protein
A_84_P810038	0,04262674				BETA CA2_CA18_CA2_carbonic anhydrase 2
A_84_P10692	0,02507846		-0,67208 AT2G36880	MAT3	MAT3methionine adenosyltransferase 3
A_84_P785818	0,03003507		-0,67292 AT3G28200		Peroxidase superfamily protein
A_84_P20345	0,02381684			AIBIAF1, BIAF1,	ATBTAF1_BTAF1_CHA16_CHR16_RGD3DNA binding;ATP binding;nucleic acid binding
A_84_P847105	0,02612251	8,31E-04 -1,5955	-0,67401 AT5G53020	ATDD07 DDD DD	Ribonuclease P protein subunit P38-related
A_84_P575740	0,03220695	0,0013898 -1,59658		ATBPC7, BBR, BP	ATBPC7_BBR_BPC7_basic pentacysteine 7
A_84_P23100	0,0485757	0,0037809 -1,59817	-0,67643 AT3G15570		Phototropic-responsive NPH3 family protein
A_84_P13702	0,0286969		-0,67643 AT3G49240	emb1796	emb1796Pentatricopeptide repeat (PPR) superfamily protein
A_84_P727905	0,04294266	0,0027434 -1,59924	-0,67739 AT1G14650		SWAP (Suppressor-of-White-APricot)/surp domain-containing protein / ubiquitin family protein
A_84_P562243	0,04831925	0,0037107 -1,60027			Pentatricopeptide repeat (PPR) superfamily protein
A_84_P10702	0,02494938	7,48E-04 -1,60028	-0,67832 AT2G18570		UDP-Glycosyltransferase superfamily protein
A_84_P17178	0,03897839	0,002162 -1,60176	-0,67966 AT1G66520;	pde194	
A_84_P527692	0,03134441	0,0013001 -1,60259	-0,6804 AT1G62110		Mitochondrial transcription termination factor family protein
A_84_P308973	0,03367786	0,0015591 -1,60346	-0,68119 AT3G52040		
A_84_P18364	0,03830155	0,0020941 -1,60363	-0,68134 AT3G12750	ZIP1	AtZIP1_ZIP1zinc transporter 1 precursor
A_84_P14396	0,0215952	4,82E-04 -1,60386	-0,68155 AT1G29950;	AJAX2, CPuORF35,	AJAX2,CPuORF34,AJAX2
A_84_P20849	0,01526442	1,73E-04 -1,60492	-0,6825 AT1G27480		alpha/beta-Hydrolases superfamily protein
A_84_P261020	0,02170344	4,91E-04 -1,60528	-0,68283 AT3G10150	ATPAP16, PAP16	ATPAP16_PAP16purple acid phosphatase 16
A_84_P854905	0,02196731	5,03E-04 -1,60722	-0,68456 AT1G68010	ATHPR1, HPR	ATHPR1_HPRhydroxypyruvate reductase
A_84_P12819	0,04017467	0,0023621 -1,60738	-0,68472 AT3G44820		Phototropic-responsive NPH3 family protein
A_84_P23779	0,0274646	9,23E-04 -1,60753	-0,68485 AT1G68520		BBX14B-box type zinc finger protein with CCT domain
A_84_P77579	0,01633501	2,11E-04 -1,60813	-0,68538 AT3G22968;	CPuORF59	
A_84_P191924	0,04994319	0,0040402 -1,60827	-0,68551 AT5G46690		bHLH071beta HLH protein 71
A_84_P832800	0.02898836	0,0010574 -1,60878			NLP2_Plant regulator RWP-RK family protein
	3,02000000	1,00010	2,00001 / 1000210		

A\_84\_P585522 0,04831925 0.0037134 -1.60881 -0.686 AT2G20980 MCM10 MCM10 minichromosome maintenance 10 A 84 P786152 0,02751567 9,31E-04 -1,60957 -0,68668 AT1G77630 LYM3 LYM3 LYP3 Peptidoglycan-binding LysM domain-containing protein A 84 P784916 0,04669211 0.0033893 -1.60997 -0.68704 AT1G10370 ATGSTU17, ERD9, ATGSTU17 ERD9 GST30 GST30B GSTU17 Glutathione S-transferase family protein A\_84\_P503065 0,04528285 0,0031241 -1,61011 -0,68716 AT3G05470 Actin-binding FH2 (formin homology 2) family protein A 84 P178514 0,01961223 3,68E-04 -1,61015 -0,68719 AT1G22030 A\_84\_P251085 0,04473491 0,003039 -1,61065 -0,68764 AT2G47840 AtTic20-II, Tic20-II AtTic20-II\_Tic20-II\_Uncharacterised conserved protein ycf60 Plastid-lipid associated protein PAP / fibrillin family protein A\_84\_P23286 0.03278848 0,0014549 -1,61139 -0,68831 AT4G22240 A\_84\_P227839 0,01156977 6,60E-05 -1,61264 -0,68942 AT1G67050 A 84 P605175 0.04312844 -1,61342 -0,69012 AT2G39370 MAKR4 0.0027638 MAKR4 A\_84\_P808801 0,04400296 0,0028932 -1,61521 -0,69172 AT2G31660 EMA1, SAD2, URM EMA1\_SAD2\_URM9\_ARM repeat superfamily protein A 84 P13999 0,03308225 0.0014754 -1,6154 -0.69189 AT5G35220 EGY1 AMOS1 EGY1 Peptidase M50 family protein A 84 P753739 0.03902999 0,0021695 -1,61616 -0.69257 AT1G51680 4CL.1, 4CL1, AT4C 4CL.1 4CL1 AT4CL1 4-coumarate:CoA ligase 1 A\_84\_P861164 0.0380066 0,0020562 -1.61661 -0.69297 AT2G23790 Protein of unknown function (DUF607) A\_84\_P540376 0,04730906 0,003474 -1,61676 -0,69311 AT3G23290 LSH4 LSH4\_Protein of unknown function (DUF640) A 84 P20759 0,04829221 0.0036885 -1,61786 -0,69409 AT5G28500 A\_84\_P16477 0,03358237 0,0015217 -1,61853 -0,69468 AT3G26590 MATE efflux family protein A 84 P19904 0.04648082 0,00335 -1,62124 -0,69709 AT1G10370 ATGSTU17, ERD9, ATGSTU17 ERD9 GST30 GST30B GSTU17 Glutathione S-transferase family protein A 84 P834131 0,01937779 3,51E-04 -1,62209 -0,69786 AT4G14650 A\_84\_P285270 0.03687182 0.0019324 -1.62361 -0.69921 AT3G51750 A\_84\_P15158 0,03913938 0,0021968 -1,62672 -0,70197 AT1G62250 A 84 P817496 0.02951533 0,001112 -1,62759 -0,70274 AT1G55960 Polyketide cyclase/dehydrase and lipid transport superfamily protein A\_84\_P15294 0,03367786 0,001556 -1,62835 -0,70341 AT1G17920 HDG12 HDG12 homeodomain GLABROUS 12 A 84 P704286 0.04465805 0,0030211 -1,6293 AtWTF1 WTF1 Ubiguitin carboxyl-terminal hydrolase family protein -0,70425 AT4G01037 AtWTF1, WTF1 LGO SMR1 LOSS OF GIANT CELLS FROM ORGANS A 84 P599010 0,02400674 6,42E-04 -1,63213 -0,70675 AT3G10525 LGO, SMR1 A\_84\_P10343 0,04857402 0,0037776 -1,63249 AtNIK1\_NIK1\_NSP-interacting kinase 1 -0,70707 AT5G16000 NIK1 violaxanthin de-epoxidase-relatedviolaxanthin de-epoxidase-related A\_84\_P869349 0,02376995 6,17E-04 -1,63255 -0,70712 AT2G21860 -0,70752 AT2G35550 ATBPC7, BBR, BP ATBPC7 BBR BPC7 basic pentacysteine 7 A 84 P756553 0.02376995 6,18E-04 -1,633 Transmembrane amino acid transporter family protein A\_84\_P148568 0.0243154 6,83E-04 -1,63473 -0,70905 AT5G02180 A\_84\_P193384 0,04311589 0,0027615 -1,63497 -0,70926 AT5G67385 Phototropic-responsive NPH3 family protein A 84 P93009 0,02991192 0.0011505 -1,63608 -0,71024 AT2G14880 SWIB/MDM2 domain superfamily protein A\_84\_P820856 0,0390369 0,0021798 -1,63918 -0,71297 AT1G64770 NDF2, NDH45, Pns NDF2\_NDH45\_PnsB2\_\_NDH-dependent cyclic electron flow 1 A 84\_P18880 0,04775265 0,0035624 -1,63928 -0,71306 AT1G35250 ALT2 Thioesterase superfamily protein A 84 P823999 0.01340841 1,14E-04 -1,63976 -0,71349 AT5G23920 A\_84\_P19078 0,04899432 0,0038642 -1,64006 -0,71375 AT1G19190 alpha/beta-Hydrolases superfamily protein A\_84\_P752571 0,01742975 2,49E-04 -1,64016 DJA7\_\_Molecular chaperone Hsp40/DnaJ family protein -0,71384 AT1G80030 A 84 P21670 0,04641205 0,0033408 -1,64036 -0,71401 AT5G63800 BGAL6, MUM2 BGAL6 MUM2 Glycosyl hydrolase family 35 protein 5,96E-04 -1,64043 -0,71407 AT2G21860 violaxanthin de-epoxidase-relatedviolaxanthin de-epoxidase-related A\_84\_P221816 0,02355661 GDSL-like Lipase/Acvlhvdrolase superfamily protein A 84 P820316 0.03270588 0.0014461 -1.64115 -0.71471 AT4G26790 A\_84\_P206348 0,04489678 0,0030788 -1,64585 -0,71883 AT1G60060 Serine/threonine-protein kinase WNK (With No Lysine)-related A 84 P67034 0.03822321 0,0020785 -1,64586 -0,71884 AT1G55910 ZIP11 ZIP11 zinc transporter 11 precursor A\_84\_P868534 0,02237896 5,20E-04 -1,64636 -0,71928 AT3G01060 A 84 P582063 0,00884543 2,42E-05 -1,6465 -0,7194 AT3G11090 LBD21 LBD21 LOB domain-containing protein 21 0 A\_84\_P760300 0,01871915 3,23E-04 -1,64706 -0,7199 AT3G28160; A\_84\_P825671 0.031304 0.0012934 -1.64847 -0.72112 AT4G08940 Ubiquitin carboxyl-terminal hydrolase family protein A\_84\_P19650 0,03828945 0,0020908 -1,64857 -0,72122 AT1G49560 Homeodomain-like superfamily protein A 84 P848405 0,01775137 2,77E-04 -1,65019 -0,72263 AT2G17695 A\_84\_P17021 0.02544358 7,85E-04 -1,65036 -0,72278 AT1G50250 FTSH1 FTSH1 FTSH protease 1 A 84 P856852 0.02849884 9,94E-04 -1,65298 -0,72507 AT3G46870 Pentatricopeptide repeat (PPR) superfamily protein A\_84\_P855921 0,03272725 0.0014484 -1,65365 -0,72566 AT1G50250 FTSH1 FTSH1 FTSH protease 1 A\_84\_P19460 YAO\_\_Transducin/WD40 repeat-like superfamily protein 0,04661662 0,0033741 -1,65387 -0,72584 AT4G05410 YAO A 84 P10051 0.03359263 0.0015332 -1.65458 -0.72646 AT4G23300 CRK22 CRK22 cvsteine-rich RLK (RECEPTOR-like protein kinase) 22

A_84_P848914 0,04811797	0,0036297 -1,6546	-0,72648 AT4G17840	
A_84_P847471 0,04320978	0,0027725 -1,65471	-0,72658 AT1G33720 CYP76C6	CYP76C6cytochrome P450, family 76, subfamily C, polypeptide 6
A_84_P589834 0,04742935	, ,	-0,72679 AT1G54385	ARM repeat superfamily proteinARM repeat superfamily protein
A_84_P20003 0,02285082	· · · ·		AtIDD14_IDD14_IDD14alpha_IDD14betaindeterminate(ID)-domain 14
A_84_P15477 0,0274646		-0,72812 AT3G10000 EDA31	EDA31Homeodomain-like superfamily protein
A_84_P19769 0,01900532	, ,	-0,72858 AT5G61570	Protein kinase superfamily protein
A_84_P23649 0,04017467		-0,72907 AT1G31050	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P12706 0,0364947	0,0018793 -1,65848	-0,72986 AT3G20240	Mitochondrial substrate carrier family protein
A_84_P763517 0,01830757	3,03E-04 -1,65852	-0,72989 AT4G07725	transposable element gene
A_84_P243195 0,03076559	0,0012382 -1,65885	-0,73018 AT4G29310	Protein of unknown function (DUF1005)
A_84_P15647 0,02364189	6,02E-04 -1,66141	-0,73241 AT3G62110	Pectin lyase-like superfamily protein
A_84_P21757 0,03828945	0,0020885 -1,66183	-0,73277 AT1G31040	PLATZ transcription factor family protein
A_84_P13934 0,03086358	0,00125 -1,66226	-0,73315 AT5G05270	AtCHIL_CHILChalcone-flavanone isomerase family protein
A_84_P755262 0,01681458	2,24E-04 -1,66277	-0,73359 AT2G18260 ATSYP112, SYP11	ATSYP112_SYP112_syntaxin of plants 112
A_84_P115622 0,01375994	1,22E-04 -1,66283	-0,73364 AT4G28230	
A_84_P610677 0,04324035	0,002778 -1,6637	-0,7344 AT3G57200	
A_84_P595350 0,03808665	0,0020649 -1,66578	-0,7362 AT2G32100 ATOFP16, OFP16	ATOFP16_OFP16ovate family protein 16
A_84_P831367 0,0364947	0,0018834 -1,6659	-0,7363 AT1G14430	glyoxal oxidase-related protein
A_84_P11394 0,03484798	0,0016778 -1,66682	-0,73709 AT1G10270 GRP23	GRP23glutamine-rich protein 23
A_84_P829677 0,01020095	4,87E-05 -1,66692	-0,73719 AT5G23420 HMGB6	HMGB6_high-mobility group box 6
A 84 P23747 0,00894727		-0,73812 AT1G10900	Phosphatidylinositol-4-phosphate 5-kinase family protein
A_84_P22031 0,0418366	, , ,	-0,739 AT2G22990 SCPL8, SNG1	SCPL8_SNG1sinapoylglucose 1
A_84_P805176 0,04461956		-0,73957 AT3G61490	Pectin lyase-like superfamily protein
A_84_P13867 0,04113878		-0,74154 AT4G30980 LRL2	LRL2_LJRHL1-like 2
A_84_P12757 0,01386093		-0,74346 AT3G49220	Plant invertase/pectin methylesterase inhibitor superfamily
A_84_P240075 0,02248672	5,33E-04 -1,67444	-0,74368 AT5G51670	Protein of unknown function (DUF668)
A_84_P123932 0,02929986		-0,74421 AT1G79510	Uncharacterized conserved protein (DUF2358)
A 84 P11458 0,02400674	, ,	-0,74534 AT1G80270 PPR596	PPR596 PENTATRICOPEPTIDE REPEAT 596
A 84 P23848 0,03569937	, ,		D AtENODL14_ENODL14_early nodulin-like protein 14
A 84 P307040 0,01608974	2,00E-04 -1,67796	-0,7467 AT4G00370 ANTR2, PHT4;4	ANTR2_PHT4;4Major facilitator superfamily protein
A_84_P521249 0,0325659	, ,	-0,74691 AT5G54400	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A 84 P853567 0,04234786	0,0026664 -1,68015	-0,74859 AT5G28500	
A_84_P755523 0,02877405	, ,	-0,74866 AT2G41950	
	0,0010659 -1,68074	-0,7491 AT5G21100	Plant L-ascorbate oxidase
A 84 P840305 0,03664915	0,001897 -1,68075	-0,74911 AT3G28960	Transmembrane amino acid transporter family protein
A 84 P845813 0,02077349	4,42E-04 -1,68098	-0,7493 AT4G14120	
A_84_P15342 0,038253	, ,	-0,74996 AT2G41820	PXC3_Leucine-rich repeat protein kinase family protein
A_84_P20023 0,00884543		-0,75015 AT1G07180 ATNDI1, NDA1	ATNDI1_NDA1alternative NAD(P)H dehydrogenase 1
A_84_P13171 0,03103756	, ,	-0,75027 AT5G66770	GRAS family transcription factor
A_84_P10220 0,01961223		-0,75198 AT5G35970	P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P809951 0,02763937	9,39E-04 -1,68432	-0,75217 AT4G23490	Protein of unknown function (DUF604)
A 84 P810121 0,04017467	0,0023738 -1,68515	-0,75288 AT1G58360 AAP1, NAT2	AAP1_AtAAP1_NAT2amino acid permease 1
A 84 P549559 0,03929059	0,0022412 -1,68521	-0,75293 AT5G38100	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P821540 0,02054225	4,23E-04 -1,68572		PUR5_phosphoribosylformylglycinamidine cyclo-ligase, chl/phosphoribosyl-aminoimidazole synthetase
A_84_P861375 0,02146564	4,74E-04 -1,68582	-0,75345 AT1G32060 PRK	PRK_phosphoribulokinase
A_84_P248025 0,03308225	0,0014823 -1,68672	-0,75422 AT2G42800 AtRLP29, RLP29	AtRLP29_RLP29_receptor like protein 29
A_84_P828254 0,02644849		-0,75422 A12G42800 AIREF29, REF29 -0,75424 AT5G63040	
A_84_P16470 0,01504028	, ,		ATSR2_ATSRPK1_CIPK7_PKS7_SnRK3.10CBL-interacting protein kinase 7
		-0,75672 AT5G04660 CYP77A4	CYP77A4cytochrome P450, family 77, subfamily A, polypeptide 4
A_84_P18661 0,04371872 A_84_P22168 0,03450806	, ,	-0,75676 AT3G21010	transposable element gene
		-	other RNA
A_84_P721249 0,03387075	0,0015759 -1,68972	-0,75678 AT5G41612	

A\_84\_P800891 0,03916847 0.0022108 -1.69015 -0.75715 AT2G32480 ARASP ARASP ARABIDOPSIS SERIN PROTEASE A 84 P12127 0.04371553 0.0028459 -1,69381 -0,76027 AT5G40820 ATATR, ATR, ATR, ATR ATATR ATR ATRAD3 Ataxia telangiectasia-mutated and RAD3-related A\_84\_P15747 0.04234786 0,0026693 -1,69383 -0,76029 AT4G28630 ABCB23, ATATM1, ABCB23\_ATATM1\_ATM1\_ABC transporter of the mitochondrion 1 A\_84\_P21618 ATFRO8 FRO8\_ferric reduction oxidase 8 0,04889452 0,0038286 -1,69394 -0,76038 AT5G50160 ATFRO8, FRO8 0,02737199 9,13E-04 -1,69467 A 84 P530419 -0,761 AT2G01990 A\_84\_P11494 0,01961223 3,70E-04 -1,69481 -0,76113 AT1G67870 glycine-rich protein A\_84\_P766073 0,04422265 0,0029496 -1,69538 -0,76161 AT5G35796 A\_84\_P787140 0.04963833 0,0039648 -1,69585 -0,762 AT5G49730; ATFRO6,FRO6,ATFRO7,FRO7 A 84 P12759 0.03916847 0,0022136 -1,69979 -0,76536 AT3G49670 BAM2 BAM2 Leucine-rich receptor-like protein kinase family protein A\_84\_P151138 0,02883385 0,0010263 -1,70155 MARD1\_Protein of unknown function (DUF581) -0,76685 AT3G63210 MARD1 A 84 P11901 0,03543342 0.0017586 -1,7035 -0,7685 AT1G06100 Fatty acid desaturase family protein A 84 P20503 0.04641205 0,003337 -1,70544 -0,77015 AT4G34760 SAUR50 SAUR-like auxin-responsive protein family A 84 P760791 0.02353903 5.92E-04 -1.70603 -0.77064 AT3G54060 A\_84\_P758763 0,02612251 8,34E-04 -1,70792 -0,77224 AT3G28160; 0 0,0015317 -1,70804 -0,77234 AT3G25570; CPuORF11 A 84 P841211 0.03359263 A\_84\_P847435 0,02740352 9,15E-04 -1,70868 -0,77289 AT3G08600 Protein of unknown function (DUF1191) A 84 P777695 0,02991192 0,0011559 -1,71528 -0,77845 AT3G23290 LSH4 LSH4 Protein of unknown function (DUF640) A 84 P22740 0,00894727 2,76E-05 -1,71737 -0,7802 AT1G77200 Integrase-type DNA-binding superfamily protein A\_84\_P579017 DVL9 RTFL3 ROTUNDIFOLIA like 3 0.04641205 0,0033409 -1,71775 -0.78052 AT1G07490 DVL9. RTFL3 A\_84\_P12616 0,01436057 1,42E-04 -1,71797 -0.78071 AT2G32220 Ribosomal L27e protein family A 84 P761712 0,03750303 0,0020003 -1,71844 -0,7811 AT3G51075 other RNA PEX11B peroxin 11B A\_84\_P12750 0,03993093 0,0023299 -1,71881 -0,78141 AT3G47430 PEX11B KRR1 family proteinKRR1 family protein A 84 P830397 0.02449762 6,94E-04 -1,71917 -0,78171 AT3G24080 A 84 P575291 0,04899432 0.0038574 -1,7192 -0,78173 AT5G36740 Acyl-CoA N-acyltransferase with RING/FYVE/PHD-type zinc finger protein A\_84\_P19829 0,04742935 0,0035099 -1,71949 Peroxidase superfamily protein -0,78198 AT5G58390 -0,7825 AT3G48160 DEL1, E2FE, E2L3 DEL1\_E2FE\_E2L3\_DP-E2F-like 1 A\_84\_P16534 0,03359263 0,0015466 -1,72011 A 84 P23531 0.01774115 2,75E-04 -1,72079 -0,78307 AT1G33240 AT-GTL1, GTL1 AT-GTL1 ATGTL1 GTL1 GT-2-like 1 A\_84\_P845749 0,01175392 7.73E-05 -1.72186 -0.78397 AT3G06770 Pectin lvase-like superfamily protein A\_84\_P826969 0,02275215 5,45E-04 -1,72208 -0,78415 AT1G65030 Transducin/WD40 repeat-like superfamily protein A 84 P12412 0.02376995 6,11E-04 -1,72218 -0,78424 AT1G60890 Phosphatidylinositol-4-phosphate 5-kinase family protein A\_84\_P22205 0,02237896 5,24E-04 -1,72289 -0,78483 AT1G48460 A\_84\_P808242 0,02732394 9.10E-04 -1.72352 -0.78535 AT1G14345 NAD(P)-linked oxidoreductase superfamily protein A 84 P750030 4,09E-04 -1,72414 -0,78588 AT1G73445 transposable element gene 0.02033809 A\_84\_P71224 PLL1\_\_poltergeist like 1 0,04488314 0,0030731 -1,72531 -0,78685 AT2G35350 PLL1 A\_84\_P23328 0,02342488 5,84E-04 -1,72568 -0,78716 AT4G31600 UTr7\_UDP-N-acetylglucosamine (UAA) transporter family A 84 P19279 0.03665218 0,0019059 -1,72793 NSN1 GTP-binding family protein -0,78904 AT3G07050 NSN1 A\_84\_P284250 0,03454188 SFP2\_Major facilitator superfamily protein 0,0016547 -1,72938 -0,79025 AT5G27360 SFP2 A 84 P600900 0.02595606 8.19E-04 -1.73042 -0.79113 AT3G56870 A\_84\_P17147 0,03786849 0,0020416 -1,73079 -0,79143 AT1G28670 ARAB-1 ARAB-1\_\_GDSL-like Lipase/Acylhydrolase superfamily protein A 84 P105006 0.03220695 0,001394 -1,73124 -0,7918 AT5G40830 S-adenosyl-L-methionine-dependent methyltransferases superfamily protein A\_84\_P824287 9,62E-05 -1,7316 -0,79211 AT1G70230 AXY4, TBL27 AXY4\_TBL27\_\_TRICHOME BIREFRINGENCE-LIKE 27 0,01246891 A 84 P17807 0,02316882 5,71E-04 -1,73257 -0,79292 AT5G43080 CYCA3;1 CYCA3;1 Cyclin A3;1 -0,79358 AT1G11545 XTH8 A\_84\_P11330 0,04611502 0,003267 -1,73336 XTH8\_\_xyloglucan endotransglucosylase/hydrolase 8 A\_84\_P18896 0.04115374 0.0025145 -1.73421 -0.79428 AT1G71480 Nuclear transport factor 2 (NTF2) family protein A\_84\_P11254 0,02226756 5,15E-04 -1,73575 -0,79556 AT5G60450 ARF4 ARF4 auxin response factor 4 A 84 P851252 0,03184718 0,0013431 -1,73627 -0,79599 AT4G31400 CTF7 AtCTF7 CTF7 ECO1 damaged DNA binding; DNA-directed DNA polymerases A\_84\_P816308 0,04572882 0,00321 -1,73696 -0,79656 AT5G05200 Protein kinase superfamily protein A 84 P20911 0.03523168 0,00172 -1,73762 -0,79711 AT1G69780 ATHB13 ATHB13 Homeobox-leucine zipper protein family A 84 P14745 0,01513611 1,69E-04 -1,73793 -0,79737 AT4G11440 Mitochondrial substrate carrier family protein A\_84\_P23234 0,00894727 2,91E-05 -1,73815 -0,79755 AT4G02630 Protein kinase superfamily protein AtSSI2 FAB2\_LDW1\_SSI2\_\_Plant stearoyl-acyl-carrier-protein desaturase family protein A 84 P21952 0.03788604 0.0020458 -1.73876 -0.79806 AT2G43710 FAB2. SSI2

A_84_P547725 0,04887474	0,0038195 -1,74083			
A_84_P812373 0,02512251	7,62E-04 -1,74112	-0,80002 AT5G01410	ATPDX1, ATPDX1	. ATPDX1_ATPDX1.3_PDX1_PDX1.3_RSR4Aldolase-type TIM barrel family protein
A_84_P15323 0,02894719	0,0010487 -1,74247	-0,80114 AT1G32240	KAN2	KAN2Homeodomain-like superfamily protein
A_84_P757718 0,01989875	3,90E-04 -1,74262	-0,80126 AT2G24755		other RNA
A_84_P15700 0,03941842	0,002258 -1,74307	-0,80163 AT4G13710		Pectin lyase-like superfamily protein
A_84_P810878 0,01520549	1,70E-04 -1,7431	-0,80166 AT3G14420		GOX1Aldolase-type TIM barrel family protein
A_84_P751365 0,01768366	2,59E-04 -1,74352	-0,802 AT1G45201	ATTLL1, TLL1	ATTLL1_TLL1triacylglycerol lipase-like 1
A_84_P606916 0,02752188	9,34E-04 -1,74457	-0,80287 AT2G30890;	TBL43	
A_84_P182154 0,03188377	0,0013513 -1,74667	-0,80461 AT5G49730	ATFRO6, FRO6	ATFRO6_FRO6_ferric reduction oxidase 6
A 84 P85719 0,04198026	0,0026241 -1,74773	-0,80548 AT3G44940	·	Protein of unknown function (DUF1635)
A_84_P587896 0,04742935	0,0035106 -1,74968	-0,80709 AT1G05420	ATOFP12, OFP12	ATOFP12_OFP12_ovate family protein 12
A_84_P542466 0,02774078	9.46E-04 -1.74995	-0,80731 AT5G36710;		
A_84_P598030 0,02883385	0,0010246 -1,75045			C ATCDT1A_CDT1_CDT1Ahomolog of yeast CDT1 A
A 84 P549710 0,00466105	3,99E-06 -1,7514		- ,- ,	
A_84_P573880 0,03122214	0,0012842 -1,75264	,		binding
A_84_P830261 0,01771716		-0,81055 AT3G05480	ATRAD9, RAD9	ATRAD9_RAD9_cell cycle checkpoint control protein family
A_84_P190804 0,04927701				B,AJAX3,CPuORF42,AJAX3,CPuORF41,AJAX3
A 84 P22043 0,03189056				ATORC2 ORC2 origin recognition complex second largest subunit 2
A_84_P232159 0,038253	0,0020836 -1,75444	,	,	L ATCSLB03_ATCSLB3_CSLB03cellulose synthase-like B3
A_84_P816662 0,01768366				AGP26_ATAGP26arabinogalactan protein 26
A_84_P825582 0,01911949		-0,81178 AT2G41040	//01/20, //1//01/20	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P21335 0,02487969		-0,81407 AT4G01030		pentatricopeptide (PPR) repeat-containing protein
A_84_P22412 0,01130293				Y ATWRKY13_WRKY13WRKY DNA-binding protein 13
A_84_P824302 0,04086231	0,0024731 -1,76437		ATWINE 15, WINK	Protein of unknown function (DUF1005)
A 84 P13857 0,04080231	0,0023693 -1,76659			GDSL-like Lipase/Acylhydrolase superfamily protein
	1,96E-04 -1,76688	-0,8212 AT4G26540		Leucine-rich repeat receptor-like protein kinase family protein
A_84_P11957 0,01592639 A 84 P815188 0,04831925		-0,8212 A14G26540		
	, ,	-0,82194 AT4G22570	,	ATGPX1_GPX1glutathione peroxidase 1 APT3adenine phosphoribosyl transferase 3
A_84_P813506 0,04465805			AF15	
A_84_P290554 0,04303499	0,0027538 -1,76814			RING/FYVE/PHD zinc finger superfamily protein
A_84_P838518 0,01642861	, ,	-0,82297 AT1G50280		Phototropic-responsive NPH3 family protein
A_84_P24138 0,00530427		-0,82438 AT3G56330		N2,N2-dimethylguanosine tRNA methyltransferase
A_84_P18868 0,00903297	3,58E-05 -1,77084	,		Pectin lyase-like superfamily protein
A_84_P610685 0,03086358		-0,82522 AT3G59670	DDV	
A_84_P848652 0,01403995		-0,82567 AT1G32060		PRK_phosphoribulokinasePRK_phosphoribulokinase
A_84_P827727 0,02156671		-0,82688 AT5G56860	GATA21, GNC	GATA21_GNCGATA type zinc finger transcription factor family protein
A_84_P12408 0,03124189	0,0012858 -1,77397			EF hand calcium-binding protein family
A_84_P21684 0,0151022		-0,82699 AT5G67200		Leucine-rich repeat protein kinase family protein
A_84_P186234 0,01202061		-0,82806 AT3G25590		
A_84_P761052 0,02103692	4,56E-04 -1,77696		ACA1, ATACA1, C	A ACA1_ATACA1_CAH1alpha carbonic anhydrase 1
A_84_P753742 0,03506512		-0,83325 AT1G04778		
A_84_P16381 0,03748751	0,0019986 -1,78434	,		Xanthine/uracil permease family protein
A_84_P231649 0,01320943		-0,83607 AT3G24080		KRR1 family proteinKRR1 family protein
A_84_P15998 0,03454188				FAS2_MUB3.9_NFB01_NFB1Transducin/WD40 repeat-like superfamily protein
A_84_P819038 0,0291012	0,0010669 -1,78872		AT-GTL1, GTL1	AT-GTL1_ATGTL1_GTL1GT-2-like 1
A_84_P22318 0,02665718	8,65E-04 -1,79012	-0,84005 AT4G12830		alpha/beta-Hydrolases superfamily protein
A_84_P13781 0,03268509	0,0014413 -1,79325	-0,84258 AT1G23340		Protein of Unknown Function (DUF239)
A_84_P15740 0,04695471	0,0034278 -1,79411	-0,84327 AT4G27030	FAD4, FADA	FAD4_FADAfatty acid desaturase A
A_84_P15126 0,02084717	4,46E-04 -1,79421	-0,84334 AT1G69160		
A_84_P540304 0,02272896	5,44E-04 -1,79606	-0,84483 AT3G02500		
A_84_P835299 0,03524169		-0,84505 AT2G41510	ATCKX1, CKX1	ATCKX1_CKX1_cytokinin oxidase/dehydrogenase 1ATCKX1_CKX1cytokinin oxidase/dehydrogenase 1
A_84_P806618 0,02384919	6,27E-04 -1,79671	-0,84536 AT2G26080	AtGLDP2, GLDP2	AtGLDP2_GLDP2_glycine decarboxylase P-protein 2
_ , ,			,	

A\_84\_P194524 0,01775137 2.77E-04 -1.79719 -0.84574 AT2G29760 OTP81 OTP81\_\_Tetratricopeptide repeat (TPR)-like superfamily protein A 84 P12173 0,01872867 3,26E-04 -1,79829 -0,84663 AT5G53730 NHL26 Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family A\_84\_P592388 0,02376995 6,14E-04 -1,7985 -0,84679 AT5G43250 NF-YC13 NF-YC13 nuclear factor Y, subunit C13 A\_84\_P21472 ATSUV3\_EDA15\_\_ATP-dependent RNA helicase, mitochondrial (SUV3) 0,04875428 0,0038017 -1,80112 -0,84889 AT4G14790 ATSUV3, EDA15 A 84 P23199 0,0288939 GIS C2H2 and C2HC zinc fingers superfamily protein 0,0010367 -1,80229 -0.84983 AT3G58070 GIS A\_84\_P539380 0,04269926 0,0027143 -1,80302 -0,85042 AT2G35075 A\_84\_P13779 0,02400674 6,47E-04 -1,80469 -0,85175 AT4G03270 CYCD6;1 CYCD6;1\_Cyclin D6;1 A\_84\_P762563 0,03930434 0,0022438 -1,80495 -0,85196 AT3G57157 other RNA A 84 P13494 0.0028213 -1.80578 0,04361687 -0,85262 AT2G36690 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein A\_84\_P788639 0,04896518 0,0038443 -1,80607 Leucine-rich repeat protein kinase family protein -0,85285 AT3G03770 A 84 P856045 0,02507846 7,56E-04 -1,80638 -0,8531 AT2G25080 ATGPX1, GPX1 ATGPX1 GPX1 glutathione peroxidase 1 A 84 P21617 0.03133682 0,0012962 -1,80737 -0.85389 AT5G49910 cpHsc70-2, HSC70 HSC70-7 cpHsc70-2 chloroplast heat shock protein 70-2 A\_84\_P12842 0,04130216 0,0025281 -1,80751 -0,854 AT4G08150 BP, BP1, KNAT1 BP BP1 KNAT1 KNOTTED-like from Arabidopsis thaliana A\_84\_P240205 0.02449762 6,97E-04 -1,80806 -0,85445 AT2G27840 HDA13, HDT04, HD HD2D\_HDA13\_HDT04\_HDT4\_histone deacetylase-related / HD-related Pentatricopeptide repeat (PPR) superfamily protein A 84 P10678 0.04091296 0,0024778 -1,80808 -0,85446 AT2G28050 A\_84\_P11318 0,03786849 0,0020407 -1,80817 -0,85453 AT1G35310 MLP168 MLP168\_MLP-like protein 168 A 84 P292434 0.01686448 2,25E-04 -1,80997 -0,85597 AT2G47930 AGP26, ATAGP26 AGP26\_ATAGP26\_arabinogalactan protein 26 A 84 P20132 0.03450806 0,0016382 -1,81012 -0,85609 AT2G21210 SAUR6 SAUR-like auxin-responsive protein family A\_84\_P843020 -0.8566 AT1G07010 AtSLP1. SLP1 AtSLP1 SLP1 Calcineurin-like metallo-phosphoesterase superfamily protein 0.02507846 7.57E-04 -1.81077 A\_84\_P802670 0,03206014 0,0013744 -1,81087 -0,85669 AT4G08150 BP, BP1, KNAT1 BP\_BP1\_KNAT1\_\_KNOTTED-like from Arabidopsis thaliana -0.86002 AT3G58120 ATBZIP61, BZIP61 ATBZIP61 BZIP61 BZIP61 Basic-leucine zipper (bZIP) transcription factor family protein A 84 P10906 0,04917615 0.0038923 -1,81506 A\_84\_P800839 0,03313515 0,0014887 -1,81524 -0,86016 AT1G22710 ATSUC2, SUC2, S ATSUC2\_SUC2\_SUT1\_sucrose-proton symporter 2 A 84 P10011 -0,86064 AT4G09350 CRRJ, NdhT CRRJ DJC75 NdhT Chaperone DnaJ-domain superfamily protein 0.01961223 3,74E-04 -1,81585 A 84 P20990 0,02998172 0,0011658 -1,81756 -0,862 AT1G71850 Ubiquitin carboxyl-terminal hydrolase family protein A\_84\_P837093 0,03818448 0,0020746 -1,8213 -0,86497 AT1G14280 PKS2 PKS2\_phytochrome kinase substrate 2PKS2\_phytochrome kinase substrate 2 A\_84\_P753729 0,04787055 0,0035844 -1,82271 -0,86608 AT1G48745 A 84 P767998 0.03172537 0.0013255 -1,82275 -0,86612 AT5G42146 A\_84\_P11240 0,04473491 0,0030409 -1,82638 -0,86899 AT1G56110 NOP56 NOP56 homolog of nucleolar protein NOP56 A\_84\_P843303 0,04156374 0,0025615 -1,82903 -0,87108 AT3G02500 A 84 P825382 0,03518269 0,001713 -1,82913 -0.87116 AT3G51895 AST12, SULTR3;1 AST12 SULTR3;1 sulfate transporter 3;1 A\_84\_P815691 0,04456152 0,0029825 -1,82926 -0,87126 AT3G49670 BAM2 BAM2\_Leucine-rich receptor-like protein kinase family protein A\_84\_P577221 ATXR6 SDG34 ARABIDOPSIS TRITHORAX-RELATED PROTEIN 6 0.02400674 6.34E-04 -1.83027 -0,87205 AT5G24330 ATXR6, SDG34 A 84 P848773 0,04176612 0,0025878 -1,83077 -0,87245 AT4G15393; CYP72A5,CYP72A6 A\_84\_P66124 ATMES12, MES12 ATMES12 MES12 methyl esterase 12 0,04418246 0,0029332 -1,83162 -0,87312 AT4G09900 A\_84\_P520907 0,04780289 0,0035725 -1,83182 -0,87328 AT3G48250 BIR6 BIR6\_Pentatricopeptide repeat (PPR) superfamily protein A 84 P16281 0,03929059 0,0022397 -1,83264 -0,87392 AT2G24820 AtTic55, Tic55, TiC AtTic55\_TIC55-II\_Tic55\_translocon at the inner envelope membrane of chloroplasts 55-II A\_84\_P19225 2,40E-04 -1,83366 -0,87472 AT1G07370 ATPCNA1, PCNA1 ATPCNA1\_PCNA1\_proliferating cellular nuclear antigen 1 0,01718834 A\_84\_P99496 0.01633501 2.12E-04 -1.83949 -0.87931 AT5G23420 HMGB6 HMGB6 high-mobility aroup box 6HMGB6 high-mobility group box 6 A\_84\_P12766 0,02582126 8,10E-04 -1,83959 -0,87938 AT3G51240 F3H, F3'H, TT6 F3'H\_F3H\_TT6\_\_flavanone 3-hydroxylase A 84 P10101 0.01835125 3,09E-04 -1,84091 -0,88042 AT4G34610 BLH6 BLH6 BEL1-like homeodomain 6 A\_84\_P11118 0,03959857 0,0022923 -1,84213 -0,88137 AT5G10390 H3.1\_\_Histone superfamily protein A 84 P127561 0,0293697 0,0010915 -1,8434 -0,88237 AT4G15680 Thioredoxin superfamily protein A\_84\_P867201 0,0457815 0,0032337 -1,84342 -0,88238 AT5G23750 Remorin family protein A\_84\_P18061 0.04057087 0.0024317 -1.84412 -0.88293 AT1G10790 A\_84\_P560726 0,03333965 0,0015041 -1,84413 -0,88294 AT3G13980 A 84 P525105 0,02894719 0,0010482 -1,84463 -0,88333 AT2G04790 A\_84\_P533182 0,04986016 0,0040208 -1,84471 -0,88339 AT2G37380 MAKR3 MAKR3 A 84 P750345 0.03278848 0,001454 -1,85026 -0,88773 AT1G49370 A\_84\_P24069 0.02612251 8,30E-04 -1,85067 -0,88805 AT3G16870 GATA17\_\_GATA transcription factor 17 GATA17 A\_84\_P525765 Cell division control, Cdc6Cell division control, Cdc6 0,04953547 0,0039456 -1,85071 -0,88808 AT1G07270 A\_84\_P21871 0.0145606 1.51E-04 -1.85105 -0.88834 AT1G66540 Cvtochrome P450 superfamily protein

A_84_P804260	0,02277204	, , ,	, ,	AJAX3,CPuORF43	AJAX3,CPuORF42,AJAX3,CPuORF41,AJAX3
A_84_P256710	0,04538051	0,0031396 -1,85527	-0,89163 AT3G57940		Domain of unknown function (DUF1726)
A_84_P17063	0,01492891	1,61E-04 -1,85666	-0,89271 AT1G30840	ATPUP4, PUP4	ATPUP4_PUP4purine permease 4
A_84_P819249	0,02720185	9,01E-04 -1,86093	-0,89603 AT5G42720		Glycosyl hydrolase family 17 protein
A_84_P65224	0,02830536	9,83E-04 -1,86545	-0,89952 AT2G28350		ARF10auxin response factor 10
A_84_P18040	0,02725007	9,04E-04 -1,86615	-0,90006 AT1G11600	CYP77B1	CYP77B1cytochrome P450, family 77, subfamily B, polypeptide 1
A_84_P23290	0,04899432	0,0038616 -1,86824	-0,90168 AT4G23290	CRK21	CRK21cysteine-rich RLK (RECEPTOR-like protein kinase) 21
A_84_P11463	0,04208476	0,0026335 -1,87075	-0,90362 AT1G73620		Pathogenesis-related thaumatin superfamily protein
A_84_P20899	0,01769782	2,63E-04 -1,87765	-0,90893 AT1G10640		Pectin lyase-like superfamily protein
A_84_P10523	0,03454188	0,0016516 -1,87887	-0,90986 AT1G34040		Pyridoxal phosphate (PLP)-dependent transferases superfamily protein
A_84_P806838	0,04826047	0,0036785 -1,87974	-0,91053 AT3G15400	ATA20	ATA20anther 20
A_84_P23582	0,04198548	0,0026254 -1,88487	-0,91446 AT5G15948;	CPuORF1	
A_84_P14983	0,01774115	2,75E-04 -1,88551	-0,91496 AT5G46740	UBP21	UBP21_ubiquitin-specific protease 21
A 84 P181494	0,02118828	4,65E-04 -1,88744			CYP71A25cytochrome P450, family 71, subfamily A, polypeptide 25
A_84_P10755	0,03543911	0,0017605 -1,88852	-0,91726 AT3G03060		P-loop containing nucleoside triphosphate hydrolases superfamily protein
A 84 P14533	0,03533791	0,0017402 -1,88942	-0,91795 AT3G05600		alpha/beta-Hydrolases superfamily protein
A_84_P15547	0,04572882	0,0031947 -1,88995	-0,91835 AT3G22560		Acyl-CoA N-acyltransferases (NAT) superfamily protein
A 84 P224349	0.04474596	0,0030498 -1,89282	-0,92054 AT2G46040		ARID/BRIGHT DNA-binding domain;ELM2 domain protein
A_84_P238483	0,03364271	0,0015506 -1,89925	-0,92543 AT5G04895		ABO6DEA(D/H)-box RNA helicase family protein
A 84 P800447	0,00895168	3,38E-05 -1,89948	-0,9256 AT3G46940		DUT1 DUTP-PYROPHOSPHATASE-LIKE 1DUT1 DUTP-PYROPHOSPHATASE-LIKE 1
A_84_P13693	0,02430013	6,80E-04 -1,89961	-0,9257 AT3G46940		DUT1DUTP-PYROPHOSPHATASE-LIKE 1DUT1DUTP-PYROPHOSPHATASE-LIKE 1
A_84_P23169	0,0394248	0,0022616 -1,90056	-0,92643 AT3G51420		ATSSL4_SSL4_strictosidine synthase-like 4
A_84_P105556	0,0394248	0,0022010 -1,90050	-0,92744 AT5G63100	A133L4, 33L4	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
		0,0012331 -1,9019			
A_84_P786857	0,02991192		-0,92759 AT4G26600	TODU	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P23359	0,02226756	5,14E-04 -1,90256	-0,92794 AT4G39770		TPPH_Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
A_84_P17249	0,04633552	0,0032928 -1,90273	-0,92807 AT2G47490	·	ATNDT1_NDT1_NAD+ transporter 1
A_84_P10370	0,03895907	0,0021575 -1,90391	-0,92897 AT5G04230		ATPAL3_PAL3_phenyl alanine ammonia-lyase 3
A_84_P19878	0,02075475	4,41E-04 -1,90716			L AtENODL8_ENODL8_early nodulin-like protein 8AtENODL8_ENODL8_early nodulin-like protein 8
A_84_P155915	0,04408735	0,0029028 -1,90771			APT1_PHT1;2_PHT2_phosphate transporter 2
A_84_P21681	0,02118828	4,65E-04 -1,91189	-0,935 AT5G66460	,	AtMAN7_MAN7Glycosyl hydrolase superfamily protein
A_84_P19887	0,02951533	0,0011137 -1,91352			NIP6_NIP6;1_NLM7NOD26-like intrinsic protein 6;1
A_84_P19966	0,04324035	0,0027808 -1,91682	-0,93872 AT1G69200	FLN2	FLN2_fructokinase-like 2
A_84_P22105	0,01592639	1,96E-04 -1,91863	-0,94007 AT3G04030		MYR2Homeodomain-like superfamily protein
A_84_P14327	0,04762339	0,0035371 -1,91956	-0,94078 AT1G78090	ATTPPB, TPPB	ATTPPB_TPPB_trehalose-6-phosphate phosphatase
A_84_P209948	0,02680561	8,73E-04 -1,92318	-0,9435 AT2G32870		TRAF-like family proteinTRAF-like family protein
A_84_P605036	0,02454009	7,15E-04 -1,92673	-0,94616 AT1G70120		Protein of unknown function (DUF1163)
A_84_P838032	0,04674462	0,0034036 -1,93005	-0,94864 AT1G68570		AtNPF3.1_NPF3.1Major facilitator superfamily protein
A_84_P18765	0,0457815	0,0032298 -1,9341	-0,95166 AT5G46280	MCM3	MCM3Minichromosome maintenance (MCM2/3/5) family protein
A_84_P281450	0,03665218	0,0019073 -1,93719	-0,95396 AT1G21270	WAK2	WAK2_wall-associated kinase 2
A_84_P819143	0,03949811	0,0022708 -1,93856	-0,95499 AT5G49910	cpHsc70-2, HSC70	) HSC70-7_cpHsc70-2chloroplast heat shock protein 70-2
A_84_P764604	0,01066107	5,66E-05 -1,93916	-0,95543 AT4G18501		
A_84_P17549	0,02581204	8,05E-04 -1,94022	-0,95622 AT4G00165		Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
A_84_P23533	0,0286969	0,0010108 -1,94194	-0,9575 AT5G57190	PSD2	PSD2_phosphatidylserine decarboxylase 2
A_84_P740964	0,03818448	0,0020739 -1,94278	-0,95812 AT5G08760		· · · · ·
A_84_P18056	0,03624299	0,0018374 -1,94482	-0,95963 AT1G77870	MUB5	MUB5membrane-anchored ubiguitin-fold protein 5 precursor
A_84_P131376	0,01771716	2,68E-04 -1,94953	-0,96312 AT5G19190		
A_84_P563710	0,03189056	0,0013553 -1,94984	-0,96336 AT5G27780		SAUR75_SAUR-like auxin-responsive protein family
A_84_P124012	0.03076559	0,0012379 -1,951	-0,96422 AT5G26790		
A 84 P826337	0,02820842	9,76E-04 -1,95334	,		
A_84_P510106	0,02609744	8.26E-04 -1.95397	-0,96641 AT2G35840;	C	
A 84 P10969	0,02184876	-, ,	-0,96649 AT4G12390	-	PME1pectin methylesterase inhibitor 1PME1pectin methylesterase inhibitor 1
//_O+_I 10303	0,02104070		0,000+0 A1+012000		

A\_84\_P14999 0,01961223 3.75E-04 -1.95445 -0.96676 AT1G64370 A 84 P566307 0.01902633 3,38E-04 -1,95447 -0,96678 AT5G06790 A\_84\_P59580 0,01782063 2,79E-04 -1,95593 -0,96786 AT4G34560 A\_84\_P812952 0,02991192 0,0011535 -1,95868 -0,96988 AT5G45350 proline-rich family protein A 84 P23341 0,02895624 0,0010515 -1,96221 SAUR3 SAUR-like auxin-responsive protein family -0,97248 AT4G34790 A\_84\_P24132 0,0274646 9,22E-04 -1,96536 A11\_AtCHI\_CFI\_CHI\_TT5\_\_Chalcone-flavanone isomerase family protein -0,97479 AT3G55120 A11, CFI, TT5 A\_84\_P23550 -0,97529 AT5G61000 ATRPA70D, RPA70 ATRPA70D\_RPA1D\_RPA70D\_Replication factor-A protein 1-related 0.02454009 7,17E-04 -1,96603 A\_84\_P808727 0,03670938 0,0019112 -1,97076 -0,97875 AT1G15760 Sterile alpha motif (SAM) domain-containing protein A 84 P770243 0.0037805 -1.97209 -0,97972 ATMG01060 ORF107G ORF107G 0.0485757 A\_84\_P17551 0,02118828 4,65E-04 -1,97252 SAUR31\_SAUR-like auxin-responsive protein family -0,98004 AT4G00880 A 84 P23806 0,04572882 0.0031974 -1.97288 -0,9803 AT1G13600 AtbZIP58, bZIP58 AtbZIP58 bZIP58 basic leucine-zipper 58 A 84 P15874 0.03307353 0,001471 -1,97656 -0,98299 AT5G24150 SQE5, SQP1 SQE5 SQP1 FAD/NAD(P)-binding oxidoreductase family protein A\_84\_P567338 0,04555509 0,0031713 -1,97721 -0.98347 AT5G55570 A\_84\_P16293 0,02551475 7,91E-04 -1,98013 -0,98559 AT2G46570 LAC6 LAC6 laccase 6 A 84 P826445 0,01163687 7,31E-05 -1,98104 -0,98626 AT4G12390 PME1 PME1 pectin methylesterase inhibitor 1PME1 pectin methylesterase inhibitor 1 A\_84\_P253555 0,0364947 0,0018828 -1,98322 -0,98784 AT4G14090 UDP-Glycosyltransferase superfamily protein -0,98908 AT5G49730; ATFRO6,FRO6,ATFRO7,FRO7 A 84 P855355 0.04294266 0.0027439 -1,98491 A\_84\_P22499 0,0409498 0.0024866 -1,98494 -0,9891 AT5G25120 CYP71B11 CYP71B11\_\_ytochrome p450, family 71, subfamily B, polypeptide 11 A\_84\_P21289 Mitochondrial substrate carrier family protein 0.01564355 1.86E-04 -1.98633 -0.9901 AT3G53940 A\_84\_P113332 0,03258517 0,0014308 -1,98887 -0,99195 AT2G31070 TCP10 TCP10 TCP domain protein 10 A 84 P556476 0.04572882 0,0032034 -1,98906 -0,99209 AT4G18750 DOT4 DOT4 Pentatricopeptide repeat (PPR) superfamily protein A\_84\_P13642 0,01769782 2,64E-04 -1,9907 -0,99328 AT3G28040 Leucine-rich receptor-like protein kinase family protein A 84 P21252 0,002446 -1,99341 Major facilitator superfamily protein 0,04066901 -0,99524 AT3G45680 A 84 P10591 0.03220695 0.0013948 -1,99503 -0,99641 AT1G25360 Pentatricopeptide repeat (PPR) superfamily protein A\_84\_P20381 0,03727133 0,0019676 -1,99657 -0,99752 AT3G63110 ATIPT3, IPT3 ATIPT3\_IPT3\_isopentenyltransferase 3 A\_84\_P805355 0,02376995 6,20E-04 -2,0018 -1,0013 AT1G12460 Leucine-rich repeat protein kinase family protein A\_84\_P752347 0.02906338 0,0010628 -2,00584 -1,00421 AT1G62835 A\_84\_P20876 0,02454009 7,11E-04 -2,00637 -1,00459 AT1G64780 AMT1;2, ATAMT1;2 AMT1;2\_ATAMT1;2\_ammonium transporter 1;2 A\_84\_P828102 0,02891722 0,0010387 -2,01778 -1,01277 AT5G62140 A 84 P820256 0.04641205 0.0033347 -2,01874 -1,01346 AT2G24820 AtTic55, Tic55, TIC AtTic55\_TIC55-II\_Tic55\_translocon at the inner envelope membrane of chloroplasts 55-II A\_84\_P792003 0,04813784 0,0036478 -2,0204 -1,01464 AT4G25845; ORP4B A\_84\_P519713 0.0153101 1,75E-04 -2,02389 -1,01713 AT1G66840 PMI2, WEB2 PMI2 WEB2 Plant protein of unknown function (DUF827) A 84 P13912 0,0364947 0,0018813 -2,02845 -1,02038 AT4G16730 TPS02 TPS02 TPS02-Ws -Ws A\_84\_P830783 -2,02983 LIP1\_Protein kinase superfamily protein 0,0286969 0,0010082 -1,02136 AT5G16500 A\_84\_P12781 0,04741487 0,003503 -2,03055 -1,02187 AT3G54400 Eukaryotic aspartyl protease family protein A 84 P515118 0,0293167 0.0010877 -2,03262 -1,02334 AT5G56200 C2H2 type zinc finger transcription factor family A\_84\_P539632 Exostosin family proteinExostosin family protein 0,03233584 0,0014049 -2,03501 -1,02504 AT4G13990 A\_84\_P273780 0.02411081 6.63E-04 -2.04066 -1.02904 AT1G49470 Family of unknown function (DUF716) A\_84\_P535510 0,0497107 0,003988 -2,04111 -1,02935 AT3G53680 Acyl-CoA N-acyltransferase with RING/FYVE/PHD-type zinc finger domain A 84 P836109 0.0390369 0.0021776 -2,04219 -1,03012 AT4G13990 Exostosin family proteinExostosin family protein A\_84\_P831659 0,0215952 4,81E-04 -2,04569 -1,03259 AT4G28680 TYRDC, TYRDC1 AtTYDC\_TYDC\_TYRDC\_TYRDC1\_L-tyrosine decarboxylase A 84 P17185 0.02532245 7,77E-04 -2,04569 -1,03259 AT1G07270 Cell division control, Cdc6Cell division control, Cdc6 A\_84\_P827163 0,03416876 0,0016018 -2,05131 -1,03654 AT1G55910 ZIP11 ZIP11\_\_zinc transporter 11 precursorZIP11\_\_zinc transporter 11 precursor A\_84\_P15502 0.03368684 0.0015619 -2.05598 -1.03982 AT3G14370 WAG2 WAG2 Protein kinase superfamily protein S-adenosyl-L-methionine-dependent methyltransferases superfamily protein A\_84\_P825578 0,01560944 1,81E-04 -2,05687 -1,04045 AT2G41040 A 84 P769220 0.04002055 0,0023422 -2,06003 -1,04267 AT5G18404 A\_84\_P110322 0,02277204 5,50E-04 -2,06171 -1,04384 AT3G14900 EMB3120 EMB3120 A 84 P292024 3,16E-04 -2,0633 -1,04495 AT4G03292 Polynucleotidyl transferase, ribonuclease H-like superfamily protein 0.01851141 A 84 P17819 0.04411166 0.0029066 -2,06614 -1,04694 AT5G46270 Disease resistance protein (TIR-NBS-LRR class) family A\_84\_P112722 S-adenosyl-L-methionine-dependent methyltransferases superfamily protein 0,01592639 1,95E-04 -2,06659 -1,04725 AT2G41040 A 84 P17526 0.04789742 0.0035963 -2.06662 -1.04727 AT3G58810 ATMTP3. ATMTPA ATMTP3 ATMTPA2 MTP3 MTPA2 metal tolerance protein A2

A_84_P788935	0,02848171	9,93E-04 -2,06742	-1,04783 AT2G20180 PIF1, PIL5	PIF1_PIL5phytochrome interacting factor 3-like 5
A_84_P161803	0,0242297	6,71E-04 -2,07139	-1,0506 AT4G29150 IQD25	IQD25_IQ-domain 25
A_84_P15258	0,03256498	0,0014223 -2,07453	-1,05279 AT1G20120	GDSL-like Lipase/Acylhydrolase superfamily protein
A_84_P22161	0,01564355	1,84E-04 -2,07782		3 AMT1;3_ATAMT1;3ammonium transporter 1;3
A_84_P12761	0,04737008	0,0034896 -2,07875	-1,05572 AT3G50130	Plant protein of unknown function (DUF247)
A_84_P202108	0,02414743	6,64E-04 -2,08193	-1,05792 AT3G20015	Eukaryotic aspartyl protease family protein
A_84_P12918	0,04830702	0,0036942 -2,08203	-1,05799 AT4G30120 ATHMA3, HMA3	ATHMA3_HMA3_heavy metal atpase 3
A_84_P14379	0,03536541	0,0017454 -2,08442		I AtHB31_FTM2_HB31_ZHD4homeobox protein 31
A_84_P14137	0,02916541	0,0010726 -2,09101		2 AtSWEET15_SAG29_SWEET15senescence-associated gene 29
A_84_P790814	0,03359263	0,0015404 -2,09429	-1,06646 AT5G05810 ATL43	ATL43RING/U-box superfamily protein
A_84_P23490	0,03367786	0,0015583 -2,0983	-1,06922 AT5G45070 AtPP2-A8, PP2-A8	AtPP2-A8_PP2-A8phloem protein 2-A8
A_84_P756272	0,04086231	0,0024738 -2,09861	-1,06944 AT2G27395	
A_84_P543622	0,02054225	4,22E-04 -2,10421	-1,07328 AT1G52530	
A_84_P15308	0,01177365	8,04E-05 -2,10465	-1,07358 AT1G16090 WAKL7	WAKL7wall associated kinase-like 7
A_84_P85229	0,02150118	4,76E-04 -2,11067	-1,0777 AT4G28310	
A_84_P15532	0,01185074	8,27E-05 -2,11283	-1,07917 AT3G14450 CID9	CID9CTC-interacting domain 9
A_84_P842922	0,04138362	0,0025408 -2,11874	-1,08321 AT3G13980	
A_84_P556074	0,01989875	3,91E-04 -2,12083	-1,08463 AT1G70985	hydroxyproline-rich glycoprotein family protein
A_84_P21737	0,02812162	9,70E-04 -2,14612	-1,10173 AT3G13065 SRF4	SRF4_STRUBBELIG-receptor family 4
A_84_P21361	0,03058917	0,0012199 -2,14979	-1,1042 AT4G10120 ATSPS4F	ATSPS4F_Sucrose-phosphate synthase family protein
A_84_P21370	0,02704814	8,94E-04 -2,15293	-1,1063 AT4G12320 CYP706A6	CYP706A6cytochrome P450, family 706, subfamily A, polypeptide 6
A_84_P845538	0,02582126	8,09E-04 -2,1533	-1,10655 AT2G36630	Sulfite exporter TauE/SafE family protein
A_84_P14418	0,02342488	5,84E-04 -2,15722	-1,10917 AT2G40230	HXXXD-type acyl-transferase family protein
A_84_P17772	0,04262674	0,0027036 -2,16194	-1,11233 AT5G25810 tny	tny_Integrase-type DNA-binding superfamily protein
A_84_P825068	0,04484528	0,0030649 -2,16261	-1,11277 AT2G41940 ZFP8	ZFP8zinc finger protein 8ZFP8zinc finger protein 8
A_84_P11644	0,03431221	0,0016139 -2,16479	-1,11422 AT2G37460	UMAMIT12nodulin MtN21 /EamA-like transporter family protein
A_84_P12717	0,04361687	0,0028206 -2,16491	-1,11431 AT3G18610 ATNUC-L2, NUC-L	ATNUC-L2_NUC-L2_NUC2_PARLL1nucleolin like 2
A_84_P22576	0,02376995	6,20E-04 -2,1669	-1,11563 AT5G53950 ANAC098, ATCUC	CANAC098_ATCUC2_CUC2_NAC No Apical Meristem domain transcriptional regulator superfamily protein
A_84_P528745	0,02586423	8,14E-04 -2,17384	-1,12024 AT2G39230 LOJ	LOJ_LATERAL ORGAN JUNCTION
A 84 P16094	0,04831884	0,0037008 -2,17819	-1,12313 AT1G23130	Polyketide cyclase/dehydrase and lipid transport superfamily protein
A_84_P861913	0,02951044	0,0011092 -2,17973		DL AtENODL8 ENODL8 early nodulin-like protein 8
A_84_P20256	0,02400674	6,45E-04 -2,19095		AtMYB10_MYB10_myb domain protein 10
A_84_P11953	0,01961223	3,65E-04 -2,19995	-1,13747 AT4G25780	CAP Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1 protein superfamily protein
A_84_P16823	0,04970967	0,0039801 -2,2006	-1,1379 AT1G01060 LHY, LHY1	LHY_LHY1_Homeodomain-like superfamily protein
A_84_P14743	0,04017467	0,002366 -2,20234		AtGH9C3_GH9C3glycosyl hydrolase 9C3
A_84_P20729	0,03929059	0,0022399 -2,20357	-1,13984 AT5G65010 ASN2	ASN2_asparagine synthetase 2
A_84_P286230	0,02160571	4,83E-04 -2,20713	-1,14217 AT3G25905 CLE27	CLE27CLAVATA3/ESR-RELATED 27
A 84 P17658	0,04017467	0,0023722 -2,21641	-	AtGLDP1_GLDP1_glycine decarboxylase P-protein 1
A_84_P10036	0,03665218	0,0019046 -2,22179	-1,15172 AT4G19590	Chaperone DnaJ-domain superfamily protein
A_84_P94599	0,04844125	0,0037583 -2,22707	-1,15515 AT3G56290	
A_84_P12595	0,04036515	0,0024119 -2,22958		L ATCSLB01_ATCSLB1_CSLB01cellulose synthase-like B1
A_84_P21423	0,04880985	0,0038083 -2,2315		AtTYDC_TYDC_TYRDC_TYRDC1_L-tyrosine decarboxylase
A_84_P819992	0,04510453	0,0030972 -2,2353	-1,16047 AT4G32340	Tetratricopeptide repeat (TPR)-like superfamily protein
A_84_P15766	0,01871661	3,22E-04 -2,24804	-1,16867 AT4G32980 ATH1	ATH1_homeobox gene 1
A_84_P15327	0,0442149	0,002947 -2,25229	-1,17139 AT2G41940 ZFP8	ZFP8zinc finger protein 8ZFP8zinc finger protein 8
A_84_P22292	0,02060719	4,30E-04 -2,25905	-1,17571 AT4G04020 FIB	FIB_PGL35fibrillinFIB_PGL35fibrillin
A_84_P761488	0,03594406	0,0018106 -2,26058	-1,1767 AT3G01329	ECA1-like gametogenesis related family protein
A_84_P18822	0,01743021	2,52E-04 -2,26516		M AtMYB28_HAG1_MYB28_PMG1myb domain protein 28
A_84_P21758	0,03959857	0,0022964 -2,27554	-1,18621 AT1G62630	Disease resistance protein (CC-NBS-LRR class) family
A_84_P21158 A_84_P21154	0,03542483	0,0022904 -2,27554 0,0017552 -2,2819	-1,19023 AT1G01390	UDP-Glycosyltransferase superfamily protein
A_84_P12740	-	0,0017552 -2,2819	-1,19148 AT1G02630	Nucleoside transporter family protein
7_04_F12140	0,03559205	0,0010407 -2,20007	-1,13140 ATTO02030	

A 84 P813422	0,0373717	0 0010811 -2 28876	-1 19456 AT3G48740		AtSWEET11_SWEET11Nodulin MtN3 family protein
A_84_P311583	0,00904516		-1,20194 AT5G42445	AGWEETT, OWE	
A_84_P21901	0,04826047	0,0036688 -2,30843			Glutaredoxin family protein
A_84_P22686	0,02695982	· · ·	-1,20091 AT1G000000		Sterile alpha motif (SAM) domain-containing proteinSterile alpha motif (SAM) domain-containing protein
A_84_P815182	0,02095982	0,0012895 -2,31089	,	ATTEN2 TEN2	ATTSO2_TSO2_Ferritin/ribonucleotide reductase-like family protein
A_84_P23195	0,0312771		-1,21414 AT3G57130		BOP1Ankyrin repeat family protein / BTB/POZ domain-containing protein
A 84 P92099	0,01454446			BOFT	• • • • •
	,		-1,21518 AT2G32880		TRAF-like family protein
A_84_P87419	0,03308225	0,0014797 -2,34244			Auxin-responsive family protein
A_84_P531612	0,03913858	0,0021945 -2,34972			
A_84_P768303	0,01642861		-1,23279 AT5G18407		Defensin-like (DEFL) family protein
A_84_P809484	0,03454188	, ,	-1,23472 AT5G15948;	CPUORF1	
A_84_P21449	0,03691384		-1,25097 AT4G34770	D.0.1.1.7	SAUR1_SAUR-like auxin-responsive protein family
A_84_P13826	0,04831925		-1,26496 AT4G21760		BGLU47_beta-glucosidase 47
A_84_P11955	0,03359263				CGA1_GATA22_GNLcytokinin-responsive gata factor 1
A_84_P566577	0,01232764		-1,26625 AT1G16730		UP6unknown protein 6
A_84_P22322	0,01771716		-1,27951 AT4G13810	AtRLP47, RLP47	AtRLP47_RLP47receptor like protein 47
A_84_P17296	0,04362199	0,0028277 -2,43572			GDSL-like Lipase/Acylhydrolase superfamily protein
A_84_P11583	0,04234786		-1,28799 AT2G40900		UMAMIT11nodulin MtN21 /EamA-like transporter family protein
A_84_P22129	0,03484798	0,0016807 -2,44812	-1,29167 AT3G27660	OLE3, OLEO4	OLE3_OLEO4oleosin 4
A_84_P90919	0,04181083	0,0025951 -2,4529	-1,29449 AT3G61950		basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P12889	0,03978182	0,0023125 -2,45669	-1,29672 AT4G23780		
A_84_P19357	0,04181817	0,0026043 -2,45844	-1,29775 AT3G44990	AtXTH31, ATXTR8	, ATXTR8_AtXTH31_XTR8xyloglucan endo-transglycosylase-related 8
A_84_P760435	0,0362616	0,0018457 -2,47559	-1,30777 AT3G31993		transposable element gene
A_84_P575477	0,02532245	7,79E-04 -2,47786	-1,3091 AT1G14630		
A_84_P571785	0,02524157	7,67E-04 -2,48436	-1,31287 AT5G51920		Pyridoxal phosphate (PLP)-dependent transferases superfamily protein
A_84_P812958	0,01526442	1,74E-04 -2,48559	-1,31359 AT4G04020	FIB	FIB PGL35 fibrillinFIB PGL35 fibrillin
A_84_P19925	0,02067666	4,34E-04 -2,48989	-1,31608 AT1G03020		Thioredoxin superfamily protein
A_84_P20159	0,04017467	0,0023769 -2,50083	-1.3224 AT2G32540	ATCSLB04, ATCSL	ATCSLB04_ATCSLB4_CSLB04cellulose synthase-like B4
A_84_P12058	0,03308225	0,0014824 -2,50956			ATFLS1_FLS_FLS1_ flavonol synthase 1
A_84_P12677	0,0484796	, ,	-1,33064 AT3G23630		ATIPT7_IPT7isopentenyltransferase 7
A_84_P21145	0,03665218	, ,	-1,34528 AT3G03480		CHATacetyl CoA:(Z)-3-hexen-1-ol acetyltransferase
A_84_P11358	0,01830757	3,06E-04 -2,56729	-1,36024 AT1G03820	0	
A_84_P14510	0,04019184			ATEXP15 ATEXPA	ATEXP15_ATEXPA15_ATHEXP ALPHA 1.3_EXP15_EXPA15expansin A15
A_84_P20589	0,02248672	5,33E-04 -2,57934	-1,367 AT5G16530		PIN5_Auxin efflux carrier family protein
A_84_P18118	0,02071601	4,39E-04 -2,59026	-1,3731 AT1G56430		ATNAS4_NAS4nicotianamine synthase 4
A_84_P11065	0,04669211		-1,39572 AT4G39480		CYP96A9Cytochrome P450, family 96, subfamily A, polypeptide 9
A_84_P210848	0,02836969		-1,39717 AT5G22930	CTFBOAB	Protein of unknown function (DUF1635)
A_84_P305070	0,02830303		-1,39896 AT2G15020		
A_84_P857350	0,00894727	0,0016803 -2,64117			Polyketide cyclase/dehydrase and lipid transport superfamily protein
					Polykelide cyclase/denydrase and ipid transport superiannity protein
A_84_P786557	0,02991109	0,0011478 -2,66592			
A_84_P10228	0,01177365		-1,42875 AT5G38820		Transmembrane amino acid transporter family protein
A_84_P584694	0,04066901	0,0024512 -2,70486			Direct investors of a still section of the listence of the listence of a still sector in
A_84_P15108	0,0241966		-1,44537 AT1G23205		Plant invertase/pectin methylesterase inhibitor superfamily protein
A_84_P12302	0,03181502	0,0013372 -2,72671			Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
A_84_P60560	0,03828945		-1,44877 AT2G25510		
A_84_P102716	0,03089017	, ,	-1,45077 AT4G18630		Protein of unknown function (DUF688)
A_84_P586938	0,04362199	0,0028265 -2,73702		ATFRO7, FRO7	ATFRO7_FRO7_ferric reduction oxidase 7
A_84_P824811	0,01731088		-1,46042 AT2G32870		TRAF-like family proteinTRAF-like family protein
A_84_P10285	0,00606654	7,02E-06 -2,75834	-1,4638 AT5G54510	DFL1, GH3.6	DFL1_GH3.6Auxin-responsive GH3 family protein
A_84_P13165	0,03569937	0,0017874 -2,76202			basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P762597	0,04636379	0,0033133 -2,77645	-1,47324 AT3G27329;	(	)

A_84_P819560	0,03418483		-1,47899 AT4G00780		TRAF-like family protein
A_84_P13124	0,02256138	5,36E-04 -2,79955	-1,4852 AT5G55340		MBOAT (membrane bound O-acyl transferase) family protein
A_84_P768492	0,03681569	0,0019258 -2,80266	-1,4868 AT5G63087		Plant thionin family protein
A_84_P255460	0,04371872	0,0028525 -2,81363	,		GATA13GATA transcription factor 13
A_84_P524292	0,04197278	0,0026219 -2,81454			ATCSLB03_ATCSLB3_CSLB03cellulose synthase-like B3
A_84_P18331	0,02884718	0,00103 -2,81674	-1,49403 AT3G04530	ATPPCK2, PEPCK	ATPPCK2_PEPCK2_pPCK2_phosphoenolpyruvate carboxylase kinase 2
A_84_P760178	0,02612251	8,31E-04 -2,81727	-1,4943 AT3G28540		P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P18215	0,0286969	0,0010059 -2,8184	-1,49487 AT2G32290	BAM6, BMY5	BAM6_BMY5beta-amylase 6
A_84_P12165	0,04603513	0,0032595 -2,86803	-1,52006 AT5G51310		2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
A_84_P761356	0,00461552	2,46E-06 -2,88402	-1,52808 AT3G61898		
A_84_P605859	0,0380408	0,0020589 -2,88648	-1,52931 AT1G47485		
A_84_P14878	0,04831925	0,0037053 -2,92152	-1,54672 AT5G04950	ATNAS1, NAS1	ATNAS1_NAS1_nicotianamine synthase 1
A 84 P757781	0,03959642		-1,58735 AT2G37555	,	other RNA
A_84_P16207	0,02477556	7.37E-04 -3.01615	-1,59271 AT1G68360		C2H2 and C2HC zinc fingers superfamily protein
A_84_P10779	0,03044238	0.0012021 -3.06119	-1.61409 AT3G30530	ATBZIP42, bZIP42	ATBZIP42_bZIP42_basic leucine-zipper 42
A_84_P599886	0,02040626		-1,64704 AT3G06145		
A_84_P767462	0,02038725		-1,64978 AT5G59990		CCT motif family proteinCCT motif family protein
A_84_P598547	0,03089017		-1,67565 AT5G55040		DNA-binding bromodomain-containing protein
A_84_P761618	0,03542483		-1,68149 AT3G26816;	MIR169L.MIR169N	
A_84_P10289	0,029931		-1,69488 AT5G55720	,	Pectin lyase-like superfamily protein
A_84_P12422	0,03897839	0,0021614 -3,29209	,		auxin-responsive GH3 family protein
A 84 P10781	0,02821329		-1,74029 AT3G15650		alpha/beta-Hydrolases superfamily protein
A_84_P16880	0,0364947	0,0018792 -3,43768	,		HXXXD-type acyl-transferase family protein
A 84 P22462	0,03497349		-1,79472 AT5G09930	ABCE2	ABCF2 ABC transporter family protein
A_84_P543942	0,03187662	0,0013467 -3,57641	,		AtGDU6_GDU6_glutamine dumper 6
A_84_P16647	0,01874703		-1,84427 AT4G13900		AtRLP49 RLP49
A_84_P13969	0,03916847				ATGSTF12_GST26_GSTF12_TT19glutathione S-transferase phi 12
A_84_P19540	0,03518269	0,0017115 -3,61873		///00// 12, 00/20	FTM1_SAD6Plant stearoyl-acyl-acrier-protein desaturase family protein
A_84_P847244	0,04730906		-1,88223 AT2G36610	<b>ATHR22 HR22</b>	ATHB22_HB22_homeobox protein 22
A_84_P788914	0,03645025	, , ,	-1,88256 AT2G40900	ATTIDZZ, TIDZZ	UMAMIT11nodulin MtN21 /EamA-like transporter family protein
A 84 P604856	0,01375994		-1,90019 AT5G59990		CCT motif family proteinCCT motif family protein
A_84_P753015	0,03439173	0,0016225 -3,77963			IDL5_inflorescence deficient in abscission (IDA)-like 5
A_84_P54840	0,01246891		-1,93098 AT3G19270		CYP707A4cytochrome P450, family 707, subfamily A, polypeptide 4
A_84_P12179	0,00894727		-1,94961 AT5G55330	OTTIONA	MBOAT (membrane bound O-acyl transferase) family protein
A 84 P846994	0,04139227	0,0025432 -3,97266			UMAMIT11 nodulin MtN21 /EamA-like transporter family protein
A_84_P592665	0,00918073	3,88E-05 -3,99169	-1,997 AT2G24762		AtGDU4_GDU4_glutamine dumper 4
A_84_P81559	0,03914714	0,0022031 -4,07614		Alob04, 0004	Wound-responsive family protein
A_84_P768727	0,01961223		-2,07468 AT5G53486		
A_84_P554758	0,0145606		-2,17898 AT4G37810		
A_64_P554756 A 84 P761647	0,04899432		-2,27449 AT3G26818		MIR169M MIR169M; miRNA
	,	, ,	,	IVITY LOAIN	P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P21430	0,01177365 0,0364524		-2,29308 AT4G30250		SPX (SYG1/Pho81/XPR1) domain-containing protein/zinc finger (C3HC4-type RING finger) protein-related
A_84_P294924 A_84_P14421	0,0364524	0,0018708 -5,13764	-2,36696 AT2G41510		ATCKX1_CKX1_cytokinin oxidase/dehydrogenase 1
A_64_P14421 A 84 P784337	0,02732394		-2,87551 AT1G65480		FT_RSB8PEBP (phosphatidylethanolamine-binding protein) family protein
	,	, ,	,	11	
A_84_P14793	0,04254959	0,0026922 -8,15501	-3,02769 AT IG21890		UMAMIT19nodulin MtN21 /EamA-like transporter family protein

## Supplemental Table 1. Differential transcript levels in TPT-RAP2.3 lines treated with -estradiol. Levels at 1h after treatment with a NO pulse (E1) versus those at 0 time (E0).

ProbeName	p (Corr) p	FC	Log FC AGI	Symbol	Corrected annoatation
A_84_P11046	0,04968483 5,50E-04	493,9474	8,948214 AT4G34410		RRTF1_redox responsive transcription factor 1
A_84_P21769	0,03177396 1,08E-04	272,0891	8,087935 AT1G61120		GES_TPS04_TPS4terpene synthase 04
A_84_P595141	0,02561561 2,76E-05	222,51181	7,797738 AT1G30135		JAZ8_TIFY5Ajasmonate-zim-domain protein 8
A_84_P21573	0,03263922 1,34E-04	111,84534	6,8053613 AT5G38120		4CL8_AMP-dependent synthetase and ligase family protein
A_84_P761749	0,01254327 4,34E-06	91,743805	6,519539 AT3G22275		
A_84_P18289	0,01203481 1,57E-06	77,07201	6,268135 AT2G44810		DAD1alpha/beta-Hydrolases superfamily protein
A_84_P851247	0,032583 1,31E-04	69,98376	6,128948 AT5G28237		Pyridoxal-5'-phosphate-dependent enzyme family protein
A_84_P521598	0,03157461 1,03E-04	63,441887	5,987364 AT2G22760		basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A 84 P610481	0,03364056 1,61E-04	57,627926	5,848696 AT2G34600		JAZ7_TIFY5B_jasmonate-zim-domain protein 7
A_84_P14748	0,03263667 1,32E-04	52,879234	5,7246294 AT1G17420	,	ATLOX3_LOX3_lipoxygenase 3ATLOX3_LOX3_lipoxygenase 3
A_84_P17584	0,02725945 4,86E-05	52,255585	5,7075133 AT1G17380	-	JAZ5_TIFY11A_jasmonate-zim-domain protein 5
A 84 P20976	0,03937708 2,68E-04	48,03467	5,5860043 AT1G19210		Integrase-type DNA-binding superfamily protein
A 84 P785475	0,02775724 6,21E-05	46,777004	5,5477276 AT1G17420		ATLOX3_LOX3_lipoxygenase 3ATLOX3_LOX3_lipoxygenase 3
A 84 P17546	0,04905209 5,24E-04	46,38127	5,5354705 AT3G44860	-	FAMTfarnesoic acid carboxyl-O-methyltransferase
A 84 P592948	0,03162822 1,06E-04	41,518867	5,375695 AT4G11911	.,	
A_84_P524680	0,01254327 4,34E-06	41,0063	5,357774 AT5G13220	JAS1 JAZ10 TIFY9	JAS1_JAZ10_TIFY9jasmonate-zim-domain protein 10
A_84_P16173	0,03545904 1,84E-04	40,13306	5,3267193 AT1G28480		GRX480_roxy19Thioredoxin superfamily protein
A_84_P10627	0,03498028 1,76E-04	37,747044	5,2382917 AT2G20880		AtERF53_ERF53Integrase-type DNA-binding superfamily protein
A_84_P18072	0,03624293 2,14E-04	36,203068	5,17804 AT1G72520		ATLOX4_LOX4_PLAT/LH2 domain-containing lipoxygenase family protein
A_84_P847119	0,02065646 1,41E-05	35,206936	5,137788 AT5G62360	-	Plant invertase/pectin methylesterase inhibitor superfamily protein
A 84 P823221	0,01417998 5,23E-06	33,437576	5,0633984 AT5G13220		JAS1_JAZ10_TIFY9jasmonate-zim-domain protein 10
A_84_P502791	0,03545904 2,03E-04	32,893456	5,0397286 AT1G32910		HXXXD-type acyl-transferase family protein
A_84_P17881	0,03265593 1,45E-04	32,795807	5,0354395 AT5G62360		Plant invertase/pectin methylesterase inhibitor superfamily protein
A_84_P11564	0,02725945 5,24E-05	32,35481	5,0159082 AT1G64160		AtDIR5_DIR5_Disease resistance-responsive (dirigent-like protein) family protein
A 84 P15993	0,01997895 1,12E-05	31,351526	4,9704638 AT5G63450		CYP94B1cytochrome P450, family 94, subfamily B, polypeptide 1
A_84_P611898	0,04834748 4,92E-04	30,85118	4,9472537 AT5G59580		UGT76E1UDP-glucosyl transferase 76E1
A_84_P788408	0,02065646 1,43E-05	30,409632	4,9264565 AT2G22760		basic helix-loop-helix (bHLH) DNA-binding superfamily protein
A_84_P20120	0,03263922 1,42E-04	25,755907	4,6868315 AT2G27690		CYP94C1cytochrome P450, family 94, subfamily C, polypeptide 1
A_84_P832235	0,04834748 4,96E-04	21,1765	4,4043922 AT3G44860;		CTF94CTCytochrome F450, family 94, Sublamily C, polypeptide T
A_84_P23179	0,00821229 2,74E-07	20,134415	4,3315916 AT3G53600		C2H2-type zinc finger family protein
A 84 P16877	0,03839359 2,45E-04	18,979265	4,246352 AT5G47240		NUDT8_atnudt8nudix hydrolase homolog 8
A_84_P18877 A_84_P18113	0,03839359 2,45E-04 0,02725945 4,88E-05	17,729317	4,148065 AT1G76640		CML39_Calcium-binding EF-hand family protein
A_84_P812834	0,02725945 4,88E-05 0,03137732 9,55E-05	17,47696	4,1273823 AT1G28480		GRX480_roxy19Thioredoxin superfamily protein
A_64_P612634 A 84 P558681	0,02065646 1,37E-05	17,238531	4,107565 AT1G53885:		
A_84_P826417	0,02065646 1,37E-05 0,03287368 1,46E-04	16,93389		, ATWRKY30, WRKY30	ATWRKY30_WRKY30WRKY DNA-binding protein 30
			3,9844198 AT1G72416	-	Chaperone DnaJ-domain superfamily protein
A_84_P789698 A_84_P853664	0,01203481 2,73E-06 0,01203481 2,41E-06	15,82814 15,815641		AtOPR3, DDE1, OPR3	AtOPR3_DDE1_OPR3oxophytodienoate-reductase 3
					Chaperone DnaJ-domain superfamily protein
A_84_P715317	0,02725945 5,34E-05	15,674281	3,9703274 AT1G72416		
A_84_P611334	0,04793091 4,71E-04	15,29613	3,9350948 AT2G22880		VQ motif-containing protein
A_84_P232439	0,03157461 9,85E-05	15,201731		ATWRKY18, WRKY18	ATWRKY18_WRKY18_WRKY DNA-binding protein 18
A_84_P17343	0,03066272 8,40E-05	15,199038			ATERF13_EREBP_ERF13ethylene-responsive element binding factor 13
A_84_P785932	0,04367585 3,50E-04	15,114456	3,9178572 AT1G25400		OD447 Integrood type DNA kinding superformity metals
A_84_P19028	0,02459617 2,06E-05	14,740016	3,8816662 AT1G74930		ORA47_Integrase-type DNA-binding superfamily protein
A_84_P76184	0,02358327 1,79E-05	14,440209	-	ATWRKY30, WRKY30	ATWRKY30_WRKY30WRKY DNA-binding protein 30
A_84_P67084	0,03983375 2,78E-04	14,163812	3,8241377 AT1G25400		
A_84_P15340	0,03545904 2,07E-04	14,054783	3,8129892 AT2G06050		AtOPR3_DDE1_OPR3oxophytodienoate-reductase 3
A_84_P818172	0,03903778 2,63E-04	13,979904	3,8052826 AT1G15040		GAT_GAT1_2.1Class I glutamine amidotransferase-like superfamily protein

A_84_P14985	0,03545904 2,01E-04	13,779772	-		ATERF-2_ATERF2_ERF2ethylene responsive element binding factor 2
A_84_P13031	0,01203481 3,23E-06	13,7481	3,7811604AT5G19110		Eukaryotic aspartyl protease family protein
A_84_P11822	0,03116852 8,89E-05	13,690612	3,775115AT3G51450		Calcium-dependent phosphotriesterase superfamily protein
A_84_P181974	0,03954257 2,72E-04	13,470726	3,7517557AT1G20310		
A_84_P852628	0,032583 1,25E-04	12,645198	3,6605177 AT5G02600	NAKR1, NPCC6	NAKR1_NPCC6Heavy metal transport/detoxification superfamily protein
A_84_P542646	0,032583 1,23E-04	12,62653	3,6583862AT1G15040		GAT_GAT1_2.1Class I glutamine amidotransferase-like superfamily protein
A_84_P217688	0,03866172 2,53E-04	12,450544	3,6381369AT1G76600		
A_84_P840907	0,03545904 2,07E-04	11,791884	3,5597224AT5G56880		
A_84_P19845	0,03866172 2,58E-04	11,764797	3,5564046AT3G27810	ATMYB21, ATMYB3, MYB2	2 ATMYB21_ATMYB3_MYB21myb domain protein 21
A_84_P586962	0,03117324 9,16E-05	11,694893	3,5478067 AT5G56880		
A_84_P529724	0,03498028 1,75E-04	11,3272085	3,5017204 AT3G20340		
A_84_P832673	0,03188318 1,09E-04	10,763261	3,4280434 AT3G20340		
A_84_P196584	0,04985619 5,54E-04	10,613109	3,4077754 AT3G48650		
A_84_P10611	0,0436378 3,44E-04	9,422673	3,2361364 AT2G43530		Scorpion toxin-like knottin superfamily protein
A_84_P137619	0,0282656 6,54E-05	8,372727	3,0656977 AT5G54490		PBP1pinoid-binding protein 1
A_84_P704033	0,02725945 3,46E-05	8,275334	3,0488176AT1G11185		other RNA
A_84_P97916	0,04715346 4,40E-04	8,26751	3,047453AT4G29780		
A_84_P236643	0,032583 1,20E-04	8,170585	3,0304394 AT1G44350	ILL6	ILL6_IAA-leucine resistant (ILR)-like gene 6
A_84_P820417	0,04292386 3,22E-04	8,059987	3,0107775AT4G17490	ATERF6, ERF6, ERF-6-6	ATERF6_ERF-6-6_ERF6ethylene responsive element binding factor 6
A_84_P15450	0,04418976 3,64E-04	7,737815	2,9519262AT2G32120	HSP70T-2	HSP70T-2_heat-shock protein 70T-2
A 84 P18859	0,04796798 4,73E-04	7,677764	2,9406862AT5G28630		alvcine-rich protein
A_84_P216248	0,03839359 2,46E-04	7,540287	2,9146194 AT1G69890		Protein of unknown function (DUF569)
A_84_P22053	0,03866172 2,51E-04	7,483406	2,903695 AT2G41100		ATCAL4_TCH3Calcium-binding EF hand family protein
A_84_P537220		7.469642	2,9010391 AT5G57510	-	3
A_84_P16821	0,03866172 2,57E-04	7,2578816	2,8595486 AT5G24470		APRR5_PRR5pseudo-response regulator 5
A 84 P17140	0,03954257 2,72E-04	7,2034364			2 ATMYC2_JAI1_JIN1_MYC2_ZBF1Basic helix-loop-helix DNA-binding protein
A_84_P169283	, ,	7,100398	2,8279AT5G06570		alpha/beta-Hydrolases superfamily protein
A_84_P13216	0,03545904 2,02E-04	6,917477	2,790246 AT1G20510		OPCL1_OPC-8:0 CoA ligase1
A_84_P18976	0,022797 1,63E-05	6,9162197	2,7899837 AT1G30730		FAD-binding Berberine family protein
A 84 P858725		6,8956404	2,7856846 AT1G72450		JAZ6_TIFY11Bjasmonate-zim-domain protein 6
A_84_P562774		6,8833942	2,7831202 AT5G12340	-	
A_84_P20831	0,02686399 3,07E-05	6,872378	2,7808094 AT1G61340		AtFBS1 FBS1 F-box family protein
A_84_P759481		6,8531885	2,7767754AT3G12145		FLOR1_FLR1_FTM4Leucine-rich repeat (LRR) family protein
A_84_P255510		6,797904	2,76509AT1G72450		JAZ6_TIFY11Bjasmonate-zim-domain protein 6
A 84 P769565		6,687007	2,7413607 AT5G44585		
A_84_P869292		6,6748314	2,7387314AT4G17500		ATERF-1_ERF-1ethylene responsive element binding factor 1
A_84_P267120		6,615539	2,7258587 AT1G74950	-	JAZ2_TIFY10BTIFY domain/Divergent CCT motif family protein
A_84_P860087	, ,	6,6047897	2,7235126 AT1G27020		
A_84_P10561	0,03973117 2,76E-04	6,524381	2,705841 AT1G51780		ILL5_IAA-leucine resistant (ILR)-like gene 5
A_84_P12419	0,03116852 8,95E-05	6,492836	2,6988487 AT1G51760		IAR3_JR3_peptidase M20/M25/M40 family protein
A_84_P811808		6,463502	2,692316AT3G12145		FLOR1_FLR1_FTM4Leucine-rich repeat (LRR) family protein
A_84_P811806		6,430457	2,6849213AT3G12145		FLOR1_FLR1_FTM4Leucine-rich repeat (LRR) family protein
A 84 P789539		6,418689	2,6822786 AT4G06746	,	DEAR5_RAP2.9_related to AP2 9
A_84_P92059	0,04866988 5,06E-04	6,3683405	2,6709175AT5G67480		ATBT4_BT4_BTB and TAZ domain protein 4
A_84_P813646	, ,	6,234517	2,6402779AT2G26530		AR781_Protein of unknown function (DUF1645)
A_84_P10039	0,03066272 8,29E-05	6,123644	2,6143904 AT1G27020		
A_84_P23398	0,03263922 1,35E-04	6,0850153	2,6052608 AT5G06870		ATPGIP2_PGIP2polygalacturonase inhibiting protein 2
A_84_P503480		6,064253	2,6003299AT5G45630	-	Protein of unknown function, DUF584
A_84_P18211	0,032583 1,30E-04	6,037292	2,5939016AT2G26530		AR781_Protein of unknown function (DUF1645)
A_84_P14882	0,03624293 2,13E-04	5,979535	2,5800333AT1G06620		2-oxoglutarate (20G) and Fe(II)-dependent oxygenase superfamily protein
/_0+_1 1+002	0,000272002,102-04	0,070000	2,000000711000020		

				0.0000	
A_84_P12418	0,02801358 6,33E-05	5,8875475	2,5576668 AT1G49530	GGPS6	GGPS6geranylgeranyl pyrophosphate synthase 6
A_84_P154615	0,0436378 3,43E-04	5,808375	2,5381346 AT1G76590		PLATZ transcription factor family protein
A_84_P503102	0,03545904 2,06E-04	5,736511	2,5201735 AT3G15440		
A_84_P19516	0,03364056 1,60E-04	5,7081337			F ATCBF2_CBF2_DREB1C_FTQ4C-repeat/DRE binding factor 2
A_84_P13376	0,03364056 1,59E-04	5,6514235	,	ATMYB95, ATMYBCP66, N	1 ATMYB95_ATMYBCP66_MYB95myb domain protein 95
A_84_P224809	0,01254327 4,16E-06	5,640257	2,495761 AT2G38790		
A_84_P515468	0,03545904 1,95E-04	5,5587335	2,4747562 AT2G45940		Protein of unknown function (DUF295)
A_84_P174151	0,032583 1,23E-04	5,4836364	2,455133 AT2G40330	-	PYL6_RCAR9PYR1-like 6
A_84_P786720	0,03209353 1,11E-04	5,4800906	2,4541998 AT5G08790	,	ATAF2_anac081NAC No Apical Meristem domain transcriptional regulator protein
A_84_P785249	0,03545904 2,07E-04	5,416209	2,4372835 AT4G31800	ATWRKY18, WRKY18	ATWRKY18_WRKY18WRKY DNA-binding protein 18
A_84_P541993	0,03263922 1,37E-04	5,3921638	2,4308643 AT2G27310		F-box family protein
A_84_P857687	0,03157461 9,96E-05	5,3083034	2,4082508 AT1G73500	ATMKK9, MKK9	ATMKK9_MKK9_MAP kinase kinase 9
A_84_P259470	0,00821229 3,79E-07	5,1318836	2,3594885 AT1G61890		MATE efflux family protein
A_84_P22892	0,02686399 3,10E-05	5,0725274	2,3427048 AT1G73500	ATMKK9, MKK9	ATMKK9_MKK9_MAP kinase kinase 9
A_84_P608981	0,03116852 8,78E-05	5,036675	2,3324716 AT4G24600		
A_84_P13913	0,03645221 2,16E-04	5,0225487	2,3284197 AT1G19380		Protein of unknown function (DUF1195)
A_84_P11731	0,04793091 4,72E-04	4,7087526	2,235345 AT3G24500	-	ATMBF1C_MBF1Cmultiprotein bridging factor 1C
A_84_P19561	0,02840631 6,81E-05	4,7005453	2,2328281 AT4G35480		RHA3BRING-H2 finger A3B
A_84_P17388	0,04589367 4,04E-04	4,5260463	2,1782513 AT3G06500	A/N-InvC	A/N-InvCPlant neutral invertase family protein
A_84_P751526	0,02686399 3,09E-05	4,4877424	2,1659899 AT1G72920		Toll-Interleukin-Resistance (TIR) domain family protein
A_84_P834641	0,02725945 3,23E-05	4,3908973	2,1345158 AT3G29000		Calcium-binding EF-hand family proteinCalcium-binding EF-hand family protein
A_84_P10171	0,03510382 1,78E-04	4,36874			S ATMES5_AtHNL_HNL_MES5methyl esterase 5
A_84_P23164	0,02995629 7,48E-05	4,366982	2,1266365 AT3G50260	ATERF#011, CEJ1, DEAR1	ATERF#011_CEJ1_DEAR1cooperatively regulated by ethylene and jasmonate 1
A_84_P15741	0,04367585 3,55E-04	4,3414803	2,118187 AT4G27280		Calcium-binding EF-hand family protein
A_84_P862058	0,03157461 1,02E-04	4,3406134	2,117899 AT3G12145	FLOR1, FLR1	FLOR1_FLR1_FTM4Leucine-rich repeat (LRR) family protein
A_84_P603728	0,04345628 3,35E-04	4,2791715	2,0973315 AT5G02600		NAKR1_NPCC6Heavy metal transport/detoxification superfamily protein
A_84_P13173	0,03364056 1,62E-04	4,248639	2,0870008 AT5G67300	ATMYB44, ATMYBR1, MYE	3 ATMYB44_ATMYBR1_MYB44_MYBR1myb domain protein r1
A_84_P210868	0,04944391 5,34E-04	4,171626	2,0606098 AT4G16146		cAMP-regulated phosphoprotein 19-related protein
A_84_P504929	0,02995629 7,57E-05	4,164958	2,058302 AT3G29000		Calcium-binding EF-hand family proteinCalcium-binding EF-hand family protein
A_84_P807259	0,02840631 6,79E-05	4,1259584	2,0447292 AT5G67300	ATMYB44, ATMYBR1, MYE	3 ATMYB44_ATMYBR1_MYB44_MYBR1myb domain protein r1
A_84_P787617	0,04905209 5,22E-04	3,9813547	1,9932594 AT5G03270	LOG6	LOG6_lysine decarboxylase family protein
A_84_P12086	0,03157461 1,04E-04	3,9647171	1,9872179 AT5G19100		Eukaryotic aspartyl protease family protein
A_84_P501308	0,02725945 5,19E-05	3,92415	1,9723802 AT3G14260		Protein of unknown function (DUF567)
A_84_P282640	0,02745376 5,67E-05	3,8140407	1,9313202 AT2G23170	GH3.3	GH3.3Auxin-responsive GH3 family protein
A_84_P23526	0,03545904 1,94E-04	3,7528946	1,9080038 AT5G55090	MAPKKK15	MAPKKK15mitogen-activated protein kinase kinase kinase 15
A_84_P765048	0,03839359 2,45E-04	3,7421644	1,9038728 AT4G33467		
A_84_P18856	0,032583 1,22E-04	3,7176847	1,8944044 AT5G17490	AtRGL3, RGL3	AtRGL3_RGL3RGA-like protein 3
A_84_P193944	0,04589367 4,04E-04	3,7046678	1,8893442 AT5G66650		Protein of unknown function (DUF607)
A_84_P10148	0,03263922 1,39E-04	3,687331	1,882577 AT5G03270	LOG6	LOG6_lysine decarboxylase family protein
A_84_P765109	0,03866172 2,56E-04	3,6716042	1,8764105 AT4G36052		other RNA
A_84_P23394	0,02725945 4,69E-05	3,6571116	1,8707047 AT5G05730	AMT1, ASA1, JDL1, TRP5,	AMT1_ASA1_JDL1_TRP5_WEI2anthranilate synthase alpha subunit 1
A_84_P10136	0,01203481 2,47E-06	3,653376	1,8692303 AT4G36670	AtPLT6, AtPMT6, PLT6, PM	1 AtPLT6_AtPMT6_PLT6_PMT6Major facilitator superfamily protein
A_84_P12969	0,03319501 1,50E-04	3,6518743	1,8686371 AT4G17230	SCL13	SCL13_SCARECROW-like 13
A_84_P230699	0,04367585 3,55E-04	3,6216953	1,8566651 AT5G05530		RING/U-box superfamily protein
A_84_P72634	0,02725945 3,40E-05	3,5925968	1,845027 AT4G36500		
A_84_P63594	0,03263922 1,39E-04	3,5852706	1,842082 AT4G18205		Nucleotide-sugar transporter family protein
A_84_P15965	0,04418976 3,63E-04	3,5069664	1,8102236 AT5G56980		
A_84_P16285	0,04292386 3,23E-04	3,41793	1,7731228 AT2G29720	CTF2B	CTF2BFAD/NAD(P)-binding oxidoreductase family protein
A_84_P784166	0,04905209 5,18E-04	3,3979995	1,7646856 AT5G45340	CYP707A3	CYP707A3cytochrome P450, family 707, subfamily A, polypeptide 3
A_84_P12726	0,0487499 5,08E-04	3,3720498	1,7536259 AT3G11020	DREB2, DREB2B	DREB2_DREB2BDRE/CRT-binding protein 2B
				-	

A_84_P146199	0,04492016 3,83E-04	3,3702714	1,7528647 AT5G05440	PYL5, RCAR8	PYL5_RCAR8Polyketide cyclase/dehydrase and lipid transport superfamily protein
A_84_P819483	0,02725945 4,12E-05	3,362552	1,7495565 AT1G72920		Toll-Interleukin-Resistance (TIR) domain family protein
A_84_P12143	0,03545904 1,98E-04	3,3614874	1,7490997 AT5G45340	CYP707A3	CYP707A3cytochrome P450, family 707, subfamily A, polypeptide 3
A_84_P786905	0,02725945 3,62E-05	3,33497	1,7376738 AT5G41120		Esterase/lipase/thioesterase family protein
A_84_P242895	0,02725945 3,90E-05	3,301954	1,72332 AT1G66160	ATCMPG1, CMPG1	ATCMPG1_CMPG1CYS, MET, PRO, and GLY protein 1
A_84_P852047	0,02725945 3,51E-05	3,3007145	1,7227783 AT5G05440		PYL5_RCAR8_Polyketide cyclase/dehydrase and lipid transport superfamily protein
A_84_P817892	0,02745376 5,57E-05	3,2935677	1,7196512 AT5G05440	PYL5, RCAR8	PYL5_RCAR8_Polyketide cyclase/dehydrase and lipid transport superfamily protein
A_84_P286390	0,04985619 5,55E-04	3,2902992	1,7182188 AT3G25780	AOC3	AOC3_allene oxide cyclase 3
A_84_P18141	0,04280318 3,19E-04	3,25511	1,7027063 AT1G55450		S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
A_84_P856402	0,04793091 4,70E-04	3,2451842	1,6983004 AT3G50950	ZAR1	ZAR1_HOPZ-ACTIVATED RESISTANCE 1
A_84_P522895	0,03545904 1,93E-04	3,2442985	1,6979065 AT5G10210		
A_84_P19214	0,04920762 5,29E-04	3,2377703	1,6950006 AT2G32510	MAPKKK17	MAPKKK17mitogen-activated protein kinase kinase kinase 17
A_84_P17683	0,03162822 1,06E-04	3,2228677	1,688345 AT4G39580		Galactose oxidase/kelch repeat superfamily protein
A_84_P762419	0,032583 1,16E-04	3,177133	1,6677254 AT3G15518		
A_84_P16568	0,03545904 1,82E-04	3,1707025	1,6648024 AT3G55840		Hs1pro-1 protein
A_84_P135945	0,04715346 4,36E-04	3,0907733	1,6279678 AT3G11840	PUB24	PUB24_plant U-box 24
A_84_P826913	0,04758364 4,52E-04	3,0673645	1,6169996 AT3G50950		ZAR1 HOPZ-ACTIVATED RESISTANCE 1
A_84_P17609	0,03263922 1,42E-04	3,0241826	1,5965452 AT4G21870		HSP20-like chaperones superfamily protein
A_84_P14664	0,04367585 3,47E-04	2,9995308	1,5847368 AT3G53160	UGT73C7	UGT73C7_UDP-glucosyl transferase 73C7
A 84 P16077	0,03984243 2,82E-04	2,9893405	1,5798272 AT1G05100		MAPKKK18mitogen-activated protein kinase kinase kinase 18
A_84_P20474	0,04715346 4,39E-04	2,9811866	1,5758867 AT4G28000		P-loop containing nucleoside triphosphate hydrolases superfamily protein
A 84 P836721	0,03364056 1,62E-04	2,9658854	1,5684628 AT4G19520		disease resistance protein (TIR-NBS-LRR class) family
A_84_P10473	0,04963683 5,48E-04	2,9100313	1	ABCG36, ATABCG36, ATP	ABCG36_ATABCG36_ATPDR8_PEN3ABC-2 Plant PDR ABC-type transporter protein
A_84_P22522	0,03995023 2,90E-04	2,9090292	1,5405378 AT5G39020		Malectin/receptor-like protein kinase family protein
A_84_P18091	0,0282656 6,58E-05	2,9027019	1,5373964 AT1G63040		
A_84_P12431	0,04492016 3,84E-04	2,9020267	1,5370607 AT1G74420	ATEUT3, EUT3	ATFUT3_FUT3_fucosyltransferase 3
A 84 P11802	0,04791181 4,66E-04	2,8997235	1,5359154 AT3G46690		UDP-Glycosyltransferase superfamily protein
A_84_P23923	0,03545904 1,90E-04	2,8560324	1,5140123 AT2G37900		Major facilitator superfamily protein
A_84_P241449	0,04680056 4,22E-04	2,8517444	1,5118446 AT1G28190		
A 84 P608304	0,03736534 2,28E-04	2,8506021	1,5112667 AT5G54710		Ankyrin repeat family protein
A_84_P11679	0,03263922 1,38E-04	2,832755	1,5022058 AT2G23680		Cold acclimation protein WCOR413 family
A_84_P596065	0,03476378 1,73E-04	2,827343	1,4994469 AT1G49520		SWIB complex BAF60b domain-containing protein
A_84_P19584	0,03319501 1,51E-04	2,818578	1,4949675 AT4G15975		RING/U-box superfamily protein
A_84_P97466	0,04605175 4,09E-04	2,8143814	1,4928178 AT2G27830		······································
A 84 P790545	0,04706127 4,32E-04	2,8054786	1,4882469 AT1G49520		SWIB complex BAF60b domain-containing protein
A_84_P857480	0,03345015 1,55E-04	2,8033059	1,4871291 AT1G20450	FRD10,   TI29,   TI45	ERD10_LTI29_LTI45Dehydrin family protein
A_84_P19057	0,02725945 4,45E-05	2,7929404	1,4817847 AT1G67970		AT-HSFA8_HSFA8_heat shock transcription factor A8
A_84_P785967	0,02995629 7,64E-05	2,792778	1,4817009 AT5G54940		Translation initiation factor SUI1 family protein
A_84_P15109	0,02725945 4,89E-05	2,7734554	1,4716845 AT1G20450	FRD10,   TI29,   TI45	ERD10_LTI29_LTI45Dehydrin family protein
A 84 P23396	0,03157461 1,04E-04	2,768429	1,4690676 AT5G06320		NHL3 NDR1/HIN1-like 3
A_84_P15958	0,04357103 3,38E-04	2,7383487	1,4533062 AT5G54940		Translation initiation factor SUI1 family protein
A_84_P17461	0,04726939 4,43E-04	2,7376068	1,4529152 AT3G43430		RING/U-box superfamily protein
A_84_P12990	0,04791181 4,65E-04	2,7213573	1	ATREC8 DIE1 REC8 SYN	AtREC8_DIF1_REC8_SYN1Rad21/Rec8-like family protein
A_84_P114372	0,01997895 1,15E-05	2,7183206	1,4427156 AT5G53050		alpha/beta-Hydrolases superfamily protein
A 84 P23419	0,04341499 3,30E-04	2,7114112	1,4390439 AT5G13200		GRAM domain family protein
A_84_P816929	0,03954257 2,71E-04	2,6990616	1,4324579 AT5G54170		Polyketide cyclase/dehydrase and lipid transport superfamily protein
A_84_P813874	0,02960254 7,30E-05	2,6988564		AtNUDT7, GFG1, NUDT7	AtNUDT7_GFG1_NUDT7MutT/nudix family protein
A_84_P16642	0,02725945 5,26E-05	2,6948738	-	AtNUDT7, GFG1, NUDT7	AtNUDT7_GFG1_NUDT7MutT/nudix family protein
A_84_P12575	0,02745376 5,76E-05	2,6869955	1,4259939 AT2G23030		SNRK2-9_SNRK2.9_SNF1-related protein kinase 2.9
A_84_P18180	0,03347359 1,57E-04	2,6693485	1,4164877 AT2G31880		EVR_SOBIR1_Leucine-rich repeat protein kinase family protein
	0,000470031,072-04	2,0030400	1,7104011 A12031000		

A_84_P19740	0,03434476 1,68E-04	2,6352463	1,3979378 AT5G54300		Protein of unknown function (DUF761)
A_84_P13235	0,04993777 5,62E-04	2,6289027	1,3944607 AT1G13260	EDF4, RAV1	AtRAV1_EDF4_RAV1related to ABI3/VP1 1
A_84_P13740	0,04680056 4,25E-04	2,5941815	1,3752794 AT3G57740		Protein kinase superfamily protein
A_84_P819884	0,04834748 4,97E-04	2,5927722	1,3744955 AT4G38550		Arabidopsis phospholipase-like protein (PEARLI 4) family
A_84_P21661	0,02065646 1,29E-05	2,581782	1,3683672 AT5G61590		Integrase-type DNA-binding superfamily protein
A_84_P16735	0,03329087 1,53E-04	2,5734754	1,3637179 AT4G39070		BBX20_BZS1B-box zinc finger family protein
A_84_P823703	0,04715346 4,39E-04	2,5656297	1,359313 AT5G52882		P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P786801	0,04605175 4,08E-04	2,5640743	1,358438 AT5G13210		Uncharacterised conserved protein UCP015417, vWA
A_84_P23992	0,02725945 5,07E-05	2,5617168	1,357111 AT3G05640		Protein phosphatase 2C family proteinProtein phosphatase 2C family protein
A_84_P166733	0,04993777 5,63E-04	2,5440683	1,3471375 AT5G17350		
A_84_P24091	0,04357103 3,39E-04	2,4709506		ATEXLA3, ATEXPL3, ATHE	ATEXLA3 ATEXPL3 ATHEXP BETA 2.3 EXLA3 EXPL3 expansin-like A3
A_84_P15703	0,032583 1,30E-04	2,453968	1,2951164 AT4G18340	,	Glycosyl hydrolase superfamily protein
A_84_P278240	0,0479263 4,67E-04	2,4509275	1,2933278 AT3G29040		Domain of unknown function (DUF26)
A 84 P17400	0,04944391 5,35E-04	2,4429362		ATRSH2 AT-RSH2 RSH2	AT-RSH2_ATRSH2_RSH2RELA/SPOT homolog 2
A_84_P19431	0,03545904 2,06E-04	2,4340978	1,2833871 AT3G62150		ABCB21_PGP21_P-glycoprotein 21
A_84_P18342	0,03839359 2,46E-04	2,408123		A37, ATPDX1.2, PDX1.2	A37_ATPDX1.2_PDX1.2_pyridoxine biosynthesis 1.2
A 84 P819063	0,04905209 5,23E-04	2,4044883	1,2657299 AT3G46640		LUX_PCL1Homeodomain-like superfamily protein
A_84_P55660	0,04903209 5,25E-04 0,01750383 6,86E-06	2,400437	1,2632971 AT3G56880	EOX, I GET	VQ motif-containing protein
A_84_P127601	0,04686994 4,27E-04	2,3992488	1,2625828 AT5G02940		Protein of unknown function (DUF1012)
		2,3992466 2,38777			Glycosyltransferase family 61 protein
A_84_P158475	0,032583 1,29E-04		1,2556639 AT2G41640		
A_84_P793477	0,032583 1,19E-04	2,3632765	1,2407885 AT5G52882		P-loop containing nucleoside triphosphate hydrolases superfamily protein
A_84_P281300	0,04968483 5,51E-04	2,3574302	1,237215 AT3G46640		LUX_PCL1Homeodomain-like superfamily protein
A_84_P564716	0,029956297,73E-05	2,3456824	1,2300076 AT1G03850	AIGRXS13, GRXS13	ATGRXS13_GRXS13Glutaredoxin family protein
A_84_P259140	0,04905209 5,22E-04	2,3317509	1,2214136 AT1G28050		BBX13_B-box type zinc finger protein with CCT domain
A_84_P19413	0,02459617 2,10E-05	2,3285053			2 ATCPK32_CDPK32_CPK32calcium-dependent protein kinase 32
A_84_P14864	0,04920762 5,30E-04	2,322025	1,2153835 AT4G37260	АТМҮВ73, МҮВ73	ATMYB73_MYB73myb domain protein 73
A_84_P796587	0,036775192,21E-04	2,283834	1,1914577 AT2G27389		
A_84_P20977	0,04418976 3,65E-04	2,2757728	1,1863565 AT1G14480		Ankyrin repeat family protein
A_84_P21825	0,03263922 1,41E-04	2,2708888	1,1832571 AT1G13210	ACA.I	ACA.Iautoinhibited Ca2+/ATPase II
A_84_P510552	0,03263922 1,38E-04	2,2424476	1,1650742 AT5G41100		
A_84_P233859	0,03545904 1,98E-04	2,2225354	1,1522064 AT2G28570		
A_84_P127291	0,01203481 1,66E-06	2,2088308	1,1432829 AT5G62865		
A_84_P828333	0,04514324 3,95E-04	2,2076542	1,1425142 AT2G29740	UGT71C2	UGT71C2_UDP-glucosyl transferase 71C2
A_84_P15729	0,04332435 3,28E-04	2,1981933	1,1363182 AT1G32460		
A_84_P17279	0,04367585 3,54E-04	2,184822	1,1275158 AT2G25090	CIPK16, SnRK3.18	AtCIPK16_CIPK16_SnRK3.18CBL-interacting protein kinase 16
A_84_P22597	0,0345411 1,70E-04	2,173714	1,120162 AT5G59550		AtRDUF2_RDUF2zinc finger (C3HC4-type RING finger) family protein
A_84_P15341	0,03117324 9,21E-05	2,149713	1,1041441 AT1G64200	VHA-E3	VHA-E3_vacuolar H+-ATPase subunit E isoform 3
A_84_P843872	0,03016915 7,86E-05	2,1364977	1,0952477 AT1G53430		Leucine-rich repeat transmembrane protein kinase
A_84_P799914	0,03545904 2,04E-04	2,1283474	1,0897336 AT3G05640		Protein phosphatase 2C family protein
A_84_P175641	0,04834748 4,83E-04	2,0755057	1,0534629 AT3G19660		
A_84_P538776	0,03839359 2,43E-04	2,0744553	1,0527326 AT4G24015		RING/U-box superfamily protein
A_84_P538274	0,02757152 5,92E-05	2,0676796	1,0480126 AT1G30755		Protein of unknown function (DUF668)
A_84_P16882	0,03866172 2,55E-04	2,0597782	1,0424889 AT5G48540		receptor-like protein kinase-related family protein
A_84_P202508	0,04834748 4,91E-04	2,0573874	1,0408134 AT1G73210		Protein of unknown function (DUF789)
A 84 P13043	0,04726939 4,45E-04	2,0551188	1,0392218 AT5G25440		Protein kinase superfamily protein
A_64_P13043 A_84_P767643	0,047209394,43E-04 0,012034811,17E-06	2,0531188	1,0381012 AT1G29620;	(	
A_84_P118232	0,012034811,17E-08 0,027379915,43E-05	2,053523	1,0305786 AT5G16360	t	NC domain-containing protein-related
A_84_P15979	0,04514324 3,92E-04	2,0288744	1,0206796 AT5G60270		LecRK-I.7_Concanavalin A-like lectin protein kinase family protein
A_84_P87289	0,04834748 4,87E-04	2,003949	1,0028458 AT1G24807;		
A_84_P16108	0,02561561 2,64E-05	2,0025756	1,0018567 AT1G09940	HEMA2	HEMA2Glutamyl-tRNA reductase family protein

A_84_P23998	0,03177396 1,07E-04	1,9864762	0,9902115 AT1G53430		Leucine-rich repeat transmembrane protein kinase
A_84_P13968	0,03319501 1,52E-04	1,9713829	0,979208 AT5G16410		HXXXD-type acyl-transferase family protein
A_84_P798820	0,03984243 2,84E-04	1,9696114	0,977911 AT2G41430	CID1, ERD15, LSR1	CID1_ERD15_LSR1dehydration-induced protein (ERD15)
A_84_P89769	0,03545904 2,05E-04	1,9542948	0,9666481 AT2G35930	PUB23	AtPUB23_PUB23plant U-box 23
A_84_P17009	0,03677519 2,21E-04	1,9475461	0,96165746 AT1G20823		RING/U-box superfamily protein
A_84_P612207	0,03545904 1,91E-04	1,9426678	0,9580392 AT2G18196		Heavy metal transport/detoxification superfamily protein
A_84_P18782	0,03995023 2,88E-04	1,9260719	0,94566154 AT1G64380		Integrase-type DNA-binding superfamily protein
A_84_P16068	0,04367585 3,50E-04	1,919927	0,9410514 AT1G33790		jacalin lectin family protein
A 84 P852460	0,04605175 4,12E-04	1,9185543	0,9400196 AT3G03020		, ,,
A_84_P20325	0,03319501 1,50E-04	1,9122261	0,9352531 AT3G50060	MYB77	MYB77myb domain protein 77
A_84_P14972	0,03839359 2,43E-04				S ARA8_ATPCS1_CAD1_PCS1phytochelatin synthase 1 (PCS1)
A_84_P828208	0,04367585 3,55E-04	1,9024699	0,9278736 AT5G53060		RCF3_SHI1RNA-binding KH domain-containing protein
A_84_P599750	0,03347359 1,57E-04	1,8927684	0,9204979 AT2G07779		0 01
A_84_P832916	0,04087757 2,99E-04	1,8911864	0,9192915 AT2G44380		Cysteine/Histidine-rich C1 domain family protein
A_84_P16701	0,02505797 2,25E-05	1,8864268	0,9156561 AT4G30350		SMXL2Double Clp-N motif-containing P-loop nucleoside triphosphate hydrolases protein
A 84 P11230	0,032583 1,24E-04	1,8629879	0,8976183 AT5G54190	PORA	PORA protochlorophyllide oxidoreductase A
A_84_P553302	0,03263667 1,32E-04	1,8568221	0,8928356 AT1G25500		Plasma-membrane choline transporter family protein
A_84_P11150	0,02725945 5,20E-05		0,89211845 AT5G24590	ANAC091 TIP	ANAC091_TIPTCV-interacting proteinANAC091_TIPTCV-interacting protein
A 84 P10316	0,03984243 2,84E-04		0,88928986 AT5G62020	-	AT-HSFB2A_HSFB2A_heat shock transcription factor B2A
A_84_P22972	0,04343475 3,33E-04	1,8432426	0,882246 AT2G42980		Eukaryotic aspartyl protease family protein
A_84_P833361	0,04292386 3,22E-04		0,87414837 AT2G30100		pentatricopeptide (PPR) repeat-containing protein
A 84 P23624	0,02725945 4,88E-05		0,87212944 AT1G71697	ATCK1 CK CK1	ATCK1_CK_CK1choline kinase 1
A 84 P839512	0,04993777 5,60E-04	1,8275504	0,8699112 AT4G10845		
A_84_P852438	0,04341499 3,32E-04	1,8163341	0,8610296 AT5G24590	ANACO91 TIP	ANAC091_TIPTCV-interacting protein
A_84_P10224	0,04357103 3,39E-04	1,7917385	0,8413601 AT5G37770	-	CML24_TCH2_EF hand calcium-binding protein family
A 84 P527116	0,03973117 2,77E-04	,	0,83870316 AT3G48180		
A_84_P20795	0,032583 1,30E-04	,	0,82505417 AT1G15890		Disease resistance protein (CC-NBS-LRR class) family
A_84_P19320	0,04196317 3,11E-04		0,82233137 AT3G16510		Calcium-dependent lipid-binding (CaLB domain) family protein
A_84_P785283	0,04190317 3,11E-04 0,03116852 8,78E-05		0,82203954 AT1G78070		Transducin/WD40 repeat-like superfamily protein
	, ,	1,754464	,		ATWRKY28_WRKY28_WRKY DNA-binding protein 28
A_84_P18540 A_84_P840152	0,04834748 4,98E-04	1,7544409	0,8110304 AT4G18170 0,8110113 AT5G13190		AtGILP_GILP_
	0,02725945 4,21E-05	,	-	-	ADR1-L1 ADR1-like 1
A_84_P19551	0,02725945 4,99E-05	1,7541347 1,7454746	0,8107595 AT4G33300 0,8036193 AT2G22300		
A_84_P272750	0,04348327 3,36E-04	,	,	,	CAMTA3_SR1signal responsive 1
A_84_P245045	0,04834748 4,84E-04	1,7222108	0,7842617 AT5G64660		ATCMPG2_CMPG2_CYS, MET, PRO, and GLY protein 2
A_84_P20605	0,04866988 5,06E-04		0,77852243 AT5G24530	DMR6	DMR6_2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
A_84_P17672	0,04993777 5,63E-04	1,7120031	0,7756853 AT4G36150		Disease resistance protein (TIR-NBS-LRR class) family
A_84_P16180	0,04430861 3,70E-04		0,77227783 AT1G11050		Protein kinase superfamily protein
A_84_P13957	0,03545904 2,04E-04	1,7032583	0,7682972 AT5G11650		alpha/beta-Hydrolases superfamily protein
A_84_P21190	0,04726939 4,44E-04		0,76679325 AT3G16350		Homeodomain-like superfamily protein
A_84_P20006	0,032583 1,23E-04	1,7005234	0,7659788 AT1G76380		DNA-binding bromodomain-containing protein
A_84_P10152	0,036775192,21E-04		0,76542753 AT5G04760		Duplicated homeodomain-like superfamily protein
A_84_P22312	0,04379721 3,57E-04	1,6839865	-		RHA1BRING-H2 finger A1B
A_84_P15820	0,04920762 5,29E-04	,	0,75063705 AT5G03630		ATMDAR2Pyridine nucleotide-disulphide oxidoreductase family protein
A_84_P21553	0,04503756 3,87E-04		0,72775555 AT5G25110	CIPK25, SnRK3.25	CIPK25_SnRK3.25CBL-interacting protein kinase 25
A_84_P159575	0,0345411 1,70E-04		0,69975466 AT4G15765		FAD/NAD(P)-binding oxidoreductase family protein
A_84_P18077	0,04963683 5,47E-04		0,69112676 AT1G67560	ATLOX6, LOX6	ATLOX6_LOX6PLAT/LH2 domain-containing lipoxygenase family protein
A_84_P156715	0,03116852 8,68E-05		0,69070244 AT5G62770		Protein of unknown function (DUF1645)
A_84_P766219	0,01997895 1,15E-05	,	0,67398834 AT5G26600		Pyridoxal phosphate (PLP)-dependent transferases superfamily protein
A_84_P12249	0,04834748 4,98E-04	1,5714763	0,65212053 AT1G28010	ABCB14, ATABCB14, MDR	ABCB14_ATABCB14_MDR12_PGP14P-glycoprotein 14
A_84_P17501	0,04605175 4,10E-04	1,5606717	0,6421671 AT3G53180	NodGS	NodGSglutamate-ammonia ligases;catalytics;glutamate-ammonia ligases

A_84_P21946	0,02725945 4,07E-05	1,5567553 0,6385422 AT1G73540		NUDT21_atnudt21nudix hydrolase homolog 21
A_84_P22448	0,03937708 2,67E-04	1,5515846 0,63374233 AT1G06640		2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
A_84_P599935	0,04963683 5,47E-04			5 NTMC2T5.2_NTMC2TYPE5.2Calcium-dependent lipid-binding (CaLB domain) protein
A_84_P18223	0,04758364 4,51E-04	1,502241 0,5871163 AT2G32800		AP4.3A_LecRK-S.2protein kinase family protein
A_84_P10812	0,032583 1,24E-04	-1,5010667 -0,58598804 AT3G13150		Tetratricopeptide repeat (TPR)-like superfamily protein
A_84_P808242	0,03545904 1,87E-04	-1,5020751 -0,5869569 AT1G14345		NAD(P)-linked oxidoreductase superfamily protein
A_84_P12750	0,04905209 5,23E-04	-1,5058012 -0,5905313 AT3G47430		PEX11Bperoxin 11B
A_84_P14719	0,04805315 4,76E-04	-1,5200204 -0,60409063 AT4G02070	ATMSH6, MSH6, MSH6-1	ATMSH6_MSH6_MSH6-1MUTS homolog 6
A_84_P557318	0,04706127 4,30E-04	-1,530575 -0,61407375 AT4G04790	1	Tetratricopeptide repeat (TPR)-like superfamily protein
A_84_P603012	0,04514324 3,95E-04	-1,5468154 -0,629301 AT5G59360	1	
A_84_P606916	0,04993777 5,63E-04	-1,5604345 -0,64194775 AT2G30890	; TBL43	
A_84_P294634	0,04430861 3,72E-04	-1,5648938 -0,64606476 AT5G53500	1	Transducin/WD40 repeat-like superfamily protein
A_84_P75044	0,04446041 3,77E-04	-1,571464 -0,65210915 AT5G02830	)	Tetratricopeptide repeat (TPR)-like superfamily protein
A_84_P22890	0,04238424 3,15E-04	-1,5726548 -0,65320206 AT1G14410	ATWHY1, PTAC1, WHY1	ATWHY1_PTAC1_WHY1_ssDNA-binding transcriptional regulator
A_84_P788949	0,04834748 4,97E-04	-1,58142 -0,6612205 AT3G28100		UMAMIT45nodulin MtN21 /EamA-like transporter family protein
A_84_P15631	0,04196317 3,10E-04	-1,5903727 -0,66936487 AT3G57830		Leucine-rich repeat protein kinase family protein
A_84_P519713	0,04805315 4,76E-04	-1,5977954 -0,6760827 AT1G66840		PMI2_WEB2Plant protein of unknown function (DUF827)
A_84_P19998	0,01822007 7,98E-06	-1,5984154 -0,67664236 AT1G70550	,	Protein of Unknown Function (DUF239)
A_84_P16203	0,01203481 2,96E-06	-1,6029801 -0,68075657 AT1G48630		RACK1B_RACK1B_ATreceptor for activated C kinase 1B
A_84_P525958	0,03670782 2,18E-04	-1,6037549 -0,68145365 AT1G77630		LYM3_LYP3Peptidoglycan-binding LysM domain-containing protein
A_84_P18915	0,04834748 4,95E-04	-1,610567 -0,68756866 AT1G51460		ABCG13_ABC-2 type transporter family protein
A_84_P814458	0,032583 1,18E-04	-1,6148211 -0,6913743 AT1G72970		EDA17_HTHGlucose-methanol-choline (GMC) oxidoreductase family protein
A_84_P756180	0,04514324 3,92E-04	-1,6263505 -0,7016382 AT2G14890	,	AGP9_arabinogalactan protein 9
A_84_P13735	0,02459617 2,04E-05	-1,6550156 -0,7268448 AT3G56370		Leucine-rich repeat protein kinase family protein
A_84_P828891	0,03302845 1,48E-04	-1,6658808 -0,73628515 AT3G28100		UMAMIT45nodulin MtN21 /EamA-like transporter family protein
A 84 P20046	0,02561561 2,78E-05	-1,6663269 -0,73667145 AT1G14280		PKS2phytochrome kinase substrate 2
A 84 P610685	0,02553658 2,42E-05	-1,6737278 -0,7430649 AT3G59670		
A_84_P23174	0,03839359 2,46E-04		ACA1, ATACA1, CAH1	ACA1_ATACA1_CAH1alpha carbonic anhydrase 1
A_84_P16459	0,03839359 2,44E-04	-1,737366 -0,79690164 AT3G14570		ATGSL04_GSL04_GSL4_atgsl4glucan synthase-like 4
A_84_P15327	0,04706127 4,31E-04	-1,7497976 -0,80718803 AT2G41940		ZFP8zinc finger protein 8
A_64_P15327 A_84_P19743	0,03545904 1,82E-04	-1,7528561 -0,8097076 AT5G55250		AtIAMT1_IAMT1_IAA carboxylmethyltransferase 1
			-	
A_84_P292434	0,03498028 1,77E-04	-1,7646543 -0,8193855 AT2G47930	-	AGP26_ATAGP26arabinogalactan protein 26
A_84_P224349	0,03407956 1,66E-04	-1,7719564 -0,82534313 AT2G46040		ARID/BRIGHT DNA-binding domain;ELM2 domain protein
A_84_P837561	0,02725945 4,04E-05	-1,7828845 -0,8342132 AT5G06790		Destais Lisson family sectors
A_84_P23459	0,02725945 5,32E-05	-1,7854155 -0,83625984 AT5G35960		Protein kinase family protein
A_84_P21962	0,04430861 3,72E-04	-1,7864089 -0,83706236 AT2G31830		5PTase14endonuclease/exonuclease/phosphatase family protein
A_84_P21617	0,02745376 5,59E-05	-1,7899876 -0,83994955 AT5G49910	•	HSC70-7_cpHsc70-2chloroplast heat shock protein 70-2
A_84_P556476	0,02553658 2,47E-05	-1,8061285 -0,8529005 AT4G18750		DOT4_Pentatricopeptide repeat (PPR) superfamily protein
A_84_P10579	0,03995023 2,86E-04	-1,8061941 -0,8529529 AT1G78970		ATLUP1_LUP1_lupeol synthase 1
A_84_P13774	0,04834748 4,95E-04	-1,8081007 -0,854475 AT4G02060		MCM7_PRLMinichromosome maintenance (MCM2/3/5) family protein
A_84_P12918	0,04845032 5,00E-04	-1,8207784 -0,8645553 AT4G30120		ATHMA3_HMA3_heavy metal atpase 3
A_84_P15930	0,04430861 3,70E-04	-1,8238839 -0,8670139 AT1G29460		SAUR65SAUR-like auxin-responsive protein family
A_84_P16879	0,04715346 4,36E-04	-1,8380351 -0,8781643 AT5G47800		Phototropic-responsive NPH3 family protein
A_84_P11901	0,03736534 2,29E-04	-1,8434998 -0,88244724 AT1G06100		Fatty acid desaturase family protein
A_84_P599886	0,04418976 3,66E-04	-1,8572689 -0,8931827 AT3G06145		
A_84_P23912	0,03263922 1,41E-04	-1,8782952 -0,90942377 AT2G38940		ATPT2_PHT1;4phosphate transporter 1;4
A_84_P14379	0,03866172 2,52E-04	-1,9066913 -0,9310713 AT1G14440	AtHB31, HB31, ZHD4	AtHB31_FTM2_HB31_ZHD4homeobox protein 31
A_84_P525765	0,04479766 3,81E-04	-1,9177188 -0,93939114 AT1G07270	1	Cell division control, Cdc6
A_84_P830586	0,03984243 2,83E-04	-1,9280196 -0,9471197 AT5G22310	1	
A_84_P13857	0,03545904 1,90E-04	-2,0062153 -1,0044764 AT4G28780	1	GDSL-like Lipase/Acylhydrolase superfamily protein
	•			

A_84_P15700 A_84_P815691 A_84_P826247 A_84_P286230 A_84_P284680 A_84_P785044 A_84_P18411 A_84_P15097 A_84_P564094	0,04834748 4,89E-04 0,04905209 5,23E-04 0,03995023 2,88E-04 0,04905209 5,16E-04 0,032583 1,29E-04 0,03954257 2,73E-04 0,03839359 2,44E-04 0,02725945 4,23E-05 0,03839359 2,43E-04	-2,0230672 -2,0243142 -2,0565464 -2,0649412 -2,0658257 -2,0822313 -2,1690044 -2,224122 -2,3109784	-1,0165442 AT4G13710 -1,0174332 AT3G49670 BAM2 -1,0402236 AT4G18960 AG -1,0461006 AT3G25905 CLE27 -1,0467185 AT3G01730 -1,0581303 AT2G27840 HDA13, HDT04, HDT4 -1,117033 AT3G44970 -1,1532359 AT1G35290 -1,2085037 AT2G10920	Pectin lyase-like superfamily protein BAM2Leucine-rich receptor-like protein kinase family protein AGK-box region and MADS-box transcription factor family protein CLE27CLAVATA3/ESR-RELATED 27 HD2D_HDA13_HDT04_HDT4histone deacetylase-related / HD- related Cytochrome P450 superfamily protein ALT1Thioesterase superfamily protein
A_84_P304094 A_84_P12766 A_84_P592665 A_84_P827741 A_84_P756272 A_84_P609989 A_84_P18634 A_84_P21135 A_84_P15502 A_84_P13212	0,030393392,43E-04 0,046051754,08E-04 0,031574619,73E-05 0,0325831,16E-04 0,047153464,40E-04 0,045143243,95E-04 0,045143243,95E-04 0,025615612,63E-05 0,049052095,22E-04 0,043595963,42E-04	-2,3103784 -2,3183165 -2,357402 -2,3619843 -2,5115683 -2,5272398 -2,7114186 -2,7293327 -2,7771978 -2,8259041	-1,2030375 AT2G10920 -1,2130775 AT3G51240 F3H, F3'H, TT6 -1,2371979 AT2G24762 AtGDU4, GDU4 -1,2399993 AT3G12820 AtMYB10, MYB10 -1,3285885 AT2G27395 -1,3375626 AT5G18720 -1,4390478 AT4G14700 ATORC1A, ORC1A -1,4485482 AT3G03820 -1,47363 AT3G14370 WAG2 -1,4987125 AT2G27395	F3'H_F3H_TT6flavanone 3-hydroxylase AtGDU4_GDU4glutamine dumper 4 AtMYB10_MYB10myb domain protein 10 Domain of unknown function (DUF3444) ATORC1A_ORC1Aorigin recognition complex 1 SAUR29SAUR-like auxin-responsive protein family WAG2Protein kinase superfamily protein
A_84_P815182 A_84_P10228 A_84_P769679 A_84_P828605 A_84_P10285 A_84_P21169	0,04367585 3,46E-04 0,04680056 4,25E-04 0,03545904 1,90E-04 0,03117324 9,41E-05 0,02725945 4,06E-05 0,04905209 5,25E-04	-3,0570955 -3,060963 -3,146212 -3,5074313 -3,8586605 -4,6126404	-1,6121616 AT3G27060 ATTSO2, TSO2 -1,6139855 AT5G38820 -1,653616 AT5G49615 TAS3b -1,8104148 AT4G25010 AtSWEET14, SWEET14 -1,9481001 AT5G54510 DFL1, GH3.6 -2,2055929 AT3G04280 ARR22, RR22	ATTSO2_TSO2_Ferritin/ribonucleotide reductase-like family protein Transmembrane amino acid transporter family protein TAS3b_TAS3b (trans-acting siRNA 3b); other RNA AtSWEET14_SWEET14_Nodulin MtN3 family protein DFL1_GH3.6_Auxin-responsive GH3 family protein ARR22_RR22_response regulator 22

Annexes