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Valenciana

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Evaluation of eggplant, wild relatives and introgression breeding materials for tolerance to abiotic stresses

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PhD Thesis

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Valencia, 2024

Advisors:

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Ph.D. dissertation by

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A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY WITHIN THE BIOTECHNOLOGY PROGRAM OF THE UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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“La felicidad se puede hallar hasta en los más oscuros momentos, si somos capaces de usar bien la luz.” - Albus Dumbledore

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¡Travesura realizada!

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ABSTRACT

Climate change, driven by human activities that increase greenhouse gas emissions and lead to extreme weather events and biodiversity loss, is intensifying abiotic stress in agriculture. Rising demands for plant products have expanded agricultural lands, often at the expense of natural ecosystems, further exacerbating climate change and desertification. In this way, drought stress severely affects crop productivity, impacting plant growth and yield. Also, excessive nitrogen fertilization, commonly used to improve crop yields, disrupts the nitrogen cycle, leading to groundwater contamination, air quality deterioration, and significant health issues.

Eggplant (*Solanum melongena* L.) is an important vegetable and leveraging genetic resources, including wild relatives, is important for breeding programs aimed at enhancing drought tolerance and nitrogen use efficiency. Advances in next-generation sequencing and genotyping have significantly improved our understanding of stress-related traits in several crops, supporting the development of resilient cultivars.

Therefore, in the present doctoral thesis, we have investigated the potential of wild eggplant relatives to improve tolerance to abiotic stresses. Our goal was to identify new sources of genetic variation for incorporation into breeding programs. This approach seeks to identify and validate valuable genetic resources, genomic regions, and candidate genes to develop more resilient eggplant varieties. By employing a multidisciplinary strategy that includes genomic, transcriptomic, and phenotypic analyses, we aim to promote more sustainable agriculture.

In the first chapter of this thesis, we focused on evaluating advanced backcrosses (ABs) of eggplant and its wild relatives to improve nitrogen use efficiency (NUE) under low nitrogen (N) conditions. This chapter includes the first comprehensive evaluation of the wild relative species *S. elaeagnifolium* from the tertiary genepool, and ABs in the eggplant genetic background, under low N cultivation conditions. We assessed *S. melongena* and *S. elaeagnifolium* parentals and their ABs for various plant, fruit, and composition traits. Significant differences were observed among the two parentals in all evaluated traits. High phenotypic diversity was observed in the ABs, with some transgressive individuals exhibiting higher yields and NUE than the cultivated parent. Additionally, we evaluated three sets of ABs from different genepools: *S. insanum* (GP1), *S. dasyphyllum* (GP2), and *S. elaeagnifolium* (GP3), under low N conditions. This evaluation provided insights into the potential of these wild species for breeding under low nitrogen input abiotic stress conditions. Low N conditions generally decreased chlorophyll content but increased flavonol and anthocyanin levels, and reduced aerial biomass, stem diameter, yield, and nitrogen and carbon content in plants and fruits. The high phenotypic diversity and wide distribution ranges in the ABs support the potential of CWRs to enhance the resilience and genetic diversity of eggplants under abiotic stress conditions, emphasizing their value in breeding programs for low nitrogen conditions and sustainable agriculture. The availability of genotyping and phenotyping data enabled the identification and validation of QTLs for plant, fruit, and composition traits. Several QTLs were identified in the ABs of *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium*. These QTLs were associated with traits such as plant growth, yield, fruit size, and nitrogen content, thereby expanding the genomic understanding of eggplant.

In the second chapter of this thesis, we investigated the drought response mechanisms in cultivated eggplant (*S. melongena*) and its wild relative *S. dasyphyllum*. We evaluated these species under hydroponic conditions with two concentrations of polyethylene glycol (PEG) (20% and 30%) at different phenological stages, using RNA sequencing (RNA-Seq) to analyze gene expression patterns. *S. dasyphyllum* demonstrated superior tolerance to osmotic stress compared to *S. melongena*, with a greater number of differentially expressed genes (DEGs) under stress. Gene ontology (GO) enrichment and KEGG pathway analyses revealed that both species activated a range of transcription factors and stress response pathways, with *S. dasyphyllum* showing more extensive gene regulation. Key pathways included abscisic acid (ABA) signaling, MAPK signaling, and various biosynthesis pathways. These findings highlight the potential of *S. dasyphyllum* as a source of genes for developing drought-tolerant eggplant varieties, emphasizing the importance of utilizing wild relatives to enhance crop tolerance and sustainability.

Overall, this doctoral thesis demonstrated the potential of wild eggplant relatives for breeding tolerance to abiotic stresses. The development of advanced backcrosses from different crop wild relatives expanded genetic variation, showing significant effects of nitrogen doses on various traits and identifying transgressive individuals with improved agronomic characteristics. The detection and validation of QTLs provided insights into eggplant productivity, nitrogen use efficiency, and drought response mechanisms, contributing to the development of more resilient and sustainable eggplant varieties.

RESUMEN

El cambio climático, impulsado por actividades humanas que incrementan las emisiones de gases de efecto invernadero y provocan eventos climáticos extremos y pérdida de biodiversidad, está intensificando el estrés abiótico en la agricultura. La creciente demanda de productos vegetales ha ampliado las tierras agrícolas, a menudo a expensas de los ecosistemas naturales, exacerbando aún más el cambio climático y la desertificación. De esta manera, el estrés por sequía afecta severamente la productividad de los cultivos, afectando el crecimiento y el rendimiento de las plantas. Además, la fertilización excesiva con nitrógeno, comúnmente utilizada para mejorar los rendimientos de los cultivos, altera el ciclo del nitrógeno, lo que lleva a la contaminación de las aguas subterráneas, el deterioro de la calidad del aire y problemas de salud significativos.

La berenjena (*Solanum melongena* L.) es una hortaliza importante y aprovechar los recursos genéticos, incluidos los parientes silvestres, es crucial para los programas de mejora genética dirigidos a mejorar la tolerancia a la sequía y la eficiencia en el uso del nitrógeno. Los avances en la secuenciación de nueva generación y el genotipado han mejorado significativamente nuestra comprensión de los caracteres relacionados con el estrés en varios cultivos, apoyando el desarrollo de cultivares resilientes.

Por lo tanto, en la presente tesis doctoral, hemos investigado el potencial de los parientes silvestres de la berenjena para mejorar la tolerancia a los estreses abióticos. Nuestro objetivo era identificar nuevas fuentes de variación genética para su incorporación en programas de mejora genética. Este enfoque busca identificar y validar recursos genéticos valiosos, regiones genómicas y genes candidatos para desarrollar variedades de berenjena más resilientes. Mediante una estrategia multidisciplinaria que incluye análisis genómicos, transcriptómicos y fenotípicos, pretendemos promover una agricultura más sostenible.

En el primer capítulo de esta tesis, nos centramos en evaluar retrocruzamientos avanzados (ABs) de berenjena y sus parientes silvestres para mejorar la eficiencia en el uso del nitrógeno (NUE) bajo condiciones de bajo nitrógeno (N). Este capítulo incluye la primera evaluación exhaustiva de la especie silvestre relacionada *S. elaeagnifolium* del pool genético terciario, y los ABs derivados con fondo genético de la berenjena, bajo condiciones de cultivo con bajo N. Evaluamos los parentales *S. melongena* y *S. elaeagnifolium* y sus ABs en diversos caracteres de planta, fruto y composición. Se observaron diferencias significativas entre los dos parentales en todos los caracteres evaluados. Se observó una alta diversidad fenotípica en los ABs, con algunos individuos transgresivos que exhibieron mayores rendimientos y NUE que el parental cultivado. Además, evaluamos tres conjuntos de ABs de diferentes pools genéticos: *S. insanum* (GP1), *S. dasyphyllum* (GP2) y *S. elaeagnifolium* (GP3), bajo condiciones de bajo N. Esta evaluación proporcionó información sobre el potencial de estas especies silvestres para la mejora genética bajo condiciones de estrés abiótico con bajo aporte de nitrógeno. Las condiciones de bajo N generalmente disminuyeron el contenido de clorofila, pero aumentaron los niveles de flavonol y antocianinas, y redujeron la biomasa aérea, el diámetro del tallo, el rendimiento y el contenido de nitrógeno y carbono en plantas y frutos. La alta diversidad fenotípica y el amplio rango de distribución en los ABs apoyan el potencial de los CWRs para mejorar la resiliencia y la diversidad genética de las berenjenas bajo condiciones de estrés abiótico, destacando su valor en los programas de mejora genética para condiciones de bajo nitrógeno y agricultura sostenible. La disponibilidad de datos de genotipado y fenotipado permitió la identificación y validación de QTLs para caracteres de planta, fruto y composición. Se identificaron varios QTLs en los ABs de *S. insanum*, *S. dasyphyllum* y *S. elaeagnifolium*. Estos QTLs se asociaron con caracteres como el crecimiento de la planta, el rendimiento, el tamaño del fruto y el contenido de nitrógeno, ampliando así la comprensión genómica de la berenjena.

En el segundo capítulo de esta tesis, investigamos los mecanismos de respuesta a la sequía en la berenjena cultivada (*S. melongena*) y su pariente silvestre *S. dasyphyllum*. Evaluamos estas especies en condiciones hidropónicas con dos concentraciones de polietilenglicol (PEG) (20% y 30%) en diferentes etapas fenológicas, utilizando la secuenciación de ARN (RNA-Seq) para analizar los patrones de expresión génica. *S. dasyphyllum* demostró una tolerancia superior al estrés osmótico en comparación con *S. melongena*, con un mayor número de genes diferencialmente expresados (DEGs) bajo estrés. Los análisis de enriquecimiento de ontología génica (GO) y las vías KEGG revelaron que ambas especies activaron una gama de factores de transcripción y vías de respuesta al estrés, con *S. dasyphyllum* mostrando una regulación génica más extensa. Las vías clave incluyeron la señalización del ácido abscísico (ABA), la señalización MAPK y varias vías de biosíntesis. Estos hallazgos resaltan el potencial de *S. dasyphyllum* como fuente de genes para desarrollar variedades de berenjena tolerantes a la sequía, destacando la importancia de utilizar parientes silvestres para mejorar la tolerancia y la sostenibilidad de los cultivos.

En general, esta tesis doctoral demostró el potencial de los parientes silvestres de la berenjena para la mejora de la tolerancia a estreses abióticos. El desarrollo de retrocruzamientos avanzados de diferentes parientes silvestres del cultivo amplió la variación genética, mostrando efectos significativos de las dosis de nitrógeno en diversos caracteres e identificando individuos transgresivos con características agronómicas mejoradas. La detección y validación de QTLs proporcionó información sobre la productividad de la berenjena, la eficiencia en el uso del nitrógeno y los mecanismos de respuesta a la sequía, contribuyendo al desarrollo de variedades de berenjena más resilientes y sostenibles.

RESUM

El canvi climàtic, impulsat per activitats humanes que incrementen les emissions de gasos d'efecte hivernacle i provoquen esdeveniments climàtics extrems i pèrdua de biodiversitat, està intensificant l'estrès abiòtic en l'agricultura. La creixent demanda de productes vegetals ha ampliat les terres agrícoles, sovint a expenses dels ecosistemes naturals, exacerbant encara més el canvi climàtic i la desertificació. D'aquesta manera, l'estrès per sequera afecta severament la productivitat dels cultius, impactant el creixement i el rendiment de les plantes. A més, la fertilització excessiva amb nitrogen, comunament utilitzada per a millorar els rendiments dels cultius, altera el cicle del nitrogen, el que porta a la contaminació de les aigües subterrànies, el deteriorament de la qualitat de l'aire i problemes de salut significatius.

L'albergina (*Solanum melongena* L.) és una hortalissa important i aprofitar els recursos genètics, inclosos els parents silvestres, és crucial per als programes de millora genètica dirigits a millorar la tolerància a la sequera i l'eficiència en l'ús del nitrogen. Els avanços en la seqüenciació de nova generació i el genotipat han millorat significativament la nostra comprensió dels caràcters relacionats amb l'estrès en diversos cultius, donant suport al desenvolupament de cultius resilients.

Per tant, en la present tesi doctoral, hem investigat el potencial dels parents silvestres de l'albergina per a millorar la tolerància als estressos abiòtics. El nostre objectiu era identificar noves fonts de variació genètica per a la seua incorporació en programes de millora genètica. Aquest enfocament busca identificar i validar recursos genètics valuosos, regions genòmiques i gens candidats per a desenvolupar varietats d'albergina més resilients. Mitjançant una estratègia multidisciplinària que inclou anàlisis genòmiques, transcriptòmiques i fenotípiques, pretenem promoure una agricultura més sostenible.

En el primer capítol d'aquesta tesi, ens vam centrar en avaluar retrocreuaments avançats (ABs) d'albergina i els seus parents silvestres per a millorar l'eficiència en l'ús del nitrogen (NUE) sota condicions de baix nitrogen (N). Aquest capítol inclou la primera avaluació exhaustiva de l'espècie silvestre relacionada *S. elaeagnifolium* del pool genètic terciari, i els ABs derivats en el fons genètic de l'albergina, sota condicions de cultiu amb baix N. Vam avaluar els parentals *S. melongena* i *S. elaeagnifolium* i els seus ABs en diversos caràcters de planta, fruit i composició. Es van observar diferències significatives entre els dos parentals en tots els caràcters avaluats. Es va observar una alta diversitat fenotípica en els ABs, amb alguns individus transgressius que van exhibir majors rendiments i NUE que la parental cultivada. A més, vam avaluar tres conjunts d'ABs de diferents pools genètics: *S. insanum* (GP1), *S. dasyphyllum* (GP2) i *S. elaeagnifolium* (GP3), sota condicions de

baix N. Aquesta avaluació va proporcionar informació sobre el potencial d'aquestes espècies silvestres per a la millora genètica sota condicions d'estrès abiòtic amb baix aport de nitrogen. Les condicions de baix N generalment van disminuir el contingut de clorofil·la però van augmentar els nivells de flavonol i antocianines, i van reduir la biomassa aèria, el diàmetre del tronc, el rendiment i el contingut de nitrogen i carboni en plantes i fruits. L'alta diversitat fenotípica i l'ampli rang de distribució en els ABs donen suport al potencial dels CWRs per a millorar la resiliència i la diversitat genètica de les albergines sota condicions d'estrès abiòtic, destacant el seu valor en els programes de millora genètica per a condicions de baix nitrogen i agricultura sostenible. La disponibilitat de dades de genotipat i fenotipat va permetre la identificació i validació de QTLs per a caràcters de planta, fruit i composició. Es van identificar diversos QTLs en els ABs de *S. insanum*, *S. dasyphyllum* i *S. elaeagnifolium*. Aquests QTLs es van associar amb caràcters com el creixement de la planta, el rendiment, la mida del fruit i el contingut de nitrogen, ampliant així la comprensió genòmica de l'albergina.

En el segon capítol d'aquesta tesi, vam investigar els mecanismes de resposta a la sequera en l'albergina cultivada (*S. melongena*) i el seu parent silvestre *S. dasyphyllum*. Vam avaluar aquestes espècies en condicions hidropòniques amb dos concentracions de polietilenglicol (PEG) (20% i 30%) en diferents etapes fenològiques, utilitzant la seqüenciació d'ARN (RNA-Seq) per a analitzar els patrons d'expressió gènica. *S. dasyphyllum* va demostrar una tolerància superior a l'estrès osmòtic en comparació amb *S. melongena*, amb un major nombre de gens diferencialment expressats (DEGs) sota estrès. Els anàlisis d'enriquiment d'ontologia gènica (GO) i les vies KEGG van revelar que ambdues espècies van activar una gamma de factors de transcripció i vies de resposta a l'estrès, amb *S. dasyphyllum* mostrant una regulació gènica més extensa. Les vies clau van incloure la senyalització de l'àcid abscísic (ABA), la senyalització MAPK i diverses vies de biosíntesi. Aquests resultats ressalten el potencial de *S. dasyphyllum* com a font de gens per a desenvolupar varietats d'albergina tolerants a la sequera, destacant la importància d'utilitzar parents silvestres per a millorar la tolerància i la sostenibilitat dels cultius.

En general, aquesta tesi doctoral va demostrar el potencial dels parents silvestres de l'albergina per a la millora de la tolerància a estressos abiòtics. El desenvolupament de retrocreuaments avançats de diferents parents silvestres del cultiu va ampliar la variació genètica, mostrant efectes significatius de les dosis de nitrogen en diversos caràcters i identificant individus transgressius amb característiques agronòmiques millorades. La detecció i validació de QTLs va proporcionar informació sobre la productivitat de l'albergina, l'eficiència en l'ús del nitrogen i els mecanismes de resposta a la sequera, contribuint al desenvolupament de varietats d'albergina més resilients i sostenibles.

Introduction

Climate change will increase abiotic stress in agriculture

The current global situation is defined by profound environmental and societal challenges, primarily driven by human activities including emissions from energy use, land-use changes, and varying consumption patterns. These activities have led to a significant accumulation of greenhouse gases in the atmosphere, especially CO_2, which has been a major driver of climate change. These changes are manifesting worldwide as unprecedented heat waves, droughts, fires, and floods, alongside significant losses in biodiversity. Concurrently, a steady increase in the global population is placing immense pressure on food production systems and water resources to meet escalating demands (Gardner, 2013; IPCC, 2023).

Over the past decades, the surge in demand for plant products has resulted in a 16% rise in the area of land harvested, a doubling of irrigated lands, and nearly a tripling of agricultural production (Cherlet et al., 2018). Much of this expansion has occurred at the expense of natural ecosystems, notably tropical forests, which are being converted into farmland. These conversions exacerbate climate change and accelerate the processes of desertification and biodiversity loss (FAO, 2023; Jaureguiberry et al., 2022). The extent and implications of these agricultural and climatic transformations are underscored by the outcomes of predictive models, which depict shifts to areas with more arid conditions (Figure 1).

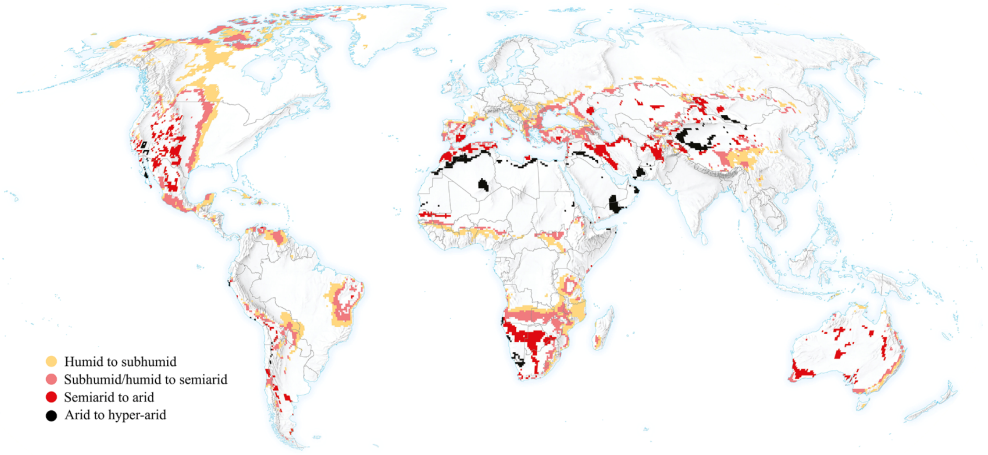

Figure 1. Projected changes to drier land types from current situation (1981–2010) to far future (2071–2100). Source: Cherlet et al., 2018.

Aridity is commonly considered as a persistent climatic trait of a region, whereas drought is recognized as an extreme event brought about by climate variability, increasingly linked to human-induced climate change (IPCC, 2023). Drought emerges as one of the most economically severe natural disasters, affecting crucial aspects of both natural and human systems. Insufficient precipitation, combined with elevated temperatures, increased evaporation, and soil water deficits, intensifies dry conditions, triggering environmental, social, and economic impacts (Cherlet et al., 2018, Figure 2).

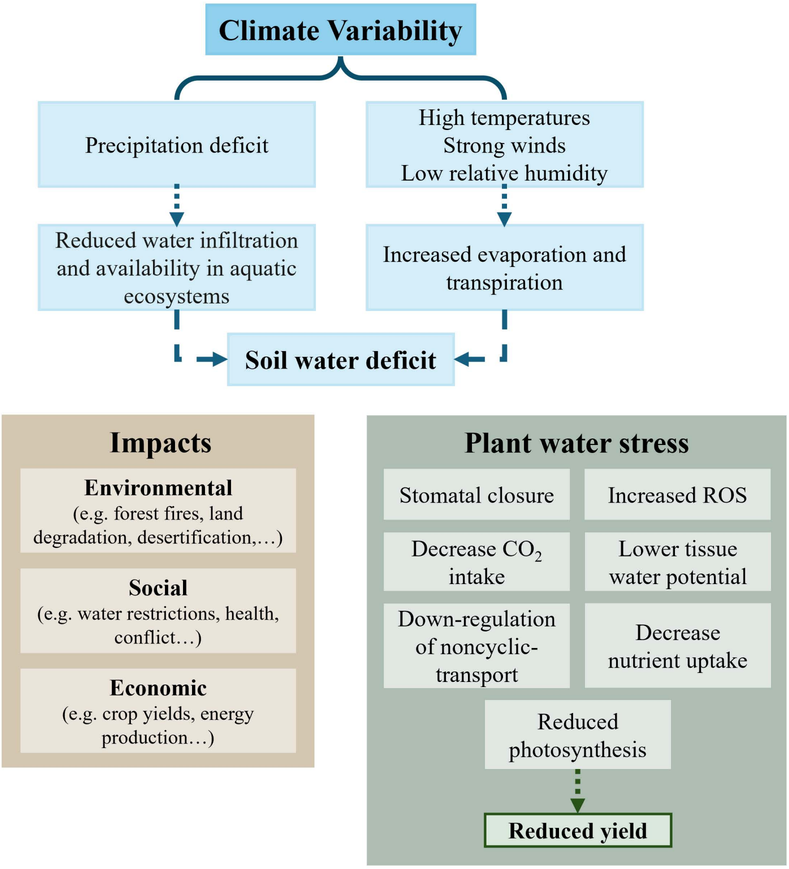

Figure 2. Diagrammatic representation of the impacts of drought on the environmental, social and economic sectors caused by climate variability, alongside the effects of water stress on plants. Adapted from Cherlet et al., 2018 and Ilyas et al., 2021.

Drought stress is widely recognized as a primary driver of decreased agricultural productivity, with effects on various morphological and physiological processes in plants. One of the most critical stages affected by drought is the initial phase of germination and seedling establishment (Li et al., 2013). Morphologically, the availability of water significantly influences cell elongation, division and differentiation, processes that are fundamental to plant development. This reduction in growth is further exacerbated by decreased turgor pressure and diminished water transport through xylem tissues and adjacent cells, which are crucial factors in maintaining cell structure and function (Farooq et al., 2009; Iqbal et al., 2020).

In response to water deficit stress, plants initially close their stomata to reduce transpiration. This stomatal closure limits the influx of CO_2, leading to an accumulation of reactive oxygen species (ROS), which causes oxidative damage and disrupts normal cellular functions. Additionally, lower tissue water potential diminishes the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Parry et al., 2002). Drought stress also triggers the downregulation of non-cyclic electron transport, further reducing ATP synthesis. These collective physiological changes reduce photosynthesis leading to diminished energy production and nutrient synthesis, which are essential for plant growth and yield (Figure 2) (Farooq et al., 2009; Hossain et al., 2016; Ilyas et al., 2021).

Understanding the mechanisms through which plants respond to drought stress is crucial for mitigating its effects. These mechanisms are generally categorized into four distinct types: (1) Drought Escape (DE), where plants complete their life cycle before the onset of drought; (2) Drought Avoidance (DA), which involves maintaining tissue hydration under high water potential; (3) Drought Tolerance (DT), the ability of plants to function while dehydrated at low water potentials; and (4) Drought Recovery, where plants regain functionality after drought stress. Each category highlights a specific strategy employed by plants to cope with water scarcity (Fang and Xiong, 2015). Morphological adaptation includes modifications in both leaf and root structures to enhance resilience under drought conditions. These adaptations include leaf size reduction, development of waxy cuticular layers, formation of xeromorphic structures, active leaf rolling and orientation, and increased trichomes and cuticle epidermis, among others. Additionally, root systems adapt by increasing early growth to access deeper soil water, developing longer and denser roots to improve water absorption, and creating extensive systems that support enhanced water potential and transpiration (Ilyas et al., 2021; Samarah, 2016).

Alongside these morphological adaptations, plants simultaneously undertake important biochemical and molecular changes to further improve their drought tolerance. The increase in production of reactive oxygen species (ROS) serves as a key signaling mechanism, triggering the activation of antioxidant enzymes including peroxidase, superoxide dismutase, ascorbate, and catalase (Impa et al., 2011). Additionally, drought stress influences the synthesis of crucial phytohormones such as abscisic acid, jasmonic acid, ethylene, gibberellins, auxins, salicylic acid, and cytokinins. These hormones activate various signaling pathways that enhance the production of antioxidant enzymes, similarly to how ROS triggers these responses, and also promote the synthesis of secondary metabolites, which affect plant responses to light, and regulate growth, development, and senescence (Iqbal et al., 2022; Salvi et al., 2021). These secondary metabolites, including protective solutes known as osmoprotectants, such as polyamines, betaines, sugars like trehalose, and amino acids like proline, along with non-enzymatic molecules like flavonoids, glutathione, and carotenoids, which collectively facilitate osmotic adjustment (Singh et al., 2015). Furthermore, microorganisms, including bacteria and fungi, play a crucial role in enhancing plant growth and development under biotic and abiotic stresses, thereby enhancing drought tolerance (Ilyas et al., 2021).

Regulation of gene expression is a fundamental part of the molecular mechanisms of plants to adapt to stress. Stress-responsive genes with potential for engineering of plants tolerant to drought include those that code for late embryogenesis abundant (LEA) proteins, aquaporins and heat shock proteins, which enhance cellular protection and water transport. Additionally, transcription factors such as the APETALA2/ethylene responsive element (AP2/ERF) superfamily and the basic leucine zipper (bZIP) play a significant role in regulating these stress-responsive genes to improve plant resilience under drought conditions (Reis et al., 2016).

Excessive nitrogen fertilization is a major environmental problem of agriculture

The application of synthetic or inorganic fertilizers, particularly nitrogen (N), has become a widespread practice in agriculture to combat deficiencies of N in the soil and to improve crop yields, significantly contributing to a global increase in food production. Recognized as a crucial nutrient for vegetative growth and crop productivity, nitrogen usage has markedly increased over the last six decades, reflecting its pivotal role in meeting agricultural demands (Zahoor et al., 2014).

Despite its widespread and effective use, nitrogen fertilization has its limitations and does not always necessarily lead to a proportional increase in crop yields (Lassaletta et al., 2014). Excessive use has become a global concern, with application rates exceeding 100 kg/ha in 26 countries (Figure 3). This excessive application of nitrogen in agriculture leads to significant environmental and health risks (Kanter et al., 2016; Zhang et al., 2015), being presented as a leverage point for climate impact mitigation, water quality, and resource conservation in agriculture to improve food security and the environment (West et al., 2014).

Human activities have significantly disrupted the global nitrogen cycle, enhancing nitrogen flows from the atmosphere into ecosystems. Nitrogen, constituting 78.1% of Earth's atmosphere, is primarily in the form of unreactive nitrogen gas (N_2), which is not biologically usable. It becomes available for biological use only when converted into reactive forms (N_r) through natural and anthropogenic processes. Reactive nitrogen includes various compounds such as nitrogen oxides (NO_x), reduced nitrogen (NH_x), nitrous oxide (N_2O), nitric acid (HNO_3), and other organic and inorganic forms, making it accessible for biological use (Stevens, 2019). Nitrogen cycle is composed of five key transformational processes: ammonification, which includes nitrogen fixation, and assimilatory and dissimilatory reduction of nitrite; nitrification; denitrification; annamox; and nitrite-nitrate interconversion (Figure 4). These processes, together with the mineralization of organic matter and its assimilation by cellular life, complete the movement of reactive nitrogen (N_r) throughout the biosphere (Stein and Klotz, 2016).

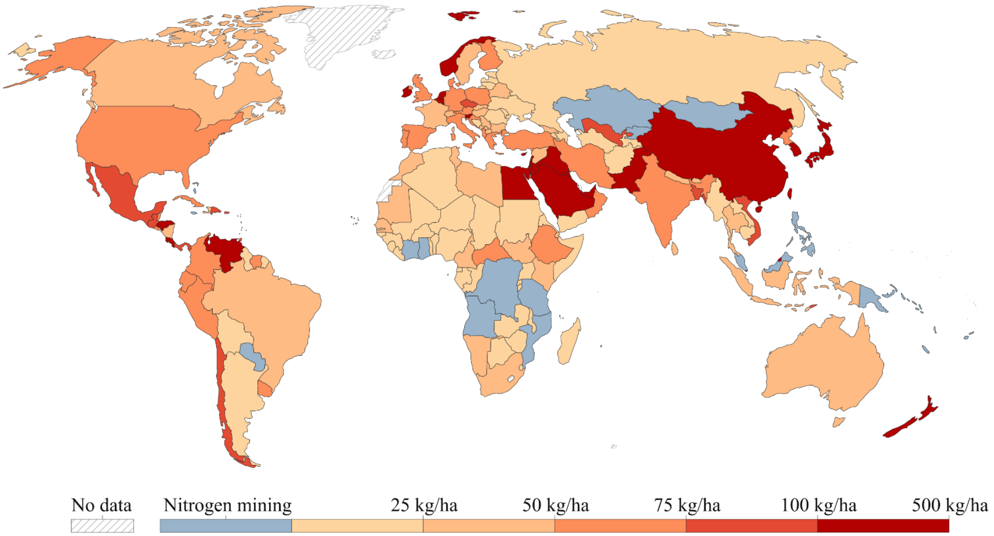

Figure 3. Excess nitrogen per hectare (kg/ha) of crop, defined as the difference between nutrient inputs and the amount of nitrogen collected in the harvested crop material. Source: West et al., 2014, processed by Our World in Data.

Half of the global fixation of total atmospheric nitrogen (N_2) into reactive nitrogen (N_r) in terrestrial and marine ecosystems is attributed to human activities. These processes encompass various anthropogenic interventions, including the use of fertilizers and the industrial Haber-Bosch process, as well as agricultural practices

(Humphreys et al., 2021). When fertilizers are applied, a portion of the Nr can be released into the atmosphere as ammonia (NH_3), influenced by the dynamic equilibrium between ambient NH_3 levels and the ammonium (NH_4^+) concentration within plant intercellular fluids. Moreover, the combustion of fossil fuel and biomass burning, along with different industrial processes, result in emissions of nitrogen oxides (NO_x) (Figure 4). These NO_x compounds are transformed from NO to NO_2 in the atmosphere, subsequently contributing to the formation of nitric acid (HNO_3). This acid serves as a precursor to various forms of oxidized nitrogen (NO_x), alongside reduced nitrogen (NH_x), which are deposited onto terrestrial surfaces and oceans through wet and dry processes. Additionally, this process leads to the generation of secondary pollutants like ozone (O_3), photochemical oxidants, and aerosols, including notable compounds such as ammonium nitrate (NH_4NO_3) and ammonium sulfate ($(NH_4)_2SO_4$). These substances can either deposit back onto the surface or remain suspended as particulate matter in the atmosphere (Figure 4). (Fowler et al., 2013).

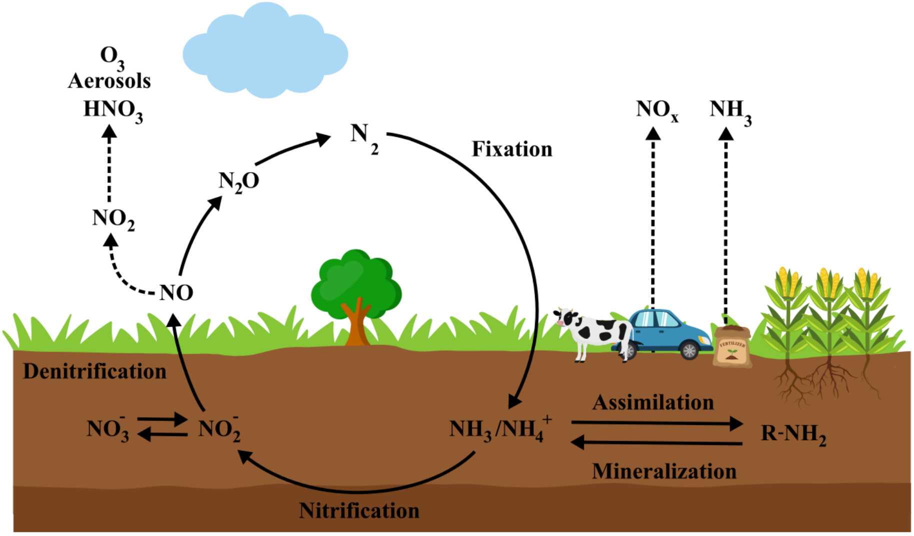

Figure 4. Simplified representation of the global nitrogen cycle. Solid arrows depict the biological processes, while dashed arrows show pathways caused by anthropogenic activities. Adapted from Fowler et al., 2013 and Stein and Klotz, 2016.

Modification of global nitrogen cycle has resulted in N_r pollution, with negative impacts for human health and well-being. These include contamination of surface and groundwater, deterioration of air quality marked by smog, particulate matter, and ground-level ozone, loss of biodiversity, climate change related to increased greenhouse gas emissions, and depletion of the ozone layer. Furthermore, the eutrophication of freshwater and coastal ecosystems originates the formation of dead zones (Erisman et al., 2013). All these factors are linked to human health risks, encompassing the consumption of nitrate-contaminated water and the intake of diets rich in root and leafy vegetables, which can accumulate nitrates from overly fertilized soils leading to certain types of cancer, hypertension, and diabetes. Exposure to airborne NO_x and NH_3 can result in various respiratory disorders (Ahmed et al., 2017).

The impact of climate change on ecosystems has notably intensified abiotic stresses such as soil aridity, drought, and the challenges associated with nitrogen fertilization. Exploring strategies to mitigate these consequences involves multidisciplinary and complementary approaches (Pascual et al., 2022). Effective resource management, including breeding strategies to enhance drought tolerance (Rauf et al., 2016) and improving nitrogen use efficiency (NUE) in plants (Ferrante et al., 2017; Sharma and Bali, 2018), aim to promote a more sustainable agriculture.

Eggplant genetic resources and tools for breeding for abiotic stress tolerance

Eggplant (*Solanum melongena* L.), also known as aubergine or brinjal, is a major vegetable crop cultivated worldwide in tropical, subtropical, and temperate regions (Aubriot et al., 2018). It belongs to the Solanaceae family and is ranked second in yield after tomato and third in total production following tomato and potato (FAO, 2022). Over the last decade, the area cultivated with eggplant has shown a modest increase of approximately 2.16%. Despite this, global production has grown by 25%, from 47.3 million tonnes in 2012 to 59.3 million tonnes in 2022, driven by a significant 22.47% increase in yield per hectare. The leading producers are China, India and Egypt (FAO, 2022).

Given the global cultivation of eggplant under diverse environmental conditions, it is crucial to understand the genetic factors that contribute to its productivity and resistance. Genetic information on variation in abiotic stress tolerance traits related to nitrogen use efficiency (NUE) and drought in eggplant is limited (Alam and Salimullah, 2021). Since productivity is sensitive to N fertilizer and water availability, various studies have investigated their effects on eggplant growth to optimize resource efficiency (Aminifard et al., 2010; Amiri et al., 2012; Maghfoer et al., 2022; Zhang et al., 2014). Several studies on model and crop species have shown a large natural variation for traits related to both genetic control and environmental adaptation (Fernie et al., 2006; Lippman and Zamir, 2007). In this way, the evaluation of phenotypic variability among genetic resources represents a fundamental tool for the selection of high NUE and drought tolerant genotypes (Mauceri et al., 2020; Plazas et al., 2019).

Originating from the Old World, the domestication of eggplant traces back to its wild ancestor, *Solanum insanum* L., in the Indian subcontinent and Southeast Asia (Page et al., 2019). It belongs to the diverse subgenus *Leptostemonum*, which includes more than 500 species (Knapp et al., 2019). Besides the widely cultivated brinjal eggplant, this genus also includes two different cultivated species, the scarlet eggplant (*S. aethiopicum* L.) and the gboma eggplant (*S. macrocarpon* L.) (Taher et al., 2017). Eggplant exhibits extensive intraspecific diversity, featuring a range of phenotypic, physiological, and biochemical traits that result in diverse growth habits and a variety of fruit shapes, sizes, and colors (Oladosu et al., 2021; Rotino et al., 2014).

Eggplant germplasm collections cover the existing diversity and serve as a vital genetic resource for breeders to develop new cultivars and elite materials with improved traits. However, the variability within cultivated species often proves limiting when addressing challenges posed by climate change, particularly in developing cultivars resilient to diverse abiotic stresses. Consequently, the creation and exploration of new genetic resources, especially through the use of crop wild relatives (CWR), have become essential. Wild species provide a broader genetic base and inherent tolerance to diverse environmental conditions such as drought, salinity, and low soil fertility. Incorporating the genetic diversity from these wild relatives expands the variability available to breeders, enabling the development of eggplant cultivars that are more adaptable to changing climates and overall, more robust (Boyaci, 2020; Plazas et al., 2016).

The CWRs are classified into primary, secondary, and tertiary genepools based on their crossability with the cultivated eggplant (Figure 5; Plazas et al., 2016). The primary genepool (GP1) consist in the cultivated eggplant *S. melongena,* and its ancestor, *S. insanum* L. (Syfert et al., 2016). This species is notably resilient, growing in a wide range of geographic, climatic and soil conditions, including infertile soils. It is naturally distributed in southern and south-eastern Asia, from eastern Pakistan, southwest to the islands of Madagascar and Mauritius in the Indian Ocean, and east to the Philippines (Ranil et al., 2017). The secondary genepool (GP2) comprises a diverse number of species, including those from the Anguivi clade, including the cultivated species *S. aethiopicum* and *S. macrocarpon,* along with their wild ancestors *S. anguivi* and *S. dasyphyllum*, respectively (Figure 5; Page et al., 2019). Additionally, this genepool includes species from the Eggplant clade such as *S. incanum* and *S. linnaeanum*, and African species such as *S. pyracanthos*, among others. These species are predominantly cultivated in, and native to, the regions of Asia and Africa, highlighting their extensive adaptation across diverse environments

(Syfert et al., 2016; Toppino et al., 2021). The tertiary genepool (GP3) comprises species that are phylogenetically more distantly related and native to the New World. This genepool includes several American species such as *S. elaeagnifolium*, *S. sisymbriifolium*, *S. torvum*, and *S. viarum* (Figure 5; Knapp et al., 2013; Syfert et al., 2016). These species are of great interest for breeding because of their tolerance to biotic and/or abiotic stresses, offering potential improvements for crop resilience (Chapman, 2020; Taher et al., 2017).

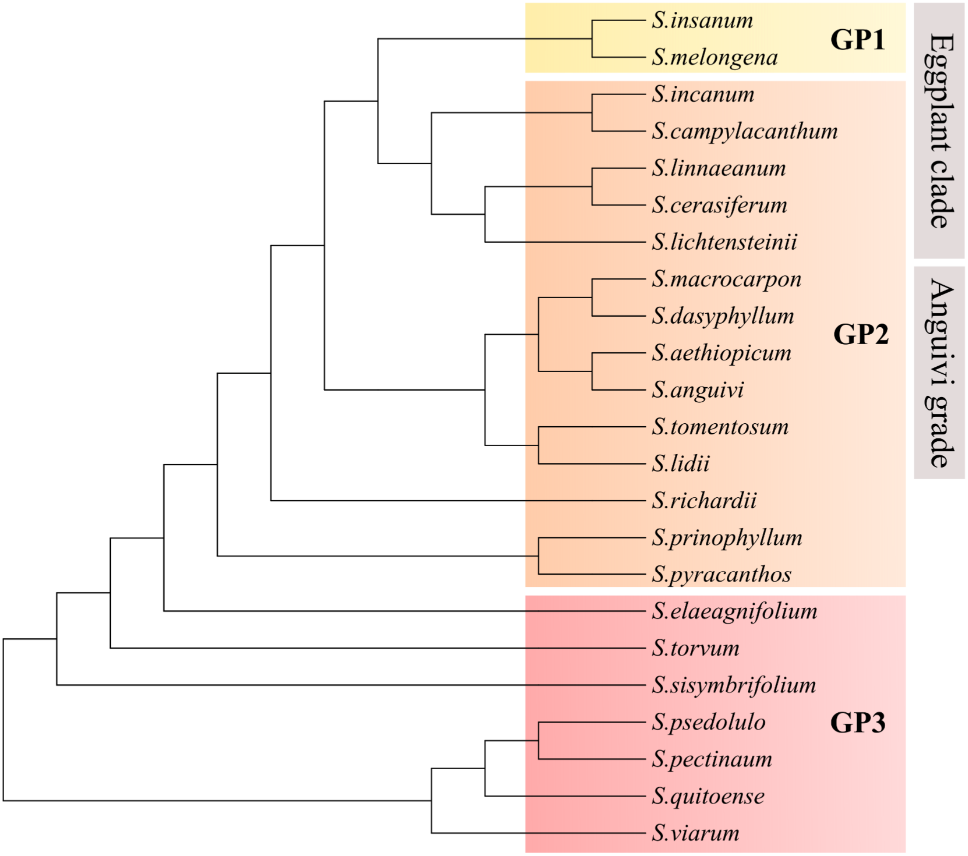

Figure 5. Phylogenetic tree of the most relevant species of the primary (GP1), secondary (GP2) and tertiary (GP3) genepools of eggplant. Adapted from Gramazio et al., 2023; Knapp et al., 2019; Knapp and Vorontsova, 2016; Syfert et al., 2016; Taher et al., 2017.

A new generation of genetic resources has emerged by the utilization of CWRs, which include mutant libraries, core collections, introgression lines (ILs), and both biparental and multiparental recombinant inbred lines (RILs) (Gramazio et al., 2023). These resources have been effectively utilized in eggplant breeding through introgression techniques (Prohens et al., 2017). The development of diverse populations incorporates species from different genepools. The first IL populations were obtained from the inter-specific cross of different *S. melongena* lines with *S. aethiopicum* (GP2) and *S. linneanum* (GP2) species (Mennella et al., 2010; Toppino et al., 2008). Further developments in ILs involved crosses of cultivated eggplant with *S. incanum* (GP2) (Gramazio et al., 2017), *S. tomentosum* (GP2) (Toppino et al., 2018) and *S. elaeagnifolium* (GP3) (García-Fortea et al., 2019), among other CWRs. Moreover, the generation of multiparental RILs, including advanced generation inter-cross (MAGIC) populations (Arrones et al., 2020; Mangino et al., 2022). Collectively, these advanced populations have significantly enriched genetic variability and enhanced the breeding potential of eggplant.

The development of new genetic and genomic tools has significantly advanced plant breeding. Next-generation sequencing (NGS) technologies have enabled high-throughput genotyping, leading to the availability of different eggplant reference genomes from high-quality genomic assemblies (Barchi et al., 2019b; Gramazio et al., 2019; Hirakawa et al., 2014; Li et al., 2021; Wei et al., 2020). In addition, pangenomes have been developed (Barchi et al., 2021), together with resequencing projects (Gramazio et al., 2019; Qian et al., 2022, 2021), which have supported the creation of eggplant-specific high-throughput genotyping platforms, such as the 5k probes single primer enrichment technology (SPET) eggplant platform (Barchi et al., 2019a). Moreover, these tools have enabled extensive genotyping of accessions and experimental populations, which, in association with phenotyping, has facilitated the construction of genetic maps with identification of quantitative trait loci (QTLs) and candidate genes associated with traits of interest (Chapman, 2020; Cockram and Mackay, 2018). These recent advances have significantly improved eggplant breeding efforts, increasing our understanding and improvement of morphological, physiological and biochemical traits, as well as those related to abiotic and biotic stresses (Mangino et al., 2020; Mishra et al., 2023; Rosa-Martínez et al., 2023a; Toppino et al., 2020, 2016).

Objectives

In the present doctoral thesis, we aimed at investigating the potential of wild eggplant relatives to improve tolerance to abiotic stresses, with the goal of identifying new sources of genetic variation for their incorporation into breeding programs. This approach seeks to identify and validate valuable genetic resources, genomic regions and candidate genes to develop more resilient eggplant varieties and promote a more sustainable agriculture through a multidisciplinary strategy that includes genomic, transcriptomic, and phenotypic analyses.

Therefore, we proposed the following specific main objectives:

1. Evaluation of morpho-agronomic and composition traits in different sets of advanced backcrosses derived from wild relatives from different genepools, including *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium,* in background of cultivated eggplant (*S. melongena*) under low nitrogen conditions.

    1.1. Evaluation of the effect of minimizing nitrogen inputs on yield, plant, fruit and composition parameters in diverse sets of parentals and advanced backcrosses.

    1.2. Identification of potential introgressed materials for eggplant breeding under low nitrogen inputs.

    1.3. Identification of genomic regions associated with plant nitrogen use efficiency and the evaluated traits.

    1.4. Validation of quantitative trait loci (QTLs) influenced by environmental factors, genotypes, and genetic backgrounds.

2. Evaluation of molecular mechanisms in response to drought stress through transcriptome analysis of eggplant (*S. melongena*) and its wild relative *S. dasyphyllum* under different levels of osmotic stress.

    2.1. Evaluation of the effects of exposure times, phenological stages of plants, and levels of osmotic stress on differential gene expression in cultivated eggplant and in the tolerant wild relative *S. dasyphyllum*.

    2.2. Identification of main pathways and genes involved in the differential drought response mechanism in eggplant and in the tolerant wild relative *S. dasyphyllum*.

Results

Chapter I:
Breeding and evaluation of eggplant and wild relatives for nitrogen use efficiency

Research article

Evaluation of advanced backcrosses of eggplant with *Solanum elaeagnifolium* introgressions under low N conditions

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Ph.D. candidate contribution

G.V. had a main role in the following activities: methodology, formal analysis, investigation, data curation, drafting manuscript, manuscript review and editing.

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Abstract

Selection and breeding of eggplant (*Solanum melongena*) materials with good performance under low nitrogen (N) fertilization inputs is a major breeding objective to reduce environmental degradation, risks for human health, and production costs. *Solanum elaeagnifolium*, an eggplant wild relative, is a potential source of variation for introgression breeding in eggplant. We evaluated 24 plant, fruit, and composition traits in a set of genotyped advanced backcrosses (BC2 and BC3) of eggplant with *S. elaeagnifolium* introgressions under low N conditions. Significant differences were found between the two parents for most traits, and a wide phenotypic diversity was observed in the advanced backcrosses, with some individuals with a much higher yield, nitrogen use efficiency (NUE), and phenolics content than the *S. melongena* parent. In general, the lower the proportion of *S. elaeagnifolium* genome introgressed in the advanced backcrosses, the higher was the general phenotypic resemblance to *S. melongena*. Putative QTLs were detected for stem diameter (*pd4*), presence of prickles in stem (*ps6*), leaf (*pl6*) and fruit calyx (*pc6*), fruit width (*fw7*), chlorogenic acid content (*cg5*), total phenolic acid peaks area (*ph6*), chlorogenic acid peak area (*ca1*), and phenolic acids pattern (*cp1*). Our results reveal that introgression breeding of eggplant with *S. elaeagnifolium* has a great interest for eggplant breeding, particularly for adaptation to low N conditions. These materials can potentially contribute to the development of improved eggplant varieties for a more sustainable agriculture.

Keywords: sustainable agriculture; *Solanum melongena*; *S. elaeagnifolium*; introgressions; low N; NUE; QTLs.

1. Introduction

The use of nitrogen (N) fertilizers is widespread in agriculture, as N has a fundamental role in increasing plant growth and crop yields and is frequently a limiting factor in soils (Marschner and Rengel, 2011). In the past six decades, intensive and generalized use of N fertilizers has resulted in an increase of global food production and a reduction of world hunger. However, a continued N over fertilization has a negative impact on the environment, including surface and groundwater contamination and eutrophication of freshwater and estuarine ecosystems (Albornoz, 2016; Xu et al., 2012). Contamination of water used for human consumption by nitrates is also a threat for human health in certain regions. Therefore, reducing N fertilization and increasing the nitrogen use efficiency (NUE) of crops is one of the main objectives to decrease environmental degradation while increasing crop productivity (Sharma and Bali, 2017; Xu et al., 2012; Zhang et al., 2015). In addition, developing new varieties able to grow and give high yields under low N conditions can extend the range of cultivation conditions suitable for a crop.

Crop wild relatives (CWRs) are a fundamental resource for plant breeding in order to develop new varieties more adapted to low input conditions and to climate change-related stresses (Dempewolf et al., 2017). In this respect, many CWRs grow under stressful natural conditions, including low N levels in the soil. Thereby, introgression breeding from CWRs into the genetic background of the crops may result in the development of more diverse, resilient, and resource efficient, including enhanced NUE (Gao et al., 2019), crops that contribute to a sustainable agriculture (Prohens et al., 2017).

Eggplant (*Solanum melongena* L.), also known as aubergine or brinjal, is an Old World domesticate from subgenus *Leptostemonum* (Knapp et al., 2013) and it is an important and widely grown vegetable crop, especially in South and Southeast Asia, the Middle East, and the Mediterranean region (Chapman, 2020). In terms of global production, eggplant is the third most widely cultivated crop in the Solanaceae family, after tomato and potato, being grown on about 1.85 million (M) ha with a total production of 55.2 M of tons in 2019 (FAO, 2019).

A wide range of CWRs from several clades within subgenus *Leptostemonum* have been used to obtain interspecific hybrids and backcrosses of eggplant, particularly Old World relatives belonging to the primary (GP1) and the secondary (GP2) gene pools (Plazas et al., 2016b; Rotino et al., 2014). New World eggplant CWRs are included in the tertiary genepool (GP3), and some of them, such as *S. elaeagnifolium* Cav., *S. sisymbriifolium* Lam., and *S. torvum* Sw., awakened an interest among breeders for their tolerance to biotic and/or abiotic stresses (Chapman, 2020; Taher et al., 2017). However, introgression breeding of eggplant with New World CWRs has remained an elusive breeding goal due to unsuccessful hybridization or high sterility of hybrids (Rotino et al., 2014). However, recently, we reported the first successful development of first backcross generations of common eggplant *(S. melongena*) with the New World species *S. elaeagnifolium* (García-Fortea et al., 2019). This wild relative of eggplant is found in deserts and dry forests in North and South America, where N levels in the soil are low (Álvarez-Yépiz et al., 2008), and it is considered a noxious invasive weed in dry areas worldwide (Knapp et al., 2017; Krigas et al., 2021). Moreover, *S. elaeagnifolium*, among other wild species, was reported to be a potential new source of variation for eggplant quality breeding due to the high levels of phenolic bioactive compounds (Kaushik et al., 2017).

Despite economic and social importance of eggplant, the development, and the use of genetic and genomic tools lags behind other Solanaceae crops such as tomato, potato, or pepper (Chapman, 2020; Gramazio et al., 2018). However, recent efforts of sequencing and resequencing eggplant accessions (Barchi et al., 2019a; Gramazio et al., 2019; Hirakawa et al., 2014; Li et al., 2021; Wei et al., 2020a) and the development of high-throughput genotyping platforms specific for eggplant, such as the 5k probes single primer enrichment technology (SPET) eggplant platform (Barchi et al., 2019b) facilitated eggplant breeding. In this way, in order to improve eggplant tolerance to abiotic stresses, the development of advanced backcrosses and introgression lines, the conservation and the study of eggplant crop-wild relatives (CWR,) and the functional analysis of adaptive traits (QTL) were highlighted as the three priority lines of introgression breeding research in this crop (Chapman, 2020).

In this study, we evaluated advanced backcrosses (AB) obtained after the backcrossing for several generations of an *S. melongena* × *S. elaeagnifolium* interspecific hybrid (García-Fortea et al., 2019) towards the recurrent *S. melongena* under low N conditions. The analysis of phenotypic and composition traits provides information of interest for breeding of eggplant materials with *S. elaeagnifolium* introgressions for cultivation under low N conditions. The high throughput genotyping of the AB individuals with the eggplant SPET platform also allowed a preliminary genome association study for the detection of QTLs for agronomic, morphological, and fruit quality traits under these conditions.

2. Materials and methods

2.1. Plant material

The *S. melongena* MEL3 and *S. elaeagnifolium* ELE2 accessions and a set of 56 advanced backcross (AB) individuals towards *S. melongena* of the interspecific hybrid between *S. melongena* MEL3 (recurrent parent) and *S. elaeagnifolium* ELE2 (donor parent) (García-Fortea et al., 2019) were used for the present study. Out of the 56 AB individuals, five were from the second backcross generation (BC2) and 51 from the third backcross generation (BC3).

2.2. Genotyping

Genomic DNA of the two parents and the 56 ABs was extracted using the SILEX DNA extraction method (Vilanova et al., 2020) and evaluated for quality and integrity by agarose gel electrophoresis and spectrophotometric ratios 260:280 and 260:230 and for concentration with a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). After dilution, the DNA samples were genotyped with the 5k SPET probes eggplant platform, which comprises 5093 probes (Barchi et al., 2019b). The whole set of 34,570 single nucleotide polymorphisms (SNPs) was filtered using as criteria a minimum count value of 95% with a minimum allele frequency (MAF) higher than 5% and a maximum heterozygosity proportion of 70% using the Tassel software (version 5.0 Standalone, (Bradbury et al., 2007)). After filtering, 851 SNPs were retained and subsequently used for the downstream analysis. The genetic similarities among the parents and the individuals of the BC2 and the BC3 segregating generations were quantified with the identity by state (IBS) distance index. Genetic relationships were described using the neighbor joining clustering method by means of Tassel software (version 5.0 Standalone, (Bradbury et al., 2007)).

2.3. Cultivation conditions

Plants were grown in an open airfield plot in Valencia (Spain) during the summer season (June to October 2019). The BC2 and the BC3 individuals, together with five plants of each of the parental lines, were distributed according to a completely randomized experimental design with 150 cm between rows and 70 cm between plants in each row. One of the plants of the recurrent parent *S. melongena* died in the early stages of the experiment and therefore was not included in the phenotyping. A drip irrigation system was used for irrigation and fertilization.

A soil physicochemical and composition analysis was performed before the transplant. Five samples consisting of five randomly selected spots of field were considered for soil analysis. For each sample, five fractions of soil 20 cm deep surrounding the selected spot were extracted, homogenized, and left to dry at room temperature. For each dried homogenate sample, 500 g were used for the analyses. Physical and chemical parameters were evaluated following the procedures described in van Reeuwijk (2002): particle size analysis, pH in water, and pH in potassium chloride, electrical conductivity, contents in total nitrogen, carbonates and organic matter, carbon:nitrogen ratio, and mineral contents of available phosphorus, potassium, calcium, magnesium, iron, zinc, and copper. Data of soil characteristics are included as Supplementary Table S1. According to the Spanish interpretation scales for each of the different elements evaluated (Yañez, 1989), the soil was moderately basic, non-saline, had low content of nitrogen, high content of carbonates, low presence of organic matter, and high carbon:nitrogen ratio. A chemical composition analysis of water was performed before adding fertilizers. Data of water analysis are included as Supplementary Table S2. The intake water was slightly basic, had low content of nitrates, phosphates, and ammonium, moderate content of sulphates and magnesium, and high content of calcium.

Based on the soil composition and the intake water analyses, a low N fertilization solution was prepared by adding 2.3 mM K_2SO_4 (Antonio Tarazona SL., Valencia, Spain) plus 0.025 l/m^3 of a microelements Welgro Hydroponic fertilizer (Química

Massó S.A., Barcelona, Spain) containing boron (BO_3^{3-}; 0.65% *p*/*v*), copper (Cu-EDTA; 0.17% *p*/*v*), iron (Fe-DTPA; 3.00% *p*/*v*), manganese (Mn-EDTA, 1.87% *p*/*v*), molybdenum (MoO_4^{2-}; 0.15% *p*/*v*), and zinc (Zn-EDTA; 1.25% p/v) to the intake water. The pH of the irrigation solution was adjusted to 5.5–5.8. The only source of N in the irrigation water came from the intake water N content (0.65 mM N). In total, 116.83 L of irrigation solution were supplied per plant with a drip irrigation system throughout the growing cycle, corresponding to 1.067 g of N supplied per plant.

2.4. Traits evaluated

A total of 24 plant and fruit traits were evaluated (Table 1). The SPAD index was measured using a SPAD-502Plus chlorophyll meter (Minolta, Osaka, Japan) as the mean of 10 measurements in five leaves of each plant. For plant traits, plant height, aerial biomass, and stem diameter were measured at the end of the trial. When plants were cut at the base of the stem, aerial shoot biomass was immediately weighed with a Sauter FK-250 dynamometer (Sauter, Balingen, Germany). Subsequently, they were dried at room temperature, and the dry weight was measured. Stem diameter was measured with a caliper at the base of the stem. Nitrogen use efficiency (NUE) was calculated as total fruit yield on a dry weight basis (yield (DM)) divided by N supply per plant (Moll et al., 1982; Samonte et al., 2006; Xu et al., 2012).

For fruit traits, pedicel length, calyx length, fruit length, and width were evaluated in at least three fruits per plant harvested at the commercially mature stage (i.e., physiologically immature). The traits evaluated as well as their abbreviations and units in which they are expressed are included in Table 1.

For leaf N and C contents, five leaves per individual were collected, frozen in liquid N2, and stored at −80 °C until lyophilized. For fruit N, C, and phenolic acids analyses, at least three fruits at the commercial maturity stage per individual plant were harvested, cleaned, peeled, and chopped, and a sample was immediately frozen in liquid N_2 and stored at −80 °C for subsequent lyophilization. Homogenized powdered tissue of leaf and fruit was used for the composition analyses.

N and C contents were measured in samples of 0.5 g of freeze-dried powder of leaves or fruit flesh. The analysis of N content was performed using the Dumas method based on a complete combustion of the sample at 950 °C in the presence of oxygen using a TruSpec CN elemental analyzer (Leco, MI, USA). Carbon content was calculated from the measurements of carbon dioxide (CO_2) using an infrared detector (Gazulla et al., 2012). Certificated reference standards of different N and C concentrations were used for the quantification.

Table 1. Plant, fruit, and composition traits evaluated in the *S. melongena* MEL3 recurrent parent, the *S. elaeagnifolium* ELE2 donor parent, and in the BC2 and the BC3 advanced backcrosses individuals, abbreviations used in tables and figures, and units in which they are expressed in the present study.

Trait	**Abbreviation**	**Units**
Plant traits		
SPAD	SPAD	-
Plant height	P-Height	cm
Aerial biomass	P-Biomass	kg FW [a]
Stem diameter	P-Diam	mm
Prickles in stem	P-StPrick	0 (absence); 1 (presence)
Prickles in leaf	P-LeafPrick	0 (absence); 1 (presence)
Yield	Yield	g plant^{-1}
Nitrogen Use Efficiency	NUE	-
Fruit traits		
Fruit pedicel length	F-PedLength	mm
Fruit calyx length	F-CaLength	mm
Fruit length	F-Length	mm
Fruit width	F-Width	mm
Prickles in calyx	F-CalPrick	0 (absence); 1 (presence)
Total number of fruits per plant	F-Number	-
Fruit mean weight	F-Weight	g
Composition traits		
Nitrogen content in leaf	N-Leaf	g kg^{-1} DM [b]
Carbon content in leaf	C-Leaf	g kg^{-1} DM
Nitrogen content in fruit	N-Fruit	g kg^{-1} DM
Carbon content in fruit	C-Fruit	g kg^{-1} DM
Total phenolics content	TPC	g kg^{-1} FW
Chlorogenic acid content	CGA	g kg^{-1} FW
Total phenolic acid peaks area	TP-Area	units
Chlorogenic acid peak area	CGA-Area	%
Phenolic acids pattern	TP-Pattern	0 (*S. melongena* pattern); 1 (*S. elaeagnifolium* pattern)

[a] FW: fresh weight. [b] DM: dry matter

Total phenolics content of fruit flesh was determined using 0.125 g of freeze-dried powdered material following the Folin–Ciocalteu method (Singleton and Rossi, 1965) after extraction with acetone (70% v/v) and acetic acid (0.5% v/v) according to Kaushik et al. (2017). Chlorogenic acid was extracted using 0.1 g of freeze-dried powdered material by ultrasonic bath according to Helmja et al. (2008). The subsequent determination of the content of chlorogenic acid (CGA) was performed by high-performance liquid chromatography (HPLC) using a 1220 Infinity LC System (Agilent 196 Technologies, CA, USA) equipped with a binary pump, an automatic injector, and a UV detector following the procedure of Plazas et al. (2014). The total area of the peaks corresponding to the different eluted phenolic acids as well as the specific area of the chlorogenic acid peak were obtained from the chromatograms of each sample. Taking the former parameter as reference, the percentage of the peak area corresponding to chlorogenic acid was calculated. As two distinct phenolic acid chromatogram patterns (TP-Pattern) were observed between the donor and the recurrent parents, plants of the ABs were classified as having a *S. melongena* or *S. elaeagnifolium* phenolic acids pattern (Table 1).

2.5. Data analysis

For each of the traits measured, the mean and its standard error (SE), range values, and coefficient of variation (CV; %) were calculated for recurrent and donor parents as well as for BC2 and BC3 generations. Normality of data within each of the two parents and BC generations was checked with a Shapiro–Wilk test. Statistical differences among the means of these four groups were assessed with an analysis of variance (ANOVA) with the Statgraphics Centurion XVII software (StatPoint Technologies, Warrenton, VA, USA). Mean separation was performed with the Student–Newman–Keuls multiple range test at $p < 0.05$. Due to the wide range of values and a positive relationship between mean and standard deviation values for P-Biomass, Yield, NUE, F-Length, F-Width, and F-Weight, these traits were subjected to a logarithmic transformation before analysis (Little and Hills, 1978). Estimation of broad-sense heritability (H^2) in the ABs population was performed for each trait using the following formula (Wricke and Weber, 1986): $H^2 = V_G/(V_G + V_E)$. The environmental variance (V_E) was estimated as the phenotypic variance of the recurrent parental *S. melongena* MEL3, while the genotypic variance (V_G) was calculated as the difference between the phenotypic variance of the advanced backcrosses BC2 and BC3 (V_P) and the environmental variance (V_E): $V_G = V_P - V_E$.

A principal components analysis (PCA) with the traits evaluated was conducted in order to globally evaluate the variation of BC2 and BC3 individuals in comparison to the recurrent parent as well as to assess the effect of the proportion of the homozygous recurrent parent genome in the distribution and the relationships of BC2 and BC3 individuals and the recurrent parent. The PCA was performed on standardized values using pairwise Euclidean distances of the two parents, BC2 and BC3 individuals, using the R package *stats* of R statistical software v3.6. 2 (R Core Team, 2021). An additional PCA was performed excluding the *S. elaeagnifolium* individuals to obtain a more detailed relationship between the recurrent *S. melongena* parent and the advanced backcrosses towards it. The PCA score and the loading plots were drawn using R package *ggplot2* (Wickham, 2016). Pearson pair-wise coefficient of correlation (r) and Spearman correlation among traits, including only the set of 56 advanced backcrosses (AB), were calculated, and the significance was assessed using a Bonferroni correction at the 0.05 probability level if significant (Hochberg, 1988) using R packages psych (Revelle, 2020) and corrplot (Wei and Simko, 2021).

2.6. QTL detection

The composite interval mapping (CIM) method was used to identify quantitative trait locus (QTL) for complex traits implemented by the R package R/qtl (Broman et al., 2003) of R statistical software v4.0.2 (R Core Team, 2021). Putative QTLs with the threshold of LOD score at the 0.05 probability level were considered as significant. The heterozygous allelic effect of each significant QTL was calculated as the difference between the mean of the trait values of the ABs carrying the wild allele in heterozygosis and the mean of the trait values of those ABs with the homozygous recurrent allele. A *t*-test ($p = 0.05$) between the two groups was performed to confirm that the QTLs effects were statistically significant. Candidate genes were searched using the Genome browser of Sol Genomics Network (Fernandez-Pozo et al., 2015) with eggplant genome consortium V4.

3. Results

3.1. Genomic characterization

After SNP marker filtering, a total of 851 markers out of the initial 34,570 markers were selected, although the number of markers in each chromosome differed (chr. 1: 150; chr. 2: 57; chr. 3: 68; chr. 4: 79; chr. 5: 41; chr 6: 99; chr. 7: 33; chr. 8: 71; chr. 9: 60; chr. 10: 90; chr. 11: 31; and chr. 12: 72). A complete coverage of the *S. elaeagnifolium* genome (in heterozygosis) was obtained with the 56 advanced backcrosses (Figure 1). The ABs had a mean recovery rate of the recurrent parent genetic background in homozygosis of 84.4% with a range of recovery among BC2 and BC3 individuals between 61.0% and 97.6%. The mean number of introgressions was 6.2 with a range between 1 and 13 (Figure 1). The frequency of heterozygosis of each marker along each chromosome was variable, the averages being higher in chromosomes 6 (0.26) and 7 (0.23) and lower in chromosomes 10 (0.06) and 9 (0.07). However, significant differences were observed in the representation among genomic regions within each chromosome (Figure 1).

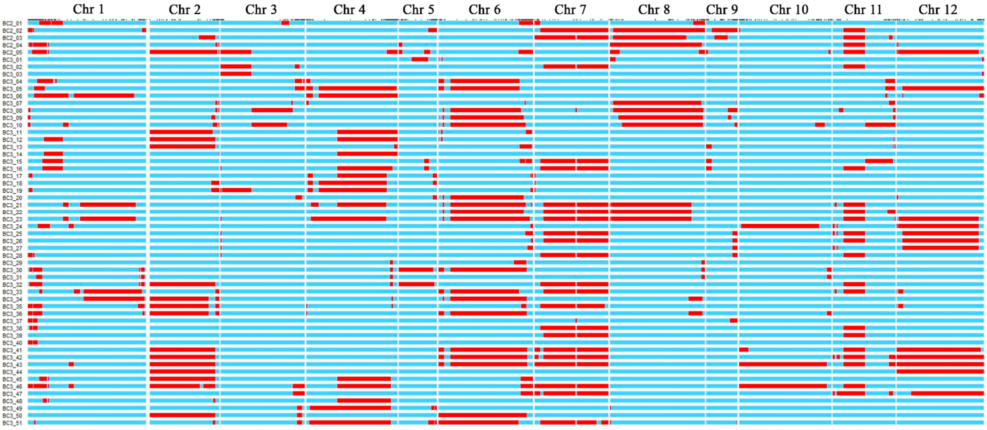


Figure 1. Graphical genotypes of 56 advanced backcross lines (ABs). The rows indicate ABs codes, and the columns indicate the chromosomes. The five first rows correspond to BC2 individuals, while the rest correspond to BC3 individuals. Introgressions of *S. elaeagnifolium* ELE2 in heterozygosis are depicted in red, and the genetic background of the recurrent parent (*S. melongena* MEL3) is depicted in blue.

The dendrogram performed on genetic data clearly separated the recurrent parent (*S. melongena*) and the BC2 and the BC3 generation individuals in a set of clusters that largely grouped individuals sharing common introgressions from *S. elaeagnifolium* (Supplementary Figure S1). In this way, BC2 and BC3 individuals were intermingled in the dendrogram grouping with other, while the recurrent parent plotted close to two BC3 individuals with few introgressed markers from *S. elaeagnifolium*.

3.2. Traits evaluated

Statistically significant differences ($p < 0.05$) between parents were detected for all plant traits (Table 2). The plants of the *S. melongena* parent were larger than those of *S. elaeagnifolium* and had a higher plant height (P-Height; 1.6-fold), aerial biomass (P-Biomass; 4.9-fold), and stem diameter (P-Diam; 2.6-fold). Additionally, due to their higher yield, *S. melongena* plants also had higher yield (Yield; 55.6-fold) and nitrogen use efficiency (NUE; 31.6-fold) than those of *S. elaeagnifolium*. In addition, significant differences were found between parents for all fruit traits, except for the number of fruits per plant (F-Number). Thus, the recurrent parent had higher mean values for the following traits: pedicel length (F-PedLength; 2.7-fold), calyx length (F-CaLength; 4.3-fold), fruit length (F-Length; 9.2-fold), fruit width (F-Width; 4.5-fold), and fruit mean weight (F-Weight; 100.0-fold) (Table 2; Figure 2). For composition traits, *S. melongena* had significantly higher N content in leaf than

S. elaeagnifolium (N-Leaf; 1.2-fold) and chlorogenic acid peak area (CGA-Area; 2.6-fold). *Solanum elaeagnifolium* displayed significantly higher values than *S. melongena* in C content in fruit (C-Fruit; 1.1-fold), total phenolics content (TPC; 3.1-fold), and total phenolic acid peak area (TP-Area; 1.9-fold) (Table 2). Few significant differences were observed among BC2 and BC3 means. In this way, only C content in fruit (C-Fruit) and total phenolics content (TPC) displayed higher mean values in BC2, although the relative differences were limited (4.6% and 32.5%, respectively), and C content in leaf (C-Leaf) displayed lower mean values in BC2 with a relatively limited difference (20.9%) (Table 2).

Table 2. Mean, range, and coefficient of variation (CV) of every trait analyzed for the donor parent (*S. elaeagnifolium*; *n* = 5), the recurrent parent (*S. melongena*; *n* = 4), the BC2 (*n* = 5) and the BC3 (*n* = 51) advanced backcrosses towards *S. melongena,* and the heritability (H^2) of the trait in the advanced backcrosses. The full name of each trait in the first column can be found in Table 1. For each trait, means with different letters are significantly different according to the Student–Newman–Keuls multiple range test ($p < 0.05$).

Trait	***S. elaeagnifolium* (*n* = 5)**			***S. melongena* (*n* = 4)**			**BC2 (*n* = 5)**			**BC3 (*n* = 51)**			
	Mean	**Range**	**CV (%)**	**Mean**	**Range**	**CV (%)**	**Mean**	**Range**	**CV (%)**	**Mean**	**Range**	**CV (%)**	**H^2**
Plant traits													
SPAD	63.7 b	55.8–71.0	9.1	46.6 a	44.0–47.9	3.9	44.2 a	41.1–48.1	6.1	44.7 a	37.0–56.9	9.0	0.79
P-Height (cm)	58.8 a	50.0–65.0	9.3	92.3 b	79.0–107.0	12.8	102.2 b	90.0–130.0	16.3	85.4 b	47.0–136.0	24.5	0.68
P-Biomass (kg FW)	0.27 a	0.16–0.40	35.4	1.32 b	0.85–2.03	38.2	1.59 b	0.51–3.80	80.7	1.42 b	0.18–4.96	76.0	0.78
P-Diam (mm)	10.5 a	9.1–11.9	10.9	27.4 b	19.3–35.4	25.4	26.3 b	17.9–36.3	28.8	24.6 b	11.0–46.2	30.9	0.15
P-StPrick	1.0 b	1.0–1.0	0.0	0.0 a	0.0–0.0	0.0	0.4 a	0.0–1.0	136.9	0.2 a	0.0–1.0	234.1	1.00
P-LeafPrick	0.0	0.0–0.0	0.0	0.0	0.0–0.0	0.0	0.2	0.0.0–1	223.6	0.2	0.0–1.0	234.1	1.00
Yield (g)	52.9 a	12.0–114.0	83.6	2891.0	1925.0–4020.0	32.5	1258.0 b	469.0–2941.0	79.4	2059.0 b	124.0–8109.0	87.1	0.71
NUE	10.3 a	1.9–22.3	85.1	325.8 b	205.6–483.2	37.1	200.5 b	67.8–533.1	94.4	272.7 b	37.4–1019.3	82.3	0.70
Fruit traits													
F-PedLength (mm)	21.3 a	20.5–22.5	4.1	58.2 c	52.6–61.5	6.8	28.4 ab	20.1–35.7	22.8	38.4 b	17.2–63.7	30.8	0.89
F-CaLength (mm)	12.2 a	11.4–13.2	6.4	52.5 c	49.2–56.7	5.9	29.8 b	24.3–34.9	15.4	36.1 b	20.2–52.6	23.8	0.87
F-Length (mm)	10.3 a	9.1–11.3	9.2	94.8 c	86.4–101.1	6.6	54.0 b	38.1–71.9	23.0	66.1 b	24.5–113.2	29.9	0.90

Trait	*S. elaeagnifolium* (*n* = 5)			*S. melongena* (*n* = 4)			BC2 (*n* = 5)			BC3 (*n* = 51)			
	Mean	**Range**	**CV (%)**	**Mean**	**Range**	**CV (%)**	**Mean**	**Range**	**CV (%)**	**Mean**	**Range**	**CV (%)**	**H^2**
F-Width (mm)	10.1 a	8.9–11.4	10.7	45.8 c	41.8–53.9	12.0	29.9 b	22.6–37.7	23.0	36.0 b	18.9–50.5	19.6	0.42
F-CalPrick	1.0 b	1.0–1.0	0.0	0.0 a	0.0–0.0	0.0	0.4 a	0.0–1.0	136.9	0.2 a	0.0–1.0	218.2	1.00
F-Number	82.8	23.0–165.0	81.1	48.5	38.0–57.0	20.5	57.2	39.0–87.0	36.2	56.8	18.0–132.0	46.1	0.85
F-Weight (g)	0.61 a	0.46–0.82	23.1	60.98 c	33.77–77.79	31.6	20.03 b	11.44–33.8	46.5	33.35 b	5.29–65.88	47.5	0.57
Composition traits													
N-Leaf (g/kg DM)	45.0 a	38.9–48.8	8.4	52.9 b	49.4–54.7	4.5	53.1 b	50.0–54.7	3.5	52.5 b	45.0–58.2	5.3	0.24
C-Leaf (g/kg DM)	439.8 a	435.0–446.0	1.0	443.3 a	439.0–448.0	0.8	446.0 a	437.0–453.0	1.6	455.5 b	423.0–469.0	1.7	0.78
N-Fruit (g/kg DM)	26.4	24.2–30.3	9.2	24.6	22.9–25.5	4.9	29.3	24.6–33.9	13.2	25.7	19.2–40.9	15.5	0.91
C-Fruit (g/kg DM)	465.8 b	461–472	1.0	428.3 a	425–430	0.5	456.8 b	438–477	3.1	435.7 a	384–479	3.7	0.98
TPC (g/kg FW)	6.12 c	4.1–7.44	20.9	1.99 a	1.7–2.34	16.7	4.09 b	3.62–5.0	13.4	2.76 a	1.48–5.75	30.8	0.86
CGA (g/kg FW)	2.53	1.79–3.41	22.8	1.78	1.61–2.09	12.1	2.53	1.44–3.74	32.5	2.27	1.02–5.02	32.8	0.92
TP-Area (units)	32931 b	31290–34898	3.9	17603 a	15639–20419	12.1	22874 a	19472–28816	17.2	20014 a	11517–32806	24.2	0.80
CGA-Area (%)	31.4 a	27.5–34.8	8.3	80.4 b	76.7–81.5	1.0	67.8 b	49.1–85.6	21.7	78.2 b	49.1–88.4	11.7	0.99
TP-Pattern	1.0 c	1.0–1.0	0.0	0.0 a	0.0–0.0	0.0	0.6 bc	0.0–1.0	91.3	0.2 ab	0.0–1.0	192.6	1.00

Regarding the comparison between the advanced backcrosses (BC2 and BC3) and *S. melongena*, significant differences were observed for pedicel, calyx, fruit lengths, fruit width, and fruit weight (F-PedLength, F-CaLength, F-Length, F-Width, and F-Weight, respectively), with *S. melongena* displaying higher mean values than the advanced backcrosses (51.2%, 43.4%, 43.0%, 34.7%, and 67.2% with BC2 and 34.1%, 31.3%, 30.3%, 21.4%, and 45.3% with BC3, respectively) than the recurrent parent (Table 2). For two composition traits (C-Fruit and TPC), significant differences between BC2 and the recurrent parent, but not between the latter and BC3, were observed. The distribution range and the coefficient of variation (CV) for traits evaluated in the advanced backcrosses were generally greater than those observed in the parents (Figure 2). For example, yield differences in BC3 were 65.4 times between the lowest (124 g) and the highest yielding (8109 g) plants, while in the parents, the differences were of only 2.1-fold in *S. melongena* and 9.5-fold in *S. elaeagnifolium* (Table 2).

A lack of overlap in the range of variation between parents was observed in all traits except for C content in leaf (C-leaf), N content in fruit (N-fruit), and chlorogenic acid content (CGA) (Table 2). All distribution ranges of advanced backcrosses overlapped with those of *S. melongena*, while with *S. elaeagnifolium,* they did not overlap for yield, nitrogen use efficiency (NUE), fruit calyx length (F-CaLength), fruit length (F-Length), fruit width (F-Width), fruit mean weight (F-Weight), and chlorogenic acid peak area (CGA-Area). For traits such as plant height (P-height), plant biomass (P-Biomass), yield, or nitrogen use efficiency (NUE), there were AB individuals with values higher than the highest for *S. melongena*, although this was not observed in case of fruit weight (F-Weight) (Table 2). In general, the coefficient of variation (CV) was higher in the BC3, being only higher in BC2 for aerial biomass (P-Biomass), nitrogen use efficiency (NUE), fruit width (F-Width), and chlorogenic acid peak area (CGA-Area) and being higher in the donor parent *S. elaeagnifolium* only for SPAD, total number of fruits per plant (F-Number), and nitrogen content in leaf (N-Leaf) (Table 2).

Broad-sense heritability (H^2) values in the BC2 and the BC3 generations ranged between 0.15 for stem diameter (P-Diam) and 1.00 for the presence of prickles in the stem (P-StPrick), in the leaf (P-Leafprick), and in the calyx (F-CalPrick) and for the pattern of phenolic acids (TP-Pattern) (Table 2). In general, heritability values were high ($H^2 \geq 0.65$), except for P-Diam ($H^2 = 0.15$), F-Width ($H^2 = 0.42$), F-Weight ($H^2 = 0.57$), and N-Leaf ($H^2 = 0.24$) (Table 2).

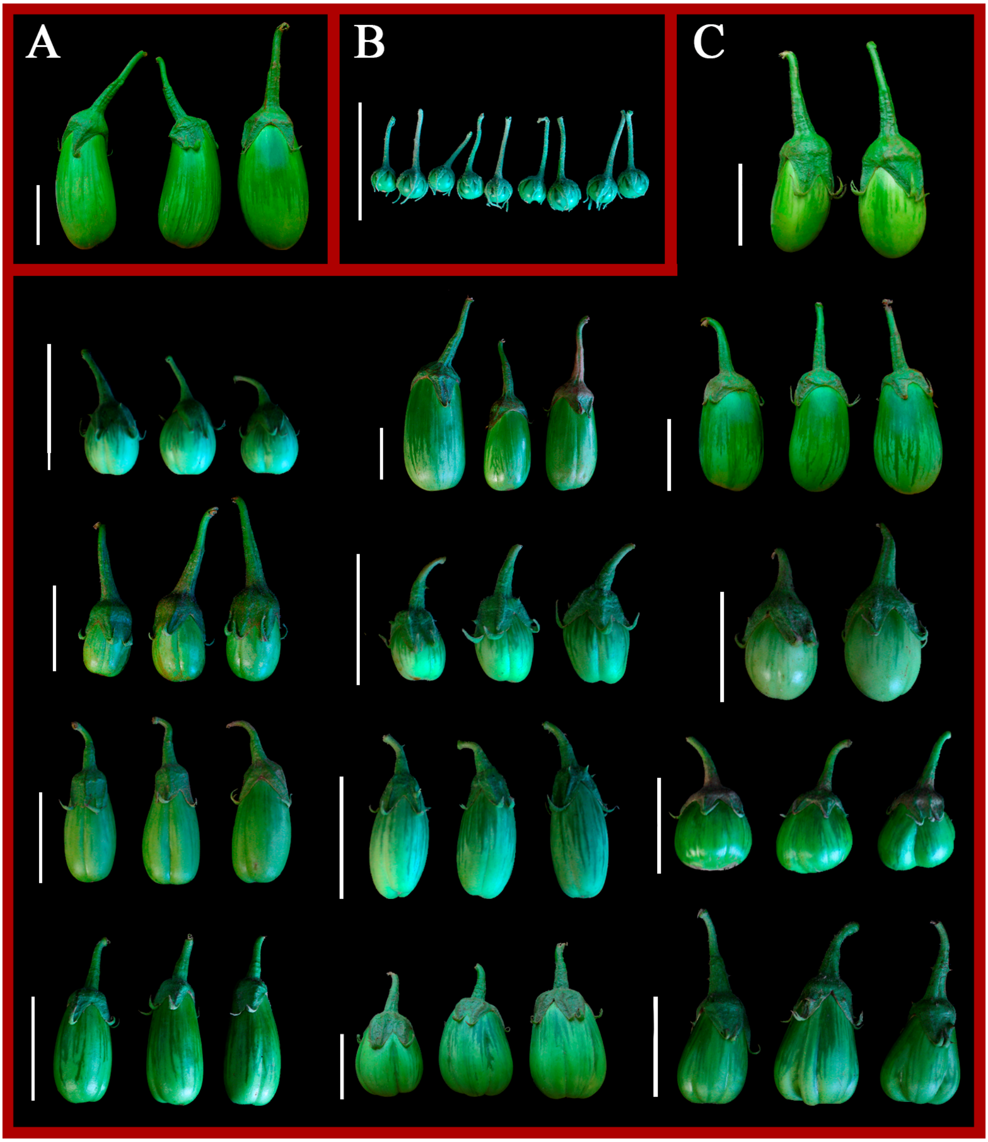


Figure 2. Fruit size and morphology of fruits of parentals *S. melongena* (**A**) and *S. elaeagnifolium* (**B**) and of a representative set of different plants of advanced backcrosses (BC2 and BC3) (**C**). Fruits are aligned with a white line scale of 5 cm for size reference.

3.3. Principal component analysis

A PCA performed with the traits evaluated and for all individuals of the two parents and the advanced backcrosses allowed to evaluate the variation within each of the parents and the two backcross segregating generations as well as to evaluate the relationships among all individuals evaluated. The PCA clearly separated the donor parent (*S. elaeagnifolium*) from the rest of materials, which clustered together (Supplementary Figure S2). In order to obtain a clearer picture of the relationships between the advanced backcrosses and the recurrent parent, a PCA was performed excluding the donor parent individuals (Figure 3). The first and the second principal components (PCs) of this PCA accounted for 33.0% and 16.2%, respectively, of the total variation observed. The first component displayed high positive correlations with phenolics content traits (total phenolics content, TPC; chlorogenic acid content, CGA; and, total phenolic acid peaks area, TP-Area) and negative ones with yield, NUE, and fruit size traits (fruit pedicel length, F-PedLength; fruit calyx length, F-CaLength; fruit length, F-Length; fruit width, F-Width; and, fruit weight, F-Weight) (Figure 3). The second component displayed high correlation values with plant vigor traits (SPAD; plant height, P-Height; aerial biomass, P-Biomass; stem diameter, P-Diam), total number of fruits (F-Number), and phenolic acids pattern (TP-Pattern). Some fruit size traits (fruit pedicel length, F-PedLength; fruit calyx length, F-CaLength; and, fruit width, F-Width) and chlorogenic acid peak area (CGA-Area) displayed a high negative correlation with PC2 (Figure 3).

The projection of the individuals in the PCA score plot showed a wide distribution over the graph area. All the individuals of the recurrent parent clustered in the lower left quadrant were associated with fruit size traits, while four out of five of the BC2 individuals plotted in the upper right quadrant were associated with high contents in phenolics and N content in the fruit. The BC3 individuals were spread over the whole PCA plot. An association between traits evaluated and percentage of recovered genetic background was observed, as individuals with higher recovery of the donor parent genetic background plotted closer to the recurrent parent and individuals with lower proportion of the genome homozygous for the recurrent parent were situated farther apart from the recurrent parent (Figure 3).

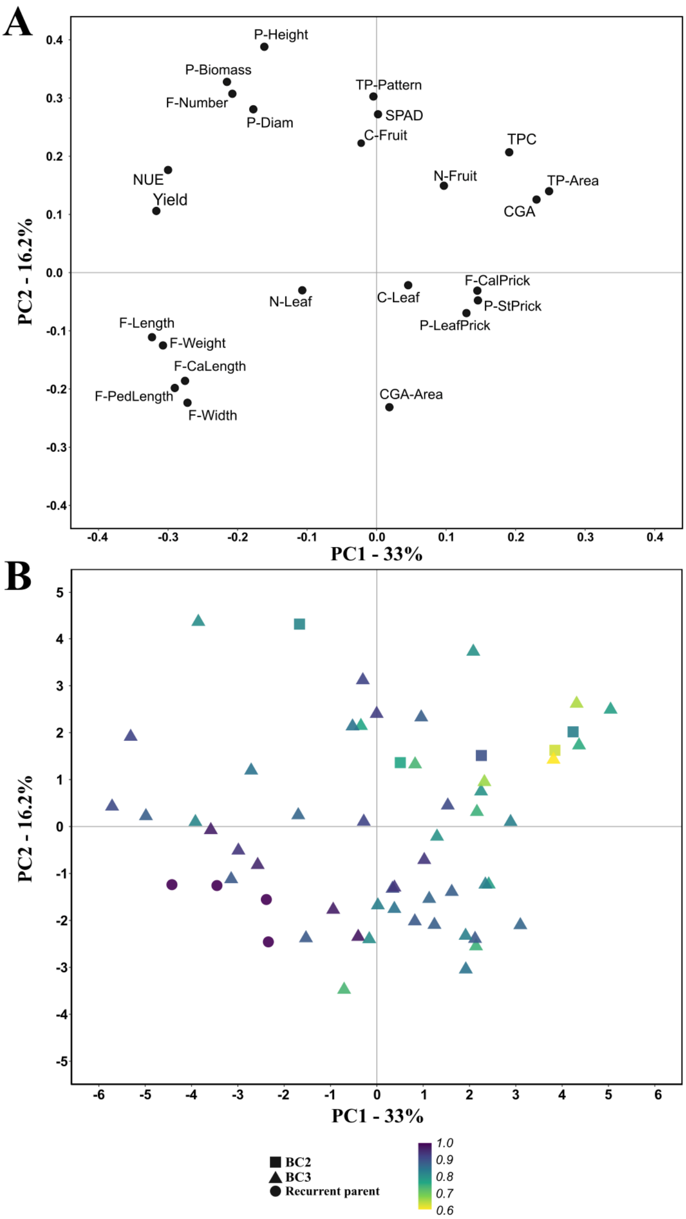
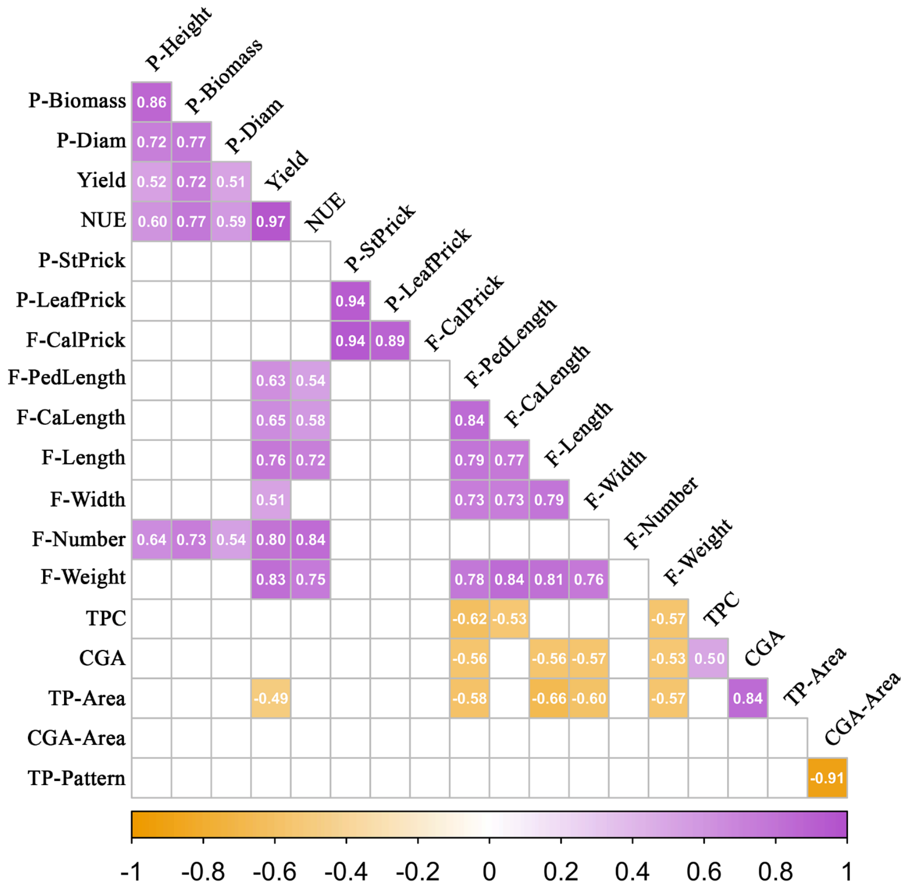


Figure 3. PCA loading plot (**A**) and score plot (**B**) on the two first principal components of PCA based on 24 plant, fruit, and composition traits of four individuals of *S. melongena* and 56 individuals of advanced backcrosses (five BC2 and 51 BC3) with *S. elaeagnifolium* introgressions. First and second components account for 33.0% and 16.2% of the total variation, respectively. The accessions are represented by different symbols according to the generation (BC2, BC3, and recurrent parent) and gradient of color according to homozygous proportion from recurrent parent of the 851 SNPs (1: dark purple to 0.6: yellow). The full name of each trait can be found in Table 1.

3.4. Correlations among traits in the ABs

Pearson's and Spearman's correlations were conducted, and in both analyses, the results were very similar. Therefore, we displayed Pearson's correlations (Figure 4). Five traits (SPAD, N-Fruit, N-Leaf, C-Fruit, and C-Leaf) were not significantly correlated ($p < 0.05$) with any other trait in the BC2 and the BC3 individuals. High positive correlations ($r > 0.7$) were observed among several traits related to plant vigor: plant height (P-Height), aerial biomass (P-Biomass), and stem diameter (P-Diam) (Figure 4). Similarly, yield showed positive correlations with plant vigor traits (P-Height, P-Biomass, and P-Diam), nitrogen use efficiency (NUE), and fruit size traits (F-PedLength, F-CaLength, F-Length, and F-Width), total number of fruits (F-Number), and fruit mean weight (F-Weight) (Figure 4). Nitrogen use efficiency (NUE) was positively correlated with plant size (P-Height, P-Biomass, and P-Diam), yield, fruit size (F-PedLength, F-CaLength, F-Length, and F-Width), total number of fruits (F-Number), and fruit mean weight (F-Weight) (Figure 4). High positive correlations were observed among fruit size traits (F-PedLength, F-CaLength, F-Length, F-Width, and F- Weight) as well as among the three traits related with the presence of prickles (F-CalPrick, P-StPrick, and P-LeafPrick). The total number of fruits (F-number) showed significant correlations with plant size traits (P-Height, P-Biomass, and P-Diam), yield, and nitrogen use efficiency (NUE) (Figure 4).

Total phenolics content (TPC), chlorogenic acid content (CGA), and total phenolic acid peaks area (TP-Area) were positively intercorrelated (Figure 4). These three traits also showed significant negative correlations with fruit size traits (F-PedLength, F-CaLength, F-Length, F-Width, and F-Weight), except for total phenolics content (TPC), which did not show significant correlations with fruit length (F-Length) and fruit width (F-Width), although it displayed a negative correlation with fruit calyx length (F-CaLength) (Figure 4). Chlorogenic acid peak area (CGA-Area) was negatively correlated with phenolic acids pattern (TP-Pattern) (Figure 4).



	P-Height	P-Biomass	P-Diam	Yield	NUE	P-StPrick	P-LeafPrick	F-CalPrick	F-PedLength	F-CaLength	F-Length	F-Width	F-Number	F-Weight	TPC	CGA	TP-Area	CGA-Area
P-Biomass	0.86																	
P-Diam	0.72	0.77																
Yield	0.52	0.72	0.51															
NUE	0.60	0.77	0.59	0.97														
P-StPrick																		
P-LeafPrick						0.94												
F-CalPrick						0.94	0.89											
F-PedLength				0.63	0.54													
F-CaLength				0.65	0.58				0.84									
F-Length				0.76	0.72				0.79	0.77								
F-Width				0.51					0.73	0.73	0.79							
F-Number	0.64	0.73	0.54	0.80	0.84													
F-Weight				0.83	0.75				0.78	0.84	0.81	0.76						
TPC									-0.62	-0.53				-0.57				
CGA									-0.56		-0.56	-0.57		-0.53	0.50			
TP-Area				-0.49					-0.58		-0.66	-0.60		-0.57		0.84		
CGA-Area																		
TP-Pattern																		-0.91

Figure 4. Pearson's correlation among traits evaluated in the 56 individuals of advanced backcrosses (five BC2 and 51 BC3) of *S. melongena* with *S. elaeagnifolium* introgressions. Only traits for which there was at least a pairwise significant correlation at $p < 0.05$ according to the Bonferroni test are included in the figure. Correlation values include only those that are significant. Orange and purple colors correspond to negative and positive correlations, respectively. The full name of each trait can be found in Table 1.

3.5. QTL detection

The genomic and the phenotypic information analysis allowed the detection of nine putative QTLs (Table 3). For plant traits, a QTL was detected for stem diameter (*pd4*) located on chromosome 4. For traits related with the presence of prickles (P-StPrick, P-LeafPrick, and F-CalPrick), three QTLs were found located in chromosome 6 at the same position (*ps6*, *pl6,* and *pc6*) with similar allelic effects. For fruit traits, a QTL for fruit width (*fw7*) was found on chromosome 7. Four QTLs associated with fruit phenolics traits were identified. They were located on chromosome 5 (*cg5*) for chlorogenic acid content, on chromosome 6 (*ph6*) for total phenolic acid peak area, and on chromosome 1 (*ca1* and *cp1*) at the same position for both chlorogenic acid peak area and phenolic acid pattern (Table 3).

Table 3. Traits for which putative QTLs were detected in advanced backcrosses of *S. melongena* with *S. elaeagnifolium*, QTL name, chromosome, and position (Mb) where they were located, *S. elaeagnifolium* heterozygous allelic effect (significant for all traits at p = 0.05 according to a t-test), and LOD score.

Trait	**QTL**	**Chr.**	**Position (Mb.)**	**Heterozygous Allelic Effect (Units)**	**LOD Score**
Plant traits					
Stem diameter (P-Diam)	*pd4*	4	21.09–68.11	−8.9 (mm)	5.82
Prickles in stem (P-StPrick)	*ps6*	6	105.06–105.56	0.91	11.10
Prickles in leaf (P-LeafPrick)	*pl6*	6	105.06–105.56	0.82	33.08
Fruit traits					
Fruit width (F-Width)	*fw7*	7	0–0.52	−9.5 (mm)	6.12
Prickles in fruit calyx (F-CalPrick)	*pc6*	6	105.06–105.56	0.89	447.96
Composition traits					
Chlorogenic acid content (CGA)	*cg5*	5	3.94–4.49	2.26 (g kg^{-1} DM)	7.15
Total phenolic acid peaks area (TP-Area)	*ph6*	6	99.76–100.78	5105	5.20
Chlorogenic acid peak area (CGA-Area)	*ca1*	1	1.05–1.42	−20.1 (%)	19.13
Phenolic acids pattern (TP-Pattern)	*cp1*	1	1.05–1.42	0.88	416.14

4. Discussion

Selecting plant materials that perform better under low N conditions is a main goal for developing a more sustainable agriculture less dependent on N fertilizers, which require significant energy for their synthesis and have a negative environmental impact (Xu et al., 2012; Zhang et al., 2015). Increasing nitrogen use efficiency (NUE) is one important pathway to enhance sustainability, and several strategies and genetic approaches were described for NUE improvement (Fernandez-Pozo et al., 2015; Han et al., 2015; van Bueren and Struik, 2017). Several efforts for NUE breeding in cereals and other field crops were published in rice (Gao et al., 2019; Kurai et al., 2011; Neeraja et al., 2019; Yang et al., 2017), wheat (Cormier et al., 2016; Lupini et al., 2021), maize (Cai et al., 2012; Ertiro et al., 2020; Semagn et al., 2015; Weber et al., 2012), barley (Rajala et al., 2017), millet (Pujarula et al., 2021), and oilseed rape (Stahl et al., 2019). In potato, breeding for NUE was also reported (Getahun, 2018; Tiwari et al., 2018). For vegetable crops, less efforts have been made, although recent contributions addressed this issue in tomato (Abenavoli et al., 2016a; Liang et al., 2019; Lupini et al., 2017) and eggplant (Mauceri et al., 2021, 2020).

In this work, advanced backcrosses of eggplant with introgressions of an American crop wild relative (*S. elaeagnifolium*) from the tertiary genepool (Syfert et al., 2016) in which the entire genome of the wild species is represented were evaluated for the first time. Genotyping with the 5k probes SPET platform (Barchi et al., 2019b) allowed us to obtain a good resolution at the genomic level of the advanced backcrosses with *S. elaeagnifolium* introgressions, revealing its utility for eggplant introgression breeding. These introgression materials are a resource of great interest for improvement of eggplant, as they widen the genetic background of the crop with exotic variation that has not been used thus far.

Solanum melongena plants developed adequately despite the low amounts of N applied. In this case, the fertilization irrigation solution did not contain any added N fertilizer, the only source of applied N being the intake water with a concentration of 0.65 mM N, much less than the recommended N concentration of 16.5 mM of N in the watering solutions for eggplant in soilless cultivation (Baixauli and Aguilar, 2002). Aside from the fact that the wild parent *S. elaeagnifolium* is a species that grows in poor environments where the N is low (Álvarez-Yépiz et al., 2008; Knapp et al., 2017), the evaluation of the advanced backcrosses allowed us to determine genetic parameters of great relevance for important traits in eggplant breeding, including the detection of putative QTLs for some of them. Similar studies were published on QTL detection in tomato under abiotic stress conditions (Asins et al., 2020; Diouf et al., 2018) but not under low N conditions.

The results showed a large difference for all plant, fruit, and composition traits between the two species (*S. melongena* and *S. elaeagnifolium*) used as parents in the development of the advanced backcrosses. This result was expected due to the high phylogenetic distance between the two species (Knapp et al., 2017; Särkinen et al., 2013). High phenotypic diversity was observed in the advanced backcrosses with much higher ranges of variation than the parents for all traits. This fact demonstrates the interest of these materials, since transgressive individuals were found for many characters that can contribute to the development of improved materials. In this way, among the advanced backcrosses, we found materials with much higher yield, NUE, and phenolics content than the cultivated parent. In fact, despite the low N content in the soil and the irrigation water (Baixauli and Aguilar, 2002; Mauceri et al., 2020), plants of some advanced backcrosses had yields over 8 kg/plant, much higher than the recurrent parent *S. melongena*, suggesting that materials with *S. elaeagnifolium* introgressions have a high potential for the development of new cultivars adapted to low N conditions.

Phenotypes that were not observed in the parents appeared in some advanced backcrosses, in particular, the presence of prickles in the leaf was observed in some backcrosses although none of the parents had prickly leaves. This is a phenomenon that was observed in other interspecific crosses in eggplant and was attributed to the presence of complementary genes in the two parents (Plazas et al., 2016b; Prohens et al., 2012). It was previously reported that some eggplant wild relatives display higher levels of phenolic acids of interest for human health than cultivated eggplant (Kaushik et al., 2017; Meyer et al., 2015; Stommel and Whitaker, 2003). In our case, we found that the eggplant wild relative *S. elaeagnifolium* displayed higher total phenolics content (TPC) as well as a different and more diverse phenolic acid chromatogram pattern (TP-Pattern). Some advanced backcrosses had higher total phenolics content than the recurrent parent (*S. melongena*), and, in addition, some of them showed the same phenolic acid chromatogram pattern of the wild parent. These results may be of interest to develop varieties with enhanced bioactive properties. Heritability values, in general, were higher or similar to other studies in which parental generations of eggplant and relatives, interspecific hybrids, and first backcrosses of eggplant were evaluated for different traits (Prohens et al., 2012). Additionally, H^2 values were higher than those observed in the evaluation of a set of introgression lines (ILs) which used *S. incanum* as donor parent (Mangino et al., 2020). This fact together with the high ranges of variation indicate that a high response to selection can be obtained, confirming the interest of these materials.

Most of the correlations among traits observed in the advanced backcrosses were expected and are in agreement with observations in other eggplant studies (Frary et al., 2014; Portis et al., 2015). In this way, we found intercorrelations among traits related to plant vigor (plant height, aerial biomass, and stem diameter) and also their correlation with yield, NUE, total number of fruits per plant, and fruit mean weight. Moreover, intercorrelations were observed among the three traits related to the presence of prickles (prickles in stem, in leaf, and in calyx) and among fruit size traits (fruit pedicel length, fruit calyx length, fruit length, fruit width, and fruit mean weight). For composition traits, correlations between total phenolics content (TPC), chlorogenic acid content (CGA), and total phenolic acid peaks area (TP-Area) were also expected (Kaushik et al., 2017; Plazas et al., 2013). In other cases, relevant correlations for breeding were found, such as the negative correlations among some fruit size traits with both total phenolics content (TPC) and chlorogenic acid content (CGA), indicating that a counterbalance between large fruits and high contents in phenolics may exist in the materials evaluated (Hanson et al., 2006; Plazas et al., 2013).

The PCA showed the wide variation observed and that a higher homozygous proportion from the recurrent parental genome resulted in greater overall phenotypic resemblance to the recurrent parent, indicating that large proportions of the *S. elaeagnifolium* genome in the genetic background of eggplant result in phenotypes that deviate from the recurrent parent. Nonetheless, there are individuals with high *S. melongena* genome recovery proportion that displayed considerable phenotypic differences among themselves, making them amenable to selection.

The availability of genotyping and phenotyping data allowed the detection of some putative QTLs for traits of interest. We detected a QTL that influenced stem diameter (*pd4*), an important trait related to vigor. Thus far, only a QTL for this trait was detected in eggplant in chromosome 2 in the ILs population of *S. incanum* in the genetic background of *S. melongena* (Mangino et al., 2020). For the three traits related to the presence of prickles (P-StPrick, F-LeafPrick, and F-CalPrick) we detected three QTLs (*ps6*, *pl6,* and *pc6*, respectively) located at the same position on chromosome 6, indicating a common genetic basis for these three traits. Previously, QTLs related to prickliness were mapped in chromosome 6 (Chapman, 2019) in an *S. linnaeanum* × *S. melongena* F2 population (Doganlar et al., 2002; Frary et al., 2014), in a BC1 population of the ILs of *S. incanum* × *S. melongena* (Gramazio et al., 2014), in an F2 intraspecific population (Portis et al., 2014), and in a panel of 191 accessions of breeding lines, old varieties, and landrace selections of eggplant (Portis et al., 2015), supporting that a major gene conserved in the eggplant group is present in this region (Miyatake et al., 2020; Zhang et al., 2021). For fruit traits, a QTL was detected for fruit width (F-Width) on chromosome 7 (*fw7*). Previously, a QTL for fruit width was also detected in chromosome 7 in an F2 intraspecific eggplant population (Portis et al., 2014). In addition, other QTLs associated with fruit width were detected scattered over six chromosomes (1, 2, 3, 8, 10, and 11) in several eggplant populations, such as the ILs of *S. incanum* (Mangino et al., 2021), an *S. linnaeanum* × *S. melongena* F2 population (Doganlar et al., 2002; Wei et al., 2020b), and the 191 eggplant accessions panel (Portis et al., 2015).

For composition traits, we identified a QTL for CGA content (*cg5*) in chromosome 5. Two QTLs related with CGA content were previously described, located in chromosomes 4 and 6 (Toppino et al., 2016). None of these three QTLs contain genes involved in the chlorogenic acid synthesis pathway (Gramazio et al., 2014; Dandan Li et al., 2021), thus, the genetic basis of CGA related traits needs to be further investigated. For total phenolic acid peaks area (TP-Area), we found a QTL on chromosome 6 (*ph6*), in which there are two putative genes involved in phenolic pathways (Günther et al., 2020; Mouradov and Spangenberg, 2014). One of them is a gene described as similar to CCR1, cinnamoyl-CoA reductase 1 (SMEL_006g261420.1.01), and it was reported to play a role in soluble phenolic content in tomato (*S. lycopersicum* L.) (van der Rest et al., 2006), and the other is a gene described as similar to 4CL2, 4-coumarate-CoA ligase 2 (SMEL_006g261630.1.01), a gene involved in the CGA synthesis pathway in eggplant (Gramazio et al., 2014). For the highly correlated traits chlorogenic acid peak area (CGA-Area) and phenolic acids pattern (TP-Pattern), we detected a QTL at the same position on chromosome 1, which was not previously reported and which is of great interest for eggplant breeding for bioactive phenolic acids content.

5. Conclusions

Overall, this work shows that introgressions of the phylogenetically distant *S. elaeagnifolium* in eggplant may be of interest for eggplant breeding, particularly for adaptation to low N conditions. These materials pave the way to the use of new exotic variation from a species that grows under low N conditions in its natural habitat for eggplant breeding. Together with the genomic information obtained from them, these materials can contribute to the development of new, dramatically improved eggplant varieties for a more sustainable agriculture. By means of selection and further backcrosses, eggplant introgression lines with improved agronomic characteristics and composition can be obtained, facilitating their incorporation in breeding pipelines.

Data availability statement: The data presented in this study are available on request from the corresponding author.

Author contributions: Conceptualization, J.P. and S.V.; methodology, G.V., E.R.-M. and A.¸S.; software, E.R.-M. and S.V.; validation, M.P., J.P. and S.V.; formal analysis, G.V., E.R.-M. and A.¸S.; investigation, G.V., E.R.-M., M.P., E.G.-F.; resources, E.G.-F. and J.P.; data curation, G.V., E.R.-M., A.¸S. and E.G.-F.; writing—original draft, G.V.; writing—review and editing, E.R.-M., M.P., J.P. and S.V.; visualization, G.V., E.R.-M. and S.V.; supervision, M.P. and J.P.; project administration, J.P.; funding acquisition, J.P. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table 1. Mean values and standard error (SE) of the soil chemical composition before transplant.

Supplementary Table 2. Values of intake water chemical composition.

Supplementary Figure 1. Neighbor-joining dendrogram based on IBS distances of 56 individuals of advanced backcrosses (BC2 and BC3) individuals with introgressions from *S. elaeagnifolium* and the recurrent parent (*S. melongena*).

Supplementary Figure 2. PCA loading plot (A) and score plot (B) evaluated in the present study based on the two first principal components of PCA. First and second components account for 40.1% and 14.7% of the total variation, respectively. The accessions are represented by different symbols according to the generation (BC2, BC3 and recurrent parent) and gradient of color according to homozygous proportion from recurrent parent (1: dark purple to 0: yellow). The full name of each trait abbreviation can be found in Table 1.

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Research article

Evaluation of three sets of advanced backcrosses of eggplant with wild relatives from different genepools under low N fertilization conditions

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Abstract

The development of new cultivars with improved nitrogen use efficiency (NUE) is key for implementing sustainable agriculture practices. Crop wild relatives (CWRs) provide valuable genetic resources for breeding programs aimed at achieving this goal. In this study, three eggplant (*Solanum melongena*) accessions together with their advanced backcrosses (ABs; BC3 to BC5 generations) were evaluated for 22 morpho-agronomic, physiological, and N use efficiency (NUE) traits under low nitrogen fertilization conditions. The ABs were developed with introgressions from the wild relatives *S. insanum*, *S. dasyphyllum*, and *S. elaeagnifolium*. The AB population comprised a total of 25, 59, and 59 genotypes, respectively, with overall donor wild relative genome coverage percentages of 58.8%, 46.3%, and 99.2%. The three *S. melongena* recurrent parents were also evaluated under control (normal) N fertilization. Reduction of N fertilization in the parents resulted in decreased chlorophyll content-related traits, aerial biomass, stem diameter and yield, and increased NUE, nitrogen uptake efficiency (NUpE), and nitrogen utilization efficiency (NUtE). However, the decrease in yield was moderate, ranging between 62.6% and 72.6%. A high phenotypic variation was observed within each of the three sets of ABs under low nitrogen conditions, with some individuals displaying improved transgressive characteristics over the recurrent parents. Using the SPET 5k probes platform for high-throughput genotyping, we observed a variable but high degree of recurrent parent genome recovery in the ABs attributable to the lines recombination, allowing the successful identification of 16 QTLs. Different allelic effects were observed for the introgressed QTL alleles. Several candidate genes were identified in the QTL regions associated with plant growth, yield, fruit size, and NUE-related parameters. Our results show that eggplant materials with introgressions from CWRs can result in a dramatic impact in eggplant breeding for a more sustainable agriculture.

Keywords: *Solanum melongena*; *S. dasyphyllum*; *S. elaeagnifolium*; *S. insanum*; advanced backcrosses; crop wild relatives; N fertilization; QTLs.

1. Introduction

Enhancing crop productivity is a fundamental objective in agriculture and remarkable advancements have been achieved in this area since the beginning of the 20th century. This has been accomplished, in part, through the widespread utilization of nitrogen (N) as a fertilizer (Zahoor et al., 2014). However, excessive use of N fertilization can lead to negative environmental impacts, such as groundwater and surface water contamination, loss of biodiversity, increased greenhouse gas emissions, and ozone layer depletion (Ahmed et al., 2017; Kanter et al., 2016; Stevens, 2019). In addition, synthetic N fertilizers require large amounts of energy to be produced (Ghavam et al., 2021). Therefore, to mitigate these negative consequences, selection and development of new varieties with improved crop nitrogen use efficiency (NUE) is a major objective of plant breeding for a more sustainable agriculture (Han et al., 2015; Zhang et al., 2015).

The utilization of plant genetic resources is essential for implementing breeding programs to address challenges associated with changes in climatic conditions. In this context, crop wild relatives (CWRs) are of great relevance, as they possess inherent adaptations to a wide range of adverse natural conditions (Dempewolf et al., 2017). However, the direct utilization of CWRs in breeding programs is often impractical due to the presence of unfavorable traits and genetic barriers. Therefore, the development of advanced backcrosses (ABs) is a viable breeding strategy that expands the available genetic diversity by incorporating genomic fragments of CWR genomes into a mostly cultivated genetic background (Prohens et al., 2017). Furthermore, ABs are useful for the detection of Quantitative Trait Loci (QTLs) by associating phenotypic variation with specific regions of the genome.

Eggplant (*Solanum melongena* L.), also known as aubergine or brinjal, is a widely cultivated vegetable crop, belonging to the subgenus *Leptostemonum* of the Solanaceae family (Chapman, 2020). It is one of the most important solanaceous crops, ranking second only to tomato (*S. lycopersicum* L.) (FAO, 2021). The recent development of genomic tools specific for eggplant, such as high-throughput genotyping platforms (Barchi et al., 2019a) and high-quality eggplant genome assemblies (Barchi et al., 2021, 2019b; Dandan Li et al., 2021; Wei et al., 2020a), among others, has facilitated genomic studies on this crop.

Eggplant wild relatives (CWRs) are classified into the primary (GP1), secondary (GP2) and tertiary (GP3) genepools, based on their level of crossability with the cultivated species. Interspecific hybrids, advanced backcrosses (ABs) and introgression lines (ILs) have been obtained by utilizing a several of these CWRs (Gramazio et al., 2017; Kouassi et al., 2016; Plazas et al., 2016b).

Among the CWRs for which ABs have been developed, *Solanum insanum* L. belongs to GP1 and is considered the wild ancestor of the common eggplant (*S. melongena*). This species grows in a wide range of environmental conditions, including infertile soils, and is naturally distributed throughout south and southeast Asia, Madagascar and Mauritius (Ranil et al., 2017). Among the many eggplant secondary genepool (GP2) species, *S. dasyphyllum* Schumach. & Thonn is part of the Anguivi clade of the *Leptostemonum* subgenus and is considered the wild progenitor of the gboma eggplant (*S. macrocarpon* L.), an African cultivated eggplant (Taher et al., 2017). Some studies have shown that *S. insanum*, *S. dasyphyllum* and their interspecific hybrids with eggplant exhibit enhanced drought (Kouassi et al., 2020; Plazas et al., 2016a) and salinity tolerance (Brenes et al., 2020; Hannachi et al., 2021; Ortega-Albero et al., 2023). Another CWR of interest is the American species *S. elaeagnifolium* Cav., which is native to Northern Mexico and the United States and that can thrive in a wide range of climatic conditions, including semiarid areas, being a globally invasive plant (Krigas et al., 2021; Roberts and Florentine, 2022). The development of backcrosses of *S. elaeagnifolium* with eggplant has been reported, making available a previously unexploited genepool for eggplant breeding (García-Fortea et al., 2019). Additionally, *S. elaeagnifolium* is a potential source for developing new varieties with enhanced drought tolerance (Fita et al., 2015) and adaptation to low N-inputs (Villanueva et al., 2021).

In the present work, we evaluated morpho-agronomic and composition traits of three *S. melongena* accessions (MEL5, MEL1 and MEL3) under two N fertigation conditions and three sets of advanced backcrosses (ABs) of these three accessions with introgressions from eggplant wild relatives *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium* under low N conditions. The study results provide valuable information in the identification of potential materials for eggplant breeding under low N fertilization. Furthermore, detection of QTLs was made possible through the association of phenotyping data and the availability of high-density genotyping data of the ABs individuals.

2. Materials and methods

2.1. Plant material

Three *S. melongena* accessions (MEL5, MEL1 and MEL3) and three sets of advanced backcrosses (ABs) of these accessions with, respectively, the eggplant wild relatives *S. dasyphyllum* DAS1, *S. elaeagnifolium* ELE2 and *S. insanum* INS1 were used for the current study (Plazas et al., 2016b). Each of the three sets of ABs has gone through several rounds of recombination in the successive backcrossings performed towards the three recurrent parents. Selection of the recombinant ABs genotypes was performed based on SPET molecular markers to maintain an overall coverage of the wild genome donor while increasing the genetic background of the recurrent domesticated parent. For the set of *S. insanum* ABs (INS1 x MEL5), 25 ABs genotypes were used, of which eight were from the fifth backcross generation (BC5) and 17 of the first selfing of the fourth backcross generation (BC4S1). In the case of the *S. dasyphyllum* ABs set (MEL1 x DAS1), a total of 59 genotypes were used, 41 of them being from the BC5 generation and 18 of the BC4S1 generation.

Finally, for the *S. elaeagnifolium* ABs set (MEL3 x ELE2), 59 ABs genotypes were used, of which 16 were from the third backcross generation (BC3) and 43 were from the fourth backcross generation (BC4).

2.2. DNA extraction and genotyping

Extraction of genomic DNA of the three recurrent parents and advanced backcrosses individuals was performed following the SILEX DNA extraction method (Vilanova et al., 2020). Isolated DNA was evaluated for quality and integrity by 0.8% agarose gel electrophoresis and spectrophotometric ratios 260:280 and 260:230 and quantified by a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Diluted DNA samples were genotyped using the eggplant 5k Single Primer Enrichment Technology (SPET) platform consisting of 5,093 probes (Barchi et al., 2019a). Single nucleotide polymorphisms (SNPs) were filtered with Tassel software (version 5.2 Standalone; (Bradbury et al., 2007)) by using a minimum count value of 97%, a minimum allele frequency (MAF) higher than 5%, a maximum heterozygosity proportion of 70% and a minimum distance between adjacent sites of 2,000 pb. After filtering, the number of discriminant SNPs between parents was 826, 1,195, 2,114 and for *S. insanum*, *S. dasyphyllum*, and *S. elaeagnifolium* ABs, respectively.

2.3. Cultivation conditions

Plants were grown during the summer season (July to October 2020) in an open field plot located on the campus of the Universitat Politècnica de València (GPS coordinates: latitude, 39° 28’ 55” N; longitude, 0° 20’ 11” W; 7 m a.s.l.). Advanced backcrosses individuals and recurrent parentals lines were randomly distributed in 17 L pots with coconut fiber, spaced 150 cm between rows and 70 cm within rows. Irrigation and fertilization were applied with a drip irrigation system.

The recurrent parents *S. melongena* MEL5, MEL1 and MEL3 were cultivated under two different nitrogen fertilization conditions, namely low (LN) and normal nitrogen (NN) treatments. Seven plants of each *S. melongena* accession together with ABs individuals of each set were cultivated under LN conditions, while seven plants of each *S. melongena* were cultivated under NN conditions.

A physicochemical and composition analysis of coconut fiber was performed before the transplant. Parameters were evaluated following the procedures described in van Reeuwijk (2002) and are shown in Table S1. A chemical composition analysis of water was performed before adding fertilizers. The intake water was slightly basic with low content of nitrates, nitrites, phosphates, ammonium, magnesium and potassium, moderate content of sulphates and calcium, and high content of sodium (Table S2).

Fertilization solutions were prepared based on the substrate composition and the intake water analyses. The low nitrogen (LN) solution was prepared by adding 1.5 mM H_3PO_4 (Antonio Tarazona SL., Valencia, Spain), 4.85 mM K_2SO_4 (Antonio Tarazona SL., Valencia, Spain), 0.58 mM $MgSO_4$ (Antonio Tarazona SL., Valencia, Spain) plus 0.025 L/m3 of a microelements Welgro Hydroponic fertilizer (Química Massó S.A., Barcelona, Spain) containing boron (BO33-; 0.65% p/v), copper (Cu-EDTA; 0.17% p/v), iron (Fe-DTPA; 3.00% p/v), manganese (Mn-EDTA, 1.87% p/v), molybdenum (MoO42-; 0.15% p/v), and zinc (Zn-EDTA; 1.25% p/v). Normal nitrogen (NN) solution included the components listed above with the addition of 7.2 mM NH4NO3 to the intake water. The pH of the solutions was adjusted to 5.5-5.8 with 23% HCl (Julio Ortega SL., Valencia, Spain).

2.4. Phenotypic trait evaluation

Plants were evaluated for a total of 22 plant, fruit and composition traits (Table 1). A DUALEX® optical leaf clip meter (Force-A, Orsay, France) was used for measuring the chlorophyll, flavonol, anthocyanin contents and Nitrogen Balance Index (NBI®) in leaves (Cerovic et al., 2012; Goulas et al., 2004). Data was obtained as the mean of 10 measurements in the upper and lower side of five leaves of each plant. At the end of the trial, stem diameter was measured with a caliper at the base of the stem and aerial biomass was immediately weighed after cutting the base of the stem with a Sauter FK-250 dynamometer (Sauter, Balingen, Germany). Subsequently, they were dried at room temperature, the leaves were separated from the stems, ground and weighed after drying in an oven at 70 ºC to constant dry weight. The total number of fruits of each plant was harvested for determining yield. Nitrogen uptake efficiency (NUpE) was calculated as the total content of nitrogen (N) in fruit, stem and leaves divided by N supplied with the irrigation solution per plant; nitrogen utilization efficiency (NUtE) was calculated as total fruit yield in dry weight (yield [DM]) divided by the total content of N in fruit, stem and leaves and nitrogen use efficiency (NUE) was the result of the multiplication of NUpE and NutE (Han et al., 2015; Jones et al., 2021; Xu et al., 2012).

For fruit traits, pedicel length, calyx length, fruit length and width were determined as the mean of at least three fruits per plant harvested at the commercially mature stage (i.e., physiologically immature). Fruits traits evaluated were measured with a caliper and their abbreviations and units are included in Table 1.

To determine fruit N and C content, at least five commercially mature fruits per plant were harvested, peeled, chopped and frozen in liquid N_2 and stored at -80 ºC.

Subsequently, the frozen samples were lyophilized, ground until turned into fine powder and homogenized. Dry powder of leaves, stem and fruits was measured in samples of 0.5 g of freeze-dried powder. The analysis of N content was performed using the Dumas method with a TruSpec CN elemental analyzer (Leco, MI, USA). Carbon content was calculated from the measurements of carbon dioxide (CO_2) using an infrared detector (Gazulla et al., 2012). Certified reference standards of different N and C concentrations were used for the quantification.

Table 1. Plant, fruit, and composition traits evaluated in the *S. melongena* MEL1, MEL3 and MEL5 recurrent parents, and their respective advanced backcrosses genotypes with *S. dasyphyllum*, *S. elaeagnifolium* and *S. insanum*, together with abbreviations and units used in the present study.

Trait	**Abbreviation**	**Units**
Plant traits		
Chlorophyll leaf content	P-Chl	$\mu g\ cm^{-2}$
Flavonol leaf content	P-Flav	-
Anthocyanin leaf content	P-Anth	-
Nitrogen Balanced Index	P-NBI	-
Aerial biomass	P-Biomass	kg FW[a]
Stem diameter	P-Diam	mm
Yield	Yield	$g\ plant^{-1}$
Nitrogen Use Efficiency	NUE	-
Nitrogen Uptake Efficiency	NUpE	-
Nitrogen Utilization Efficiency	NUtE	-
Fruit traits		
Fruit pedicel length	F-PedLength	mm
Fruit calyx length	F-CaLength	mm
Fruit length	F-Length	mm
Fruit width	F-Width	mm
Total number of fruits per plant	F-Number	-
Fruit mean weight	F-Weight	g
Composition traits		
Nitrogen content in leaf	N-Leaf	$g\ kg^{-1}$ DM[b]
Carbon content in leaf	C-Leaf	$g\ kg^{-1}$ DM
Nitrogen content in fruit	N-Fruit	$g\ kg^{-1}$ DM
Carbon content in fruit	C-Fruit	$g\ kg^{-1}$ DM
Nitrogen content in stem	N-Stem	$g\ kg^{-1}$ DM
Carbon content in stem	C-Stem	$g\ kg^{-1}$ DM

[a] FW: fresh weight. [b] DM: dry matter

2.5. Data analysis

For plant, fruit and composition data of each ABs set and the recurrent parents (*S. melongena* MEL5, MEL1 and MEL3) mean, standard deviation (SD), range values and coefficient of variation (CV, %) were calculated. Analysis of variance (ANOVA) was performed to detect significant mean differences between the two N cultivation conditions in the recurrent parents, and between each set of ABs and its corresponding recurrent parent in the LN conditions. Significant differences were detected with the Student-Newman-Keuls multiple range test at $p < 0.05$ using Statgraphics Centurion 18 software (StatPoint Technologies, Warrenton, VA, USA).

For each set of ABs and its recurrent parent cultivated in the same conditions (low nitrogen, LN) a principal component analysis (PCA) was performed. Pairwise Euclidean distances were calculated for the analysis of each PCA using R package stats (R Core Team, 2016) of the R statistical software (R Core Team, 2021). The PCA score and loading plots were drawn using R packages *ggplot2* (Wickham, 2016) and RColorConesa (Salguero, 2022). In addition, Pearson pair-wise correlation coefficients were calculated among traits for each set of ABs and *S. melongena* parents cultivated under LN conditions. Their statistical significance was evaluated using a Bonferroni correction at $p < 0.01$ (Hochberg, 1988) using R packages psych (Revelle, 2020) and corrplot (Wei and Simko, 2021).

2.6. Quantitative trait loci detection and candidate gene identification

Detection of quantitative trait loci (QTLs) was performed for each set of ABs using the single QTL model for genome-wide scanning of the R package *R/qtl* (Broman et al., 2003) of R statistical software v4.1.0 (R Core Team, 2021). The threshold of LOD score was established at the 0.05 probability level for significant QTLs. For each putative QTL detected, allelic effects were calculated by establishing significant differences between the means of each genotype with the Student-Newman-Keuls multiple range test ($p < 0.05$).

To identify potential candidate genes within each QTL region, a search was conducted using the '67/3' eggplant reference genome assembly (V3 version) (Barchi et al., 2019b). This search was performed through the Sol Genomics Network database (http://www.solgenomics.net).

3. Results

3.1. Genomic characterization

Genome coverage of the donor wild relatives in the whole sets of ABs was of 58.8% for *S. insanum*, 46.3% for *S. dasyphyllum*, and 99.2% *S. elaeagnifolium* when both heterozygous and homozygous introgressions are considered (Figure 1). Selection of ABs set of *S. insanum* prioritized a proper representation of chromosomes 1 (86.8%), 3 (80.9%), 6 (100%), 9 (78.4%), 10 (100%) and a region of chromosome 11 (34.8%) with several individuals per introgression for its evaluation (Figure 1A). The set of 59 selected ABs of *S. dasyphyllum* included most of chromosomes 1 (84.8%), 5 (86.3%), 6 (89.9%), 8 (89.1%) and. 12 (98.9%), plus an introgression at the end of chromosome 2 (11.5%) and another one at the beginning of chromosome 7 (20.2%) (Figure 1B). For the 59 ABs of *S. elaeagnifolium*, a high percentage of total genome coverage (in heterozygosis) was present for chromosomes 1, 2, 4, 6, 7, 8, 10 and 12 with several individuals for each large introgression (Figure 1C).

The percentage of recovered genetic background from the recurrent parent is on average higher in the set of ABs of *S. insanum* (between 88.3% and 98.3%) and *S. dasyphyllum* (between 97.1% and 99.6%), while in the ABs of *S. elaeagnifolium* the percentage of recovery of the recurrent parent is lower (between 69.3% and 98.7%). These differences in the percentages of recovery can be attributed to the varying generations of backcrossing utilized in each set of ABs. The ABs of *S. insanum* and *S. dasyphyllum* involve individuals from the BC5 and BC4S1 generations, respectively, which are further along in the backcrossing process compared to the ABs of *S. elaeagnifolium*, which consist of individuals from the BC3 and BC4 generations.

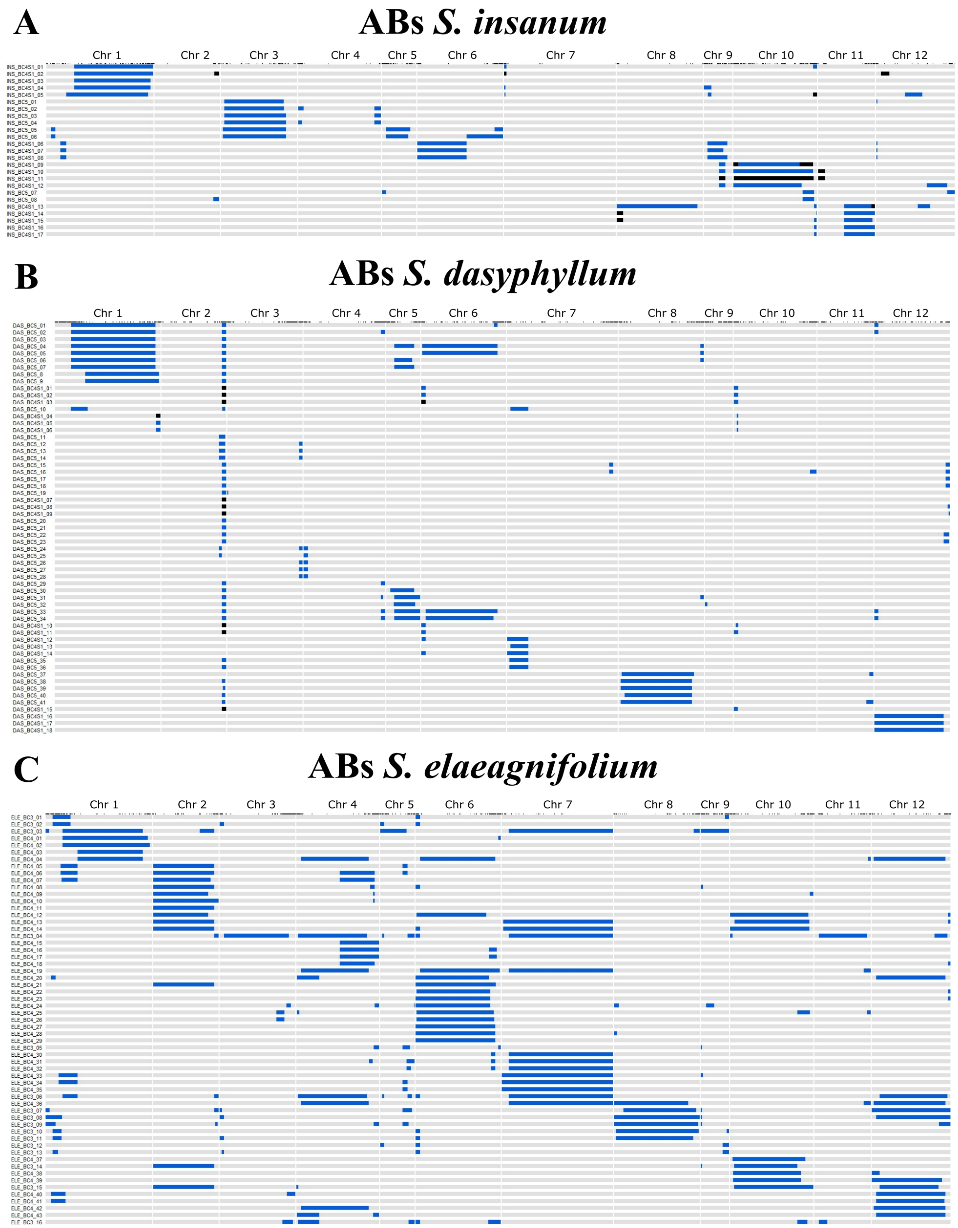


Figure 1. Graphical genotypes of advanced backcross (ABs) lines of *S. insanum* (**A**; n=25), *S. dasyphyllum* (**B**; n=59), and *S. elaeagnifolium* (**C**; n=59) assessed for the present experiment. Each row corresponds to the ABs codes and genotypes and the columns indicate the chromosomes. Heterozygous introgressions are colored in blue, homozygous introgressions are colored in black and the genetic background of each recurrent parent (*S. melongena* MEL5, MEL1 and MEL3, respectively) are colored in grey.

3.2. Characterization of recurrent parents and ABs

Overall, significant differences were detected between N treatments in *S. melongena* recurrent parents (MEL5, MEL1 and MEL3) for plant and composition traits, except for anthocyanin content in leaves (P-Anth) in MEL5 and carbon content in stem (C-Stem) in MEL1 (Tables 2, 3 and 4). *Solanum melongena* individuals cultivated under normal N conditions had higher chlorophyll content in leaf (P-Chl), nitrogen balanced index (P-NBI), aerial biomass (P-Biomass), stem diameter (P-Diam), yield (Yield), total number of fruits per plant (F-Number) and N and C content in leaves, fruits, and stem (N-Leaf, C-Leaf, N-Fruit, C-Fruit, N-Stem, C-Stem) than plants under low N conditions (LN). In addition, a significant decrease in values of flavonol and anthocyanin content in leaves (P-Flav, P-Anth), NUE, NUpE and NUtE were observed in NN plants (Table 2). MEL5 showed the highest significant differences between treatments in yield (Yield, 3.7-fold) and total number of fruits (F-Number, 5.0-fold) with higher values under NN conditions, and the lowest differences in NUE (13.2-fold), NUpE (10.0-fold) and NUtE (1.3-fold) with highest values under LN conditions (Table 2). On the other hand, MEL1 presented the greatest differences between treatments for N content in the different plant parts, with higher values in NN than in LN for leaf (N-Leaf, 3.0-fold), fruit (N-Fruit, 2.0-fold) and stem (N-Stem, 3.6-fold) (Table 4).

Some differences were observed between recurrent parents in N treatments for fruit shape and size traits (Table 3). Fruit pedicel length (F-PedLength) was statistically higher in MEL 5 cultivated under LN conditions than under NN conditions, and the same was observed for fruit length (F-Length) and fruit mean weight (F-Weight) in MEL1 and MEL5. For fruit calyx length (F-CaLength) and fruit width (F-Width) no statistically significant differences were detected (Table 3).

No significant differences were observed between each set of ABs and its corresponding recurrent parent cultivated under low N conditions for plant traits, except for P-Anth in the set of ABs of *S. elaeagnifolium*, being the values significantly higher in MEL3 (1.1-fold) (Table 2). The same results were observed for fruit traits, except for F-Weight in the set of ABs of *S. dasyphyllum*, which displayed significantly lower mean values than its recurrent parent MEL1 individuals in fruit mean weight (F-Weight; 1.2-fold) (Table 3). For composition traits, no statistically significant differences were detected (Table 4).

The distribution ranges for traits evaluated in the three sets of ABs were wider than those observed in the recurrent parents cultivated under low N conditions and transgressive individuals were found for all traits. The recurrent parents MEL5, MEL1 and MEL3 cultivated under NN conditions showed a wider distribution range for P-Biomass, P-Diam and yield (Table 2). In addition, the same results were observed in MEL5 for F-Number and nitrogen content in fruit and stem (N-Fruit and N-Stem), in MEL1 for N-Stem, and in MEL3 for F-Number (Table 3, Table 4).

3.3. Principal components analysis

A PCA was performed with the traits evaluated for each of the sets of ABs with *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium* (Figure 2). Three groups of traits can be observed in common, one includes chlorophyll content-related traits (P-Chl, P-NBI) and nitrogen content in plant (N-Leaf and N-Stem), another group of correlated traits includes plant vigor traits (P-Biomass, P-Diam), yield, NUE and F-Number, and the third group involves fruit size traits (F-PedLength, F-CaLength, F-Length, F-Width and F-Weight).

The PCA performed for the set of ABs of *S. insanum* and its recurrent parent *S. melongena* MEL5 revealed that the first two components accounted for 48.8% of the total variation observed, with PC1 and PC2 accounting for 29.5% and 19.3%, respectively (Figure 2A). The distribution of individuals in the PCA score plot showed that recurrent parent individuals of MEL5 were positioned along the central axis of PC1. Individuals with higher recovery percentages were located closer to the recurrent parentals. The first principal component displayed high negative correlation values with chlorophyll content-related traits (P-Chl, P-NBI), and positive correlations with P-Flav, P-Anth and F-CaLength. NUpE, yield, NUE and F-Number were highly negatively correlated with PC2, and P-Anth and F-CaLength were positively correlated to PC2 (Figure 2A, Table S3).

For the set of ABs of *S. dasyphyllum* and its recurrent parent *S. melongena* MEL1, the first and the second principal components (PCs) accounted for 27.2% and 18.3%, respectively, of the variation (Figure 2B). The projection of individuals in the PCA score plot showed that the individuals of the recurrent parent MEL1 displayed a wide distribution, being intermingled with some ABs individuals. Individuals with different recovery percentages were distributed all over the graph. The first component was highly negatively correlated with yield, NUE, plant vigor traits (P-Biomass, P-Diam) and F-Number, and positively with chlorophyll content-related traits (P-Chl, P-NBI). The second component was highly negatively correlated with flavonol and anthocyanin content in leaves (P-Flav and P-Anth), and positively with P-NBI, and F-Length (Figure 2B, Table S3).

Regarding PCA performed for ABs of *S. elaeagnifolium* and its recurrent parent *S. melongena* MEL3, the first and the second components accounted, respectively, for 23.7% and 16.7%, of the observed variation (Figure 2C). The distribution of the individuals in the PCA score plot revealed a wide overall dispersion over the plot area, with most of the individuals with the lowest percentage of the recovered genetic background of the recurrent parent plotting apart from the recurrent parent MEL3 individuals. The composition traits carbon and nitrogen content in leaf (C-Leaf and N-Leaf) and C-Fruit were positively correlated with PC1, whereas some size-related fruit traits (F-Weight, F-CaLength, F-PedLength and F-Length) were highly negatively correlated with PC1. On the other hand, the second component was highly positively correlated with P-Flav and F-Number, and negatively correlated with P-NBI and N-Leaf (Figure 2C, Table S3).

Table 2. Mean values and range of plant traits of *S. melongena* MEL5, MEL1 and MEL3 in low nitrogen (LN) and normal nitrogen (NN) cultivation conditions and advanced backcrosses (ABs) of *S. insanum* (INS; n=25), *S. dasyphyllum* (DAS; n=59) and *S. elaeagnifolium* (ELE; n=59) in low nitrogen (LN) cultivation conditions. The full name of each trait in the first column can be found in Table 1. For each trait, means with different letters are significant according to the Student–Newman–Keuls multiple range test ($p < 0.05$).

Plant traits	**Mean/ range**	***S. melongena* MEL5 (n=7)**		**ABs *S. insanum* INS (n=25)**	***S. melongena* MEL1 (n=7)**		**ABs *S. dasyphyllum* DAS (n=59)**	***S. melongena* MEL3 (n=7)**		**ABs *S. elaeagnifolium* ELE (n=59)**
		NN	**LN**	**LN**	**NN**	**LN**	**LN**	**NN**	**LN**	**LN**
P-Chl	Mean	45.8 b	37.7 a	36.1 a	38.2 b	29.9 a	30.2 a	37.4 b	25.4 a	27.7 a
	Range	41.0-49.7	35.8-41.7	30.2-42.4	35.7-41.7	27.5-33.7	24.3-37.1	33.4-43.6	24.4-27.4	20.7-33.6
P-Flav	Mean	1.5 a	1.9 b	1.9 b	1.7 a	2.5 b	2.4 b	1.7 a	2.5 b	2.5 b
	Range	1.3-1.6	1.8-2.2	1.5-2.4	1.5-1.8	2.3-2.6	1.9-2.8	1.4-1.8	2.4-2.6	2.2-2.8
P-Anth	Mean	0.20 a	0.23 ab	0.25 b	0.18 a	0.27 b	0.25 b	0.19 a	0.30 c	0.27 b
	Range	0.16-0.24	0.21-0.26	0.18-0.39	0.16-0.23	0.22-0.30	0.18-0.33	0.16-0.21	0.28-0.35	0.21-0.37
P-NBI	Mean	31.0 b	19.8 a	19.7 a	23.1 b	12.2 a	12.9 a	23.2 b	10.1 a	11.2 a
	Range	28.7-32.4	16.6-21.5	12.6-26.2	20.4-27.8	11.0-13.6	8.7-17.6	18.7-26.9	9.4-11.3	8.3-14.3
P-Biomass	Mean	1.9 b	0.4 a	0.4 a	2.2 b	0.4 a	0.4 a	1.4 b	0.3 a	0.3 a
	Range	1.2-2.4	0.2-0.6	0.2-0.6	1.5-3.0	0.2-0.6	0.1-0.7	0.7-2.5	0.2-0.4	0.1-1.0
P-Diam	Mean	20.4 b	11.2 a	11.9 a	22.6 b	13.3 a	13.1 a	19.2 b	12.7 a	13.1 a
	Range	15.9-29.7	9.5-12.2	9.0-14.5	16.2-33.0	11.3-15.0	7.9-20.4	14.6-24.3	10.7-14.5	9.5-18.5
Yield	Mean	5881.9 b	1609.7 a	1609.6 a	6914.1 b	2584.6 a	2116.2 a	5464.4 b	1934.3 a	1564.6 a
	Range	4979.0-7464.0	994.0-2163.0	840.0-2264.0	5185.0-10601.0	1552.0-3553.0	793.0-4328.0	1612.0-8014.0	1257.0-2478.0	145.0-3395.0
NUE	Mean	7.9 a	104.1 b	103.7 b	7.5 a	144.2 b	114.8 b	7.6 a	122.3 b	97.9 b
	Range	5.8-10.6	57.8-142.1	52.3-167.1	5.9-11.4	88.7-216.2	38.8-297.9	2.5-11.1	74.9-157.1	12.9-203.4
NUpE	Mean	0.3 a	3.3 b	3.4 b	0.4 a	4.2 b	4.5 b	0.3 a	3.9 b	4.4 b
	Range	0.2-0.5	1.4-4.8	1.8-5.2	0.3-0.4	3.1-5.7	2.2-14.2	0.2-0.5	2.6-6.9	1.9-15.1
NUtE	Mean	24.2 a	32.5 b	30.3 b	21.5 a	34.5 b	27.1 ab	22.9 a	33.2 b	25.0 ab
	Range	20.2-30.4	25.6-42.6	20.7-43.0	16.9-26.9	28.9-42.0	7.0-46.4	15.3-28.5	22.8-45.2	2.9-56.3

Table 3. Mean values and range of fruit traits of *S. melongena* MEL5, MEL1 and MEL3 in low nitrogen (LN) and normal nitrogen (NN) cultivation conditions and advanced backcrosses (ABs) of *S. insanum* (INS; n=25), *S. dasyphyllum* (DAS; n=59) and *S. elaeagnifolium* (ELE; n=59) in low nitrogen (LN) cultivation conditions. The full name of each trait in the first column can be found in Table 1. For each trait, means with different letters are significant according to the Student–Newman–Keuls multiple range test ($p < 0.05$).

Fruit traits	**Mean/range**	***S. melongena* MEL5 (n=7)**		**ABs *S. insanum* INS (n=25)**	***S. melongena* MEL1 (n=7)**		**ABs *S. dasyphyllum* DAS (n=59)**	***S. melongena* MEL3 (n=7)**		**ABs *S. elaeagnifolium* ELE (n=59)**
		NN	**LN**	**LN**	**NN**	**LN**	**LN**	**NN**	**LN**	**LN**
F-PedLength (mm)	Mean	35.7 a	43.3 b	39.3 ab	39.0	44.7	39.5	41.6	46.8	40.5
	Range	32.1-40.9	34.7-50.4	14.3-51.8	27.2-48.1	40.3-54.5	19.7-58.4	32.0-49.1	37.9-55.6	20.6-66.7
F-CaLength (mm)	Mean	25.9	27.3	26.9	35.7	40.1	35.4	45.0	41.9	37.5
	Range	21.8-37.5	24.3-29.1	11.7-33.5	31.9-39.7	35.6-43.0	16.1-44.0	38.0-47.7	30.7-51.6	17.3-51.9
F-Length (mm)	Mean	76.3 a	97.3 b	87.2 ab	71.8 a	90.6 b	84.7 b	100.1	93.8	85.0
	Range	64.0-86.5	71.4-118.2	33.5-106.0	57.3-87.5	69.0-114.5	30.3-114.3	84.6-122.2	70.6-115.5	37.0-132.4
F-Width (mm)	Mean	41.1	37.7	36.0	53.5	52.9	47.8	54.8	42.7	39.6
	Range	33.4-49.3	31.8-44.0	17.0-41.8	43.3-61.7	46.0-60.3	18.4-56.7	50.8-58.4	34.0-54.5	17.9-54.4
F-Number	Mean	198.7 b	39.7 a	42.7 a	115.7 b	35.7 a	36.5 a	87.7 b	32.9 a	32.6 a
	Range	126.0-268.0	27.0-58.0	22.0-66.0	93.0-143.0	25.0-49.0	17.0-78.0	32.0-118.0	19.0-49.0	12.0-82.0
F-Weight (g)	Mean	30.3 a	40.8 b	38.2 b	59.5 a	71.9 b	58.7 a	61.7	60.7	49.3
	Range	24.1-40.4	32.9-48.1	24.4-54.2	43.2-74.1	59.7-80.8	34.6-82.3	50.4-69.6	48.0-77.7	12.1-81.5

Table 4. Mean values and range of composition traits of *S. melongena* MEL5, MEL1 and MEL3 in low nitrogen (LN) and normal nitrogen (NN) cultivation conditions and advanced backcrosses (ABs) of *S. insanum* (INS; n=25), *S. dasyphyllum* (DAS; n=59) and *S. elaeagnifolium* (ELE; n=59) in low nitrogen (LN) cultivation conditions. The full name of each trait in the first column can be found in Table 1. For each trait, means with different letters are significant according to the Student–Newman–Keuls multiple range test ($p < 0.05$).

Composition traits	**Mean/ range**	***S. melongena* MEL5 (n=7)**		**ABs *S. insanum* INS (n=25)**	***S. melongena* MEL1 (n=7)**		**ABs *S. dasyphyllum* DAS (n=59)**	***S. melongena* MEL3 (n=7)**		**ABs *S. elaeagnifolium* ELE (n=59)**
		NN	**LN**	**LN**	**NN**	**LN**	**LN**	**NN**	**LN**	**LN**
N-Leaf (g/kg DM)	Mean	58.9 b	27.2 a	26.7 a	61.4 b	20.7 a	24.4 a	57.4 b	28.2 a	28.4 ± 5.0 a
	Range	51.1-64.0	22.7-33.8	16.3-34.5	52.8-67.4	17.5-23.8	14.2-33.2	51.6-63.4	25.1-33.6	17.6-40.4
C-Leaf (g/kg DM)	Mean	453.1 b	414.7 a	413.8 a	455.9 b	410.1 a	415.3 a	465.7 b	423.1 a	425.6 ± 18.3 a
	Range	436.0-464.0	400.0-433.0	388.0-435.0	450.0-463.0	398.0-429.0	394.0-437.0	458.0-480.0	407.0-436.0	385.0-471.0
N-Fruit (g/kg DM)	Mean	33.3 b	20.3 a	20.5 a	37.4 b	19.0 a	19.8 a	34.6 b	18.6 a	19.7 ± 3.3 a
	Range	28.4-40.1	17.4-22.6	15.9-24.0	31.6-44.8	14.6-22.1	13.8-27.2	31.9-37.6	14.4-27.2	13.6-31.2
C-Fruit (g/kg DM)	Mean	422.3 b	403.0 a	407.0 a	413.4 b	402.3 a	398.5 a	425.1 b	396.6 a	405.7 ± 13.4 a
	Range	410.0-436.0	384.0-428.0	368.0-419.0	409.0-425.0	382.0-420.0	357.0-424.0	402.0-446.0	348.0-415.0	376.0-441.0
N-Stem (g/kg DM)	Mean	31.8 b	10.3 a	10.8 a	37.1 b	10.2 a	10.3 a	29.9 b	10.2 a	11.2 ± 2.7 a
	Range	28.0-35.6	6.6-13.4	7.3-14.4	32.2-45.4	7.2-13.3	7.0-19.3	24.3-33.8	8.6-12.5	7.9-18.6
C-Stem (g/kg DM)	Mean	416.4 b	395.1 a	400.0 a	424.1	413.0	411.2	424.0 b	412.4 a	409.3 ± 9.2 a
	Range	414.0-420.0	389.0-410.0	387.0-415.0	417.0-431.0	403.0-419.0	388.0-494.0	415.0-432.0	398.0-423.0	387.0-430.0

3.4. Correlations among traits

Significant Pearson's linear correlations among traits evaluated were found in the three sets of ABs of eggplant with *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium* (Figure 3). For plant traits, negative correlations common to all ABs sets were observed among pigment content in leaves (P-Chl, P-Anth), and between P-NBI and anthocyanin and flavonol content in leaf (P-Anth, P-Flav). Positive correlations were detected between P-Chl and P-NBI. Traits related to plant vigor P-Biomass and P-Diam were positively correlated (r > 0.6). In addition, yield, NUE and total number of fruits (F- Number) were positively correlated. Regarding fruit shape and size traits, shared positive correlations were found among F-PedLength and F-Width with F-CaLength and F-Length. For composition traits, N-Leaf showed a common significant negative correlation with P-Flav (Figure 3).

The set of ABs of *S. insanum* shared different significant correlations with the set of ABs of *S. dasyphyllum*. In this way, a positive correlation was observed between P-Flav and P-Anth (r > 0.6) in both sets (Figure 3A, B). Yield, in addition to showing significant positive correlations with NUE, was also correlated with P-Biomass and NUpE. N-Leaf and N-Stem also displayed a shared positive correlation in both ABs sets. On the other hand, several correlations among traits were shared between the ABs of *S. dasyphyllum* and the ABs of *S. elaeagnifolium*. In both sets, yield showed a positive correlation with NUtE (r > 0.5) (Figure 3B, C), whereas for fruit traits, F-length was positively correlated with F-CaLength and F-Weight, and F-PedLength was positively correlated with F-Width.

In addition, each set of ABs showed specific correlations. In this way, for ABs of *S. insanum*, N-Leaf displayed a positive correlation with P-Anth and a negative with P-NBI (Figure 3A). Regarding specific significant correlations found in ABs of *S. dasyphyllum*, NUE and F-Number were positively correlated with plant vigor-related traits (P-Biomass and P-Diam) (r > 0.7) (Figure 3B). NUE was also correlated with F-CaLength and C-Fruit, and F-Number showed a positive correlation with NUpE. In addition, yield showed positive correlations among F-CaLength and C-Fruit in this set of ABs. Finally, for ABs set of *S. elaeagnifolium*, NUtE showed positive correlations with other nitrogen use efficiency parameters (NUE and NUpE) and with F-Number, and a negative correlation with N-Fruit (Figure 3C). F-Weight was positively correlated with yield and traits related to fruit size (F-PedLength, F-CaLength, F-Length and F-Width), and negatively correlated with C-Leaf. In addition, in this set of ABs F-PedLenght was negatively correlated with C-Fruit.

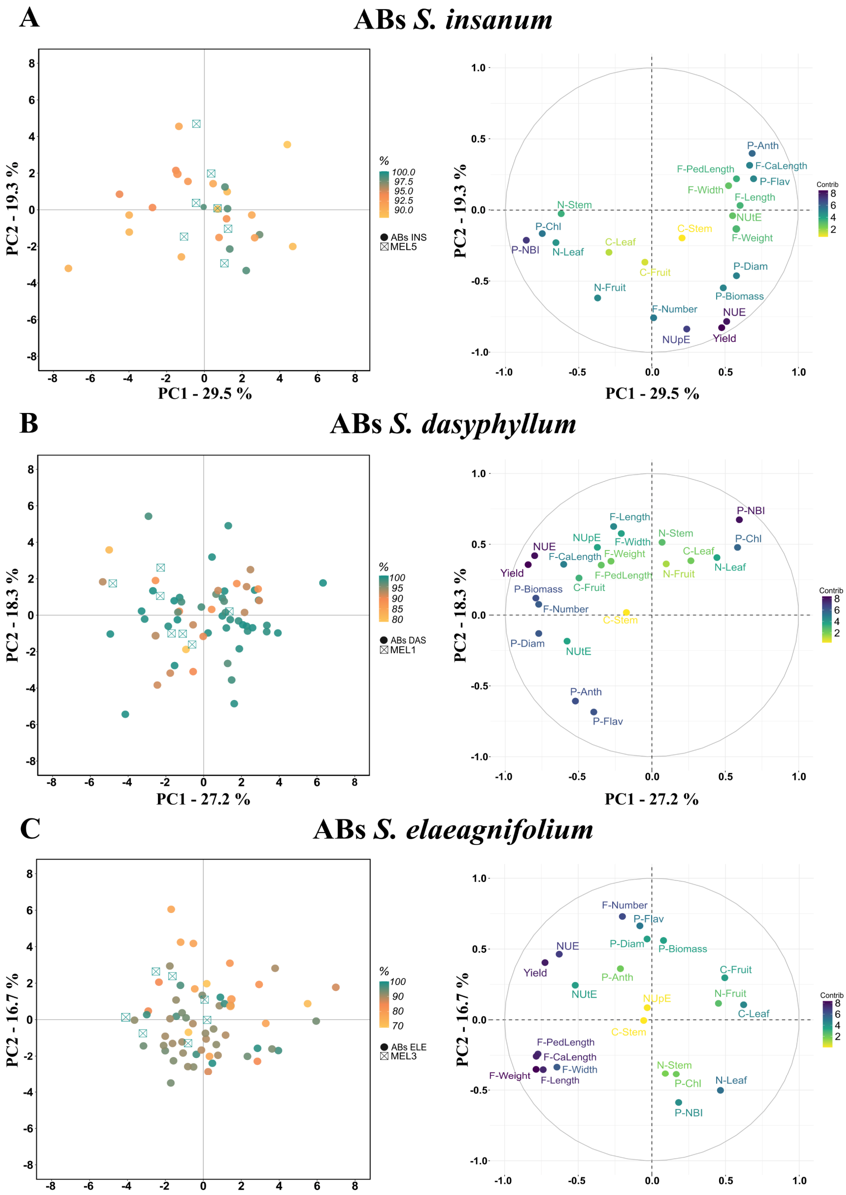
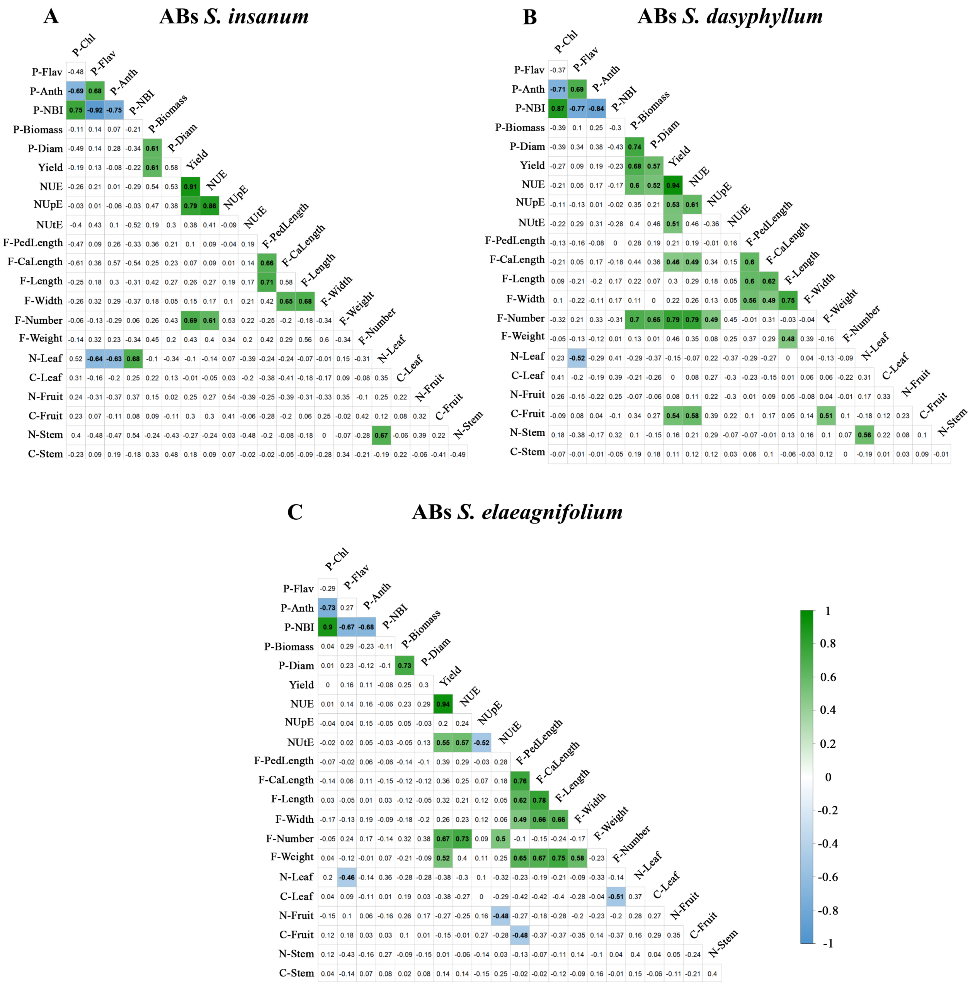


Figure 2. PCA score plot and loading plot based on the two first principal components of PCA performed for all traits of advanced backcrosses (ABs) of lines of *S. insanum* (**A**; n=25), *S. dasyphyllum* (**B**; n=59) and *S. elaeagnifolium* (**C**; n=59) and recurrent parents MEL5 (n=7), MEL1 (n=7) and MEL3 (n=7). The accessions are represented by different symbols according to the recurrent parent (MEL5, MEL1 and MEL3, respectively) and ABs of each line. Gradient of color in PCA score plot according to recovery percentage (%) from recurrent parent (100%: green to 80% (A); 70% (B); 90% (C): yellow). Gradient of color in PCA loading plot according to contribution proportion of each trait (8: dark blue to 2: yellow). The full name of each trait can be found in Table 1.



A ABs *S. insanum*

	P-Chl	P-Flav	P-Anth	P-NBI	P-Biomass	P-Diam	Yield	NUE	NUpE	NUtE	F-PedLength	F-CaLength	F-Length	F-Width	F-Weight	F-Number	N-Leaf	C-Leaf	N-Fruit	C-Fruit	N-Stem
P-Flav	-0.48																				
P-Anth	-0.69	0.68																			
P-NBI	0.75	-0.92	-0.75																		
P-Biomass	-0.11	0.14	0.07	-0.21																	
P-Diam	-0.49	0.14	0.28	-0.34	0.61																
Yield	-0.19	0.13	-0.08	-0.22	0.61	0.58															
NUE	-0.26	0.21	0.01	-0.29	0.54	0.53	0.91														
NUpE	-0.03	0.01	-0.06	-0.03	0.47	0.38	0.79	0.86													
NUtE	-0.4	0.43	0.1	-0.52	0.19	0.3	0.38	0.41	-0.09												
F-PedLength	-0.47	0.09	0.26	-0.33	0.36	0.21	0.1	0.09	-0.04	0.19											
F-CaLength	-0.61	0.36	0.57	-0.54	0.25	0.23	0.07	0.09	0.01	0.14	0.66										
F-Length	-0.25	0.18	0.3	-0.31	0.42	0.27	0.26	0.27	0.19	0.17	0.71	0.58									
F-Width	-0.26	0.32	0.29	-0.37	0.18	0.05	0.15	0.17	0.1	0.21	0.42	0.65	0.68								
F-Number	-0.06	-0.13	-0.29	0.06	0.26	0.43	0.69	0.61	0.53	0.22	-0.25	-0.2	-0.18	-0.34							
F-Weight	-0.14	0.32	0.23	-0.34	0.45	0.2	0.43	0.4	0.34	0.2	0.42	0.29	0.56	0.6	-0.34						
N-Leaf	0.52	-0.64	-0.63	0.68	-0.1	-0.34	-0.1	-0.14	0.07	-0.39	-0.24	-0.24	-0.07	-0.01	0.15	-0.31					
C-Leaf	0.31	-0.16	-0.2	0.25	0.22	0.13	-0.01	-0.05	0.03	-0.2	-0.38	-0.41	-0.18	-0.17	0.09	-0.08	0.35				
N-Fruit	0.24	-0.31	-0.37	0.37	0.15	0.02	0.25	0.27	0.54	-0.39	-0.25	-0.39	-0.31	-0.33	0.35	-0.1	0.25	0.22			
C-Fruit	0.23	0.07	-0.11	0.08	0.09	-0.11	0.3	0.3	0.41	-0.06	-0.28	-0.2	0.06	0.25	-0.02	0.42	0.12	0.08	0.32		
N-Stem	0.4	-0.48	-0.47	0.54	-0.24	-0.43	-0.27	-0.24	0.03	-0.48	-0.2	-0.08	-0.18	0	-0.07	-0.28	0.67	-0.06	0.39	0.22	
C-Stem	-0.23	0.09	0.19	-0.18	0.33	0.48	0.18	0.09	0.07	-0.02	-0.02	-0.05	-0.09	-0.28	0.34	-0.21	-0.19	0.22	-0.06	-0.41	-0.49

B ABs *S. dasyphyllum*

	P-Chl	P-Flav	P-Anth	P-NBI	P-Biomass	P-Diam	Yield	NUE	NUpE	NUtE	F-PedLength	F-CaLength	F-Length	F-Width	F-Weight	F-Number	N-Leaf	C-Leaf	N-Fruit	C-Fruit	N-Stem
P-Flav	-0.37																				
P-Anth	-0.71	0.69																			
P-NBI	0.87	-0.77	-0.84																		
P-Biomass	-0.39	0.1	0.25	-0.3																	
P-Diam	-0.39	0.34	0.38	-0.43	0.74																
Yield	-0.27	0.09	0.19	-0.23	0.68	0.57															
NUE	-0.21	0.05	0.17	-0.17	0.6	0.52	0.94														
NUpE	-0.11	-0.13	0.01	-0.02	0.35	0.21	0.53	0.61													
NUtE	-0.22	0.29	0.31	-0.28	0.4	0.46	0.51	0.46	-0.36												
F-PedLength	-0.13	-0.16	-0.08	0	0.28	0.19	0.21	0.19	-0.01	0.16											
F-CaLength	-0.21	0.05	0.17	-0.18	0.44	0.36	0.46	0.49	0.34	0.15	0.6										
F-Length	0.09	-0.21	-0.2	0.17	0.22	0.07	0.3	0.29	0.18	0.05	0.6	0.62									
F-Width	0.1	-0.22	-0.11	0.17	0.11	0	0.22	0.26	0.13	0.05	0.56	0.49	0.75								
F-Number	-0.32	0.21	0.33	-0.31	0.7	0.65	0.79	0.79	0.49	0.45	-0.01	0.31	-0.03	-0.04							
F-Weight	-0.05	-0.13	-0.12	0.01	0.13	0.01	0.46	0.35	0.08	0.25	0.37	0.29	0.48	0.39	-0.16						
N-Leaf	0.23	-0.52	-0.29	0.41	-0.29	-0.37	-0.15	-0.07	0.22	-0.37	-0.29	-0.27	0	0.04	-0.13	-0.09					
C-Leaf	0.41	-0.2	-0.19	0.39	-0.21	-0.26	0	0.08	0.27	-0.3	-0.23	-0.15	0.01	0.06	0.06	-0.22	0.31				
N-Fruit	0.26	-0.15	-0.22	0.25	-0.07	-0.06	0.08	0.11	0.22	-0.3	0.01	0.09	0.05	-0.08	0.04	-0.01	0.17	0.33			
C-Fruit	-0.09	0.08	0.04	-0.1	0.34	0.27	0.54	0.58	0.39	0.22	0.1	0.17	0.05	0.14	0.51	0.1	-0.18	0.12	0.23		
N-Stem	0.18	-0.38	-0.17	0.32	0.1	-0.15	0.16	0.21	0.29	-0.07	-0.07	-0.01	0.13	0.16	0.1	0.07	0.56	0.22	0.08	0.1	
C-Stem	-0.07	-0.01	-0.01	-0.05	0.19	0.18	0.11	0.12	0.12	0.03	0.06	0.1	-0.06	-0.03	0.12	0	-0.19	0.01	0.03	0.09	-0.01

C ABs *S. elaeagnifolium*

	P-Chl	P-Flav	P-Anth	P-NBI	P-Biomass	P-Diam	Yield	NUE	NUpE	NUtE	F-PedLength	F-CaLength	F-Length	F-Width	F-Weight	F-Number	N-Leaf	C-Leaf	N-Fruit	C-Fruit	N-Stem
P-Flav	-0.29																				
P-Anth	-0.73	0.27																			
P-NBI	0.9	-0.67	-0.68																		
P-Biomass	0.04	0.29	-0.23	-0.11																	
P-Diam	0.01	0.23	-0.12	-0.1	0.73																
Yield	0	0.16	0.11	-0.08	0.25	0.3															
NUE	0.01	0.14	0.16	-0.06	0.23	0.29	0.94														
NUpE	-0.04	0.04	0.15	-0.05	0.05	-0.03	0.2	0.24													
NUtE	-0.02	0.02	0.05	-0.03	-0.05	0.13	0.55	0.57	-0.52												
F-PedLength	-0.07	-0.02	0.06	-0.06	-0.14	-0.1	0.39	0.29	-0.03	0.28											
F-CaLength	-0.14	0.06	0.11	-0.15	-0.12	-0.12	0.36	0.25	0.07	0.18	0.76										
F-Length	0.03	-0.05	0.01	0.03	-0.12	-0.05	0.32	0.21	0.12	0.05	0.62	0.78									
F-Width	-0.17	-0.13	0.19	-0.09	-0.18	-0.2	0.26	0.23	0.12	0.06	0.49	0.66	0.66								
F-Number	-0.05	0.24	0.17	-0.14	0.32	0.38	0.67	0.73	0.09	0.5	-0.1	-0.15	-0.24	-0.17							
F-Weight	0.04	-0.12	-0.01	0.07	-0.21	-0.09	0.52	0.4	0.11	0.25	0.65	0.67	0.75	0.58	-0.23						
N-Leaf	0.2	-0.46	-0.14	0.36	-0.28	-0.28	-0.38	-0.3	0.1	-0.32	-0.23	-0.19	-0.21	-0.09	-0.33	-0.14					
C-Leaf	0.04	0.09	-0.11	0.01	0.19	0.03	-0.38	-0.27	0	-0.29	-0.42	-0.42	-0.4	-0.28	-0.04	-0.51	0.37				
N-Fruit	-0.15	0.1	0.06	-0.16	0.26	0.17	-0.27	-0.25	0.16	-0.48	-0.27	-0.18	-0.28	-0.2	-0.23	-0.2	0.28	0.27			
C-Fruit	0.12	0.18	0.03	0.03	0.1	0.01	-0.15	-0.01	0.27	-0.28	-0.48	-0.37	-0.37	-0.35	0.14	-0.37	0.16	0.29	0.35		
N-Stem	0.12	-0.43	-0.16	0.27	-0.09	-0.15	0.01	-0.06	-0.14	0.03	-0.13	-0.07	-0.11	0.14	-0.1	0.04	0.4	0.04	0.05	-0.24	
C-Stem	0.04	-0.14	0.07	0.08	0.02	0.08	0.14	0.14	-0.15	0.25	-0.02	-0.02	-0.12	-0.09	0.16	-0.01	0.15	-0.06	-0.11	-0.21	0.4

Figure 3. Pearson's correlations among traits evaluated advanced backcrosses (ABs) of *S. insanum* (**A**; n=25), *S. dasyphyllum* (**B**; n=59) and *S. elaeagnifolium* (**C**; n=59). Only significant correlations at $p<0.01$ according to the Bonferroni tests are colored. Color scale from green (positive correlations) to blue (negative correlations). The full name of each trait can be found in Table 1.

3.5. *Detection and effect of putative QTLs*

A total of 16 putative significant QTLs were found in the analysis of the three different sets of ABs (Table 5). Five QTLs (flavonol leaf content, *fv-9*; nitrogen balanced index, *nb-9*; fruit mean weight, *fw-9*; nitrogen content in leaf, *nl-9*; and nitrogen content in stem; *ns-9*) were found in ABs of *S. insanum* at the same position on chromosome 9 (Table 5, Figure 4A). QTLs for flavonol leaf content (*fv-9*) and for mean fruit weight (*fw-9*) presented similar effects (Figure 5A, C). In contrast, opposing effects were observed for QTLs associated with nitrogen balance index (*nb-9*), nitrogen leaf content (*nl-9*) and nitrogen stem content (*ns-9*), which presented significant higher values in individuals with heterozygous introgression (Figure 5B, D, E). Regarding ABs of *S. dasyphyllum*, eight of them were identified. Three QTLs were detected at the same position on chromosome 1 (Table 5, Figure 4B). QTL for flavonol leaf content (*fl-1*) presented significant higher values in individuals with heterozygous introgression, whereas an opposite QTL effect was observed for nitrogen balanced index (*nb-1*) (Figure 5G, H). For stem diameter (*di-1*), a dominance of the *S. melongena* allele decreasing the values was observed (Figure 5J). On chromosome 2, five QTLs were located at the same position (Table 5, Figure 4C). Chlorophyll leaf content (*ch-2*) presented dominance of the *S. dasyphyllum* allele, which displayed significant higher values while an opposite QTL effect was observed for biomass (*bi-2*) and yield (*yd-2*) (Figure 5F, I, K). For fruit pedicel length (*fp*-2) and fruit mean weight (*fd-2*) incomplete dominance was observed with a negative allelic effect of *S. dasyphyllum* introgression on the values (Figure 5L, M). The analysis of ABs of *S. elaeagnifolium* allowed the detection of two QTLs for fruit traits, namely fruit calyx length (*fc-2*) and fruit mean weight (*fw-2*) located at the same position on chromosome 2 (Table 5, Figure 4D), showing very similar effects with significant lower values in individuals with heterozygous introgression (Figure 5N, O). A QTL was also detected on chromosome 8 (Figure 4E), displaying significant higher carbon content in leaf (*cl-8*) corresponding to the *S. elaeagnifolium* heterozygous introgression (Figure 5P).

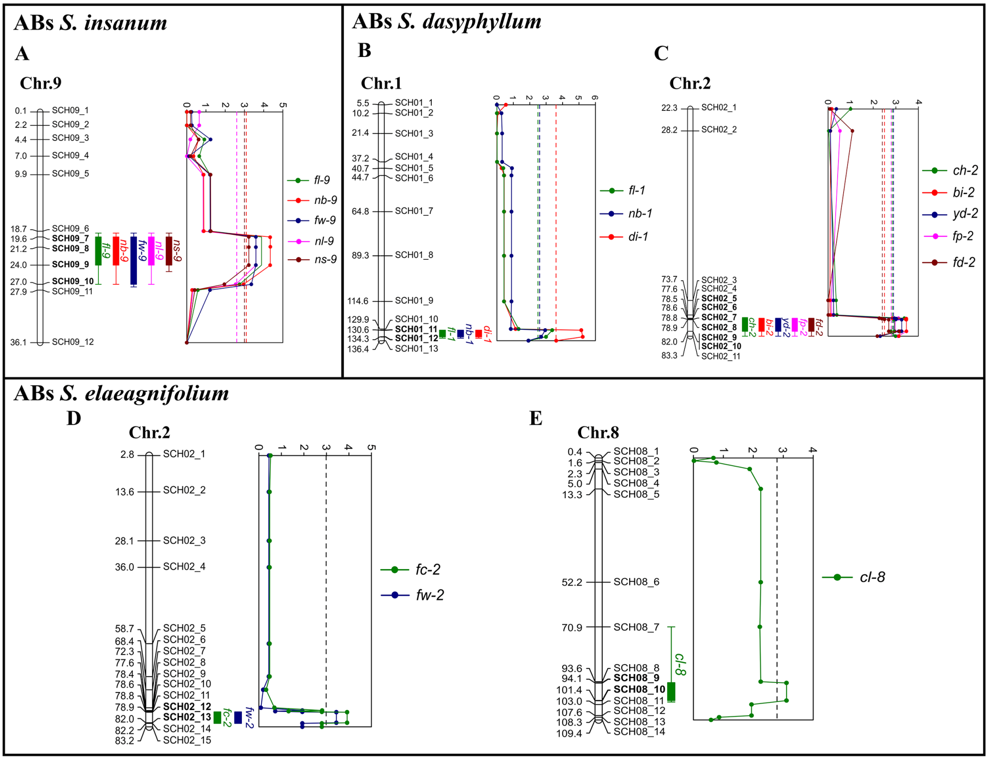


Figure 4. Physical map and LOD score of chromosomes with putative QTLs. A, ABs of *S. insanum*; B-C, ABs of *S. dasyphyllum*; and D-E, ABs of S. *elaeagnifolium*. Dotted lines indicate the LOD score thresholds of each QTL, with corresponding chromosome positions indicated on the physical map.

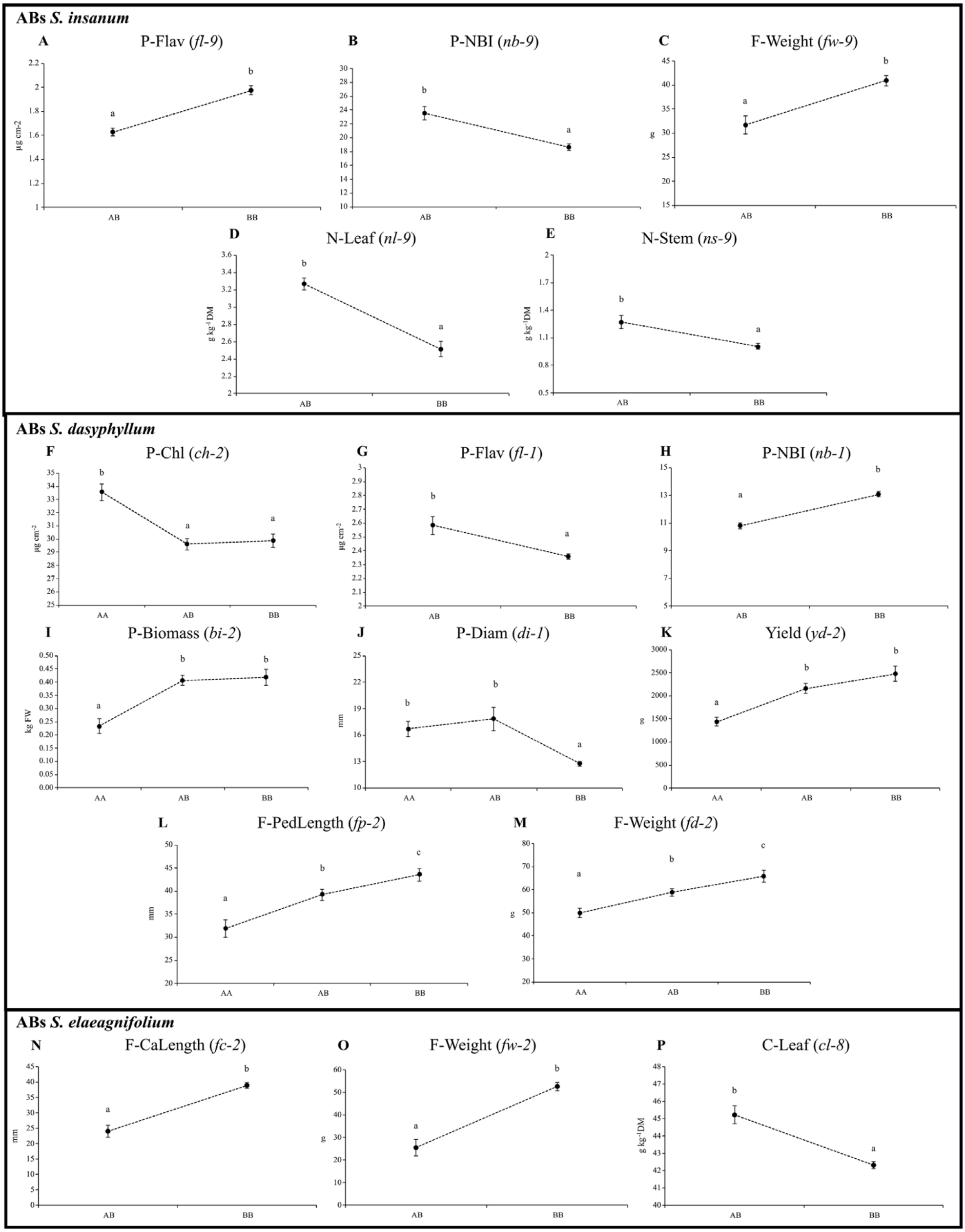


Figure 5. Effect plots for putative QTLs based on allelic distributions determined at the peak marker of the QTL. Three possible genotypes are indicated on the x-axis (A= wild parent, B= recurrent parent, AB= hybrid). A-E, ABs of *S. insanum*; F-M, ABs of *S. dasyphyllum*; and N-P, ABs of S. *elaeagnifolium*. The y-axis represents mean values of each trait for each genotype. Error bars represent standard error of the mean (SEM). For each trait, means with different letters are significant according to the Student–Newman–Keuls multiple range test ($p < 0.05$).

3.6. Identification of candidate genes

The search for candidate genes in the '67/3' eggplant reference genome assembly (V3 version) (Barchi et al., 2019b) allowed identification of several potential candidate genes that may be associated to putative QTLs detected in this study. For the QTLs detected in ABs of *S. insanum* related to nitrogen content in plants, a gene encoding NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (NRT1/PRT) FAMILY (NPF) proteins (SMEL_009g328470) was identified and mapped to the corresponding region of the detected QTL on chromosome 9. Regarding the QTLs detected on chromosome 2 associated with plant growth, yield, and fruit size parameters in ABs of *S. dasyphyllum* and *S. elaeagnifolium*, two distinct potential candidate genes were found to be possibly associated to these traits. One gene (SMEL_002g164700) encodes a PIN-FORMED (PIN) 8 auxin efflux transporter, while another gene (SMEL_002g167520) encodes a Myb-related protein 306 (MYB306), which is a transcription factor (TF) involved in anthocyanin regulation (Wang et al., 2022). Additionally, within the same region on chromosome 2, a gene (SMEL_002g164340) was detected that encodes an NPF protein, which could also be associated with the same traits.

Table 5. List of putative QTLs detected for traits in advanced backcrosses (ABs) of *S. melongena* (MEL5, MEL1 and MEL3) with *S. insanum*, *S. dasyphyllum*, *S. elaeagnifolium*. QTL name, chromosome, position (Mb) their genomic location, and LOD score.

ABs	Trait	QTL	Chr.	Position (Mb.)	LOD Score
ABs *S. insanum*	*Plant traits*				
	Flavonol leaf content (P-Flav)	*fv-9*	9	19.6-27.0	3.89
	Nitrogen Balanced Index (P-NBI)	*nb-9*	9	19.6-27.0	4.35
	Fruit traits				
	Fruit mean weight (F-Weight)	*fw-9*	9	19.6-27.0	3.6
	Composition traits				
	Nitrogen content in leaf (N-Leaf)	*nl-9*	9	19.6-27.0	3.6
	Nitrogen content in stem (N-Stem)	*ns-9*	9	19.6-27.0	3.23
ABs *S. dasyphyllum*	*Plant traits*				
	Chlorophyll leaf content (P-Chl)	*ch-2*	2	78.5-83.3	3.38
	Flavonol leaf content (P-Flav)	*fl-1*	1	130.6-134.3	3.37
	Nitrogen Balanced Index (P-NBI)	*nb-1*	1	130.6-134.3	2.94
	Aerial biomass (P-Biomass)	*bi-2*	2	78.5-83.3	3.47
	Stem diameter (P-Diam)	*di-1*	1	130.6-134.3	5.23
	Yield	*yd-2*	2	78.5-83.3	3.26
	Fruit traits				
	Fruit pedicel length (F-PedLength)	*fp-2*	2	78.5-83.3	3.48
	Fruit mean weight (F-Weight)	*fd-2*	2	78.5-83.3	3.15
ABs *S. elaeagnifolium*	*Fruit traits*				
	Fruit calyx length (F-CaLength)	*fc-2*	2	78.4-83.2	3.92
	Fruit mean weight (F-Weight)	*fw-2*	2	78.4-83.2	3.44
	Composition traits				
	Carbon content in leaf (C-Leaf)	*cl-8*	8	94.0-101.4	3.11

4. Discussion

The utilization of populations with CWR introgressions, such as advanced backcrosses, enables the utilization of variation present in the CWRs, facilitating the development of crop varieties that are suitable for sustainable agriculture practices (Prohens et al., 2017). ABs populations increase the precision in detection of QTLs, as the different genotypes share a common genetic background that differs only in one or a few introgressed genomic fragments of the donor species (Villanueva et al., 2021). Effective fertilizer management is crucial for improving sustainability, with nitrogen use efficiency (NUE) being a critical breeding goal due to its significant impact on economic and environmental factors (Langholtz et al., 2021; Sutton et al., 2020; Zhang et al., 2015). Several studies have been conducted to improve NUE thought breeding in different crops, particularly in cereals and potato (van Bueren and Struik, 2017). However, until recently, few efforts have been made to address this issue in eggplant recent years (Mauceri et al., 2022, 2021, 2020; Rosa-Martínez et al., 2023; Villanueva et al., 2021).

In this study, three sets of advanced backcrosses (ABs) of eggplant wild relatives from different genepools, namely *S. insanum* (GP1), *S. dasyphyllum* (GP2) and S. *elaeagnifolium* (GP3) were evaluated under the same conditions for the first time. The availability of eggplant ABs with introgressions in different chromosomes provided an overview of the potential of wild species for breeding when evaluated under low nitrogen input abiotic stress conditions. In addition, recurrent parental lines of *S. melongena* (MEL5, MEL1 and MEL3) were tested under low and normal N input, providing insights into the effect of N in the different traits in cultivated eggplant.

Previous studies evaluated nitrogen use efficiency (NUE) by conducting hydroponic culture in different Solanaceae species, including tomato (Abenavoli et al., 2016b; Aci et al., 2021), potato (Sharifi et al., 2007; Xie et al., 2018) and eggplant (Mauceri et al., 2022, 2021). Other works investigated NUE using soil cultivation methods (Aminifard et al., 2010; Du et al., 2019; Souza et al., 2019; Villanueva et al., 2021), or combining different abiotic stresses (Badr et al., 2012; Henrique et al., 2018; Zhang et al., 2014). Also, different approaches have been documented for the evaluation of NUE (Sharma and Bali, 2017). The use of pots and the automatic N fertilization system employed here allowed for more controlled conditions to evaluate the impact on different traits under low nitrogen input. This approach allows greater control of the experimental conditions, as all plants are subjected to the same fertilization and substrate, that soil cultivation.

The study revealed significant differences in plant and composition traits of *S. melongena* recurrent parents cultivated under different N treatments. Generally, plants grown under normal N conditions showed higher values in chlorophyll content and lower values in flavonol and anthocyanin content. These traits, which have been reported to correlate with nitrogen content in plant leaves, can be effectively measured using proximal optical sensors for nitrogen, thereby enabling optimized management of vegetable crop cultivation (Padilla et al., 2018; Thompson et al., 2017). The results also indicated that plants grown under normal N conditions displayed higher values for the studied traits such as aerial biomass, stem diameter, yield, total number of fruits per plant, and nitrogen and carbon content in plant and fruits. These findings are consistent with previous reports that demonstrated the impact of different nitrogen fertilization treatments on eggplant (Mauceri et al., 2020; Rosa-Martínez et al., 2023; Ulas et al., 2018). In contrast to Mauceri et al.

(2020), the LN treatment resulted in much higher NUE, NupE and NUtE values than under NN. Similar results were found by Rosa-Martínez et al. (2023) suggesting that established fertilization practices may not always be the most efficient or sustainable approach to eggplant cultivation. Instead, carefully managed fertigation with reduced nitrogen inputs can improve NUE, resulting in higher yield per unit of N fertilization applied. Moreover, no significant differences were observed for most traits between each set of ABs and their respective recurrent parents under low N conditions. However, the wider distribution ranges for some traits observed within sets of ABs, which is in agreement with Villanueva et al. (2021), suggests that these materials may be of interest for enhancing the overall performance and variability of eggplant. The set of ABs used in this study do not cover the entire genome of wild eggplant relatives, indicating that further investigation into unexplored regions could result in significant findings for eggplant breeding under low nitrogen conditions.

The results of the PCA analysis revealed a relatively wide distribution of the advanced backcrosses in the PCA plot. Despite the general trend of ABs genotypes with lower recovery percentages being distributed separately from the recurrent parents, it is also observed that some accessions with high recovery percentages are distributed separately while others with low recovery percentages are closely located to the recurrent parents. This suggests that, in general, a high recovery of the recurrent genome background in advanced backcrosses is needed to have a general phenotype similar to the recurrent parent, although there are exceptions that may have great interest for breeding.

Breeding programs can benefit from correlations observed among similar traits in each set of ABs as these can help in predicting the phenotype of specific traits, requiring the assessment of fewer traits. Correlations established between traits evaluated by leaf clip meter Dualex® are consistent with results in previous studies (Cerovic et al., 2015; Liu et al., 2021). Similarly, associations identified between traits linked to plant vigor, such as plant biomass and stem diameter, and those related to yield, NUE and number of fruits per plant, as well as those associated with fruit morphology, are in agreement with several studies (Mangino et al., 2021; Patel et al., 2017; Rosa-Martínez et al., 2023; Villanueva et al., 2021). Notably, some relevant correlations related to nitrogen content in plant were observed, including negative correlations between nitrogen content and flavonol and anthocyanin content in leaves, as well as positive intercorrelation between nitrogen content in leaves and stem. In addition, differences in correlations among NUE, NUpE and NUtE, and other traits, such as fruit calyx length or those related to plant vigor were, found between sets of ABs.

The identification of 16 putative significant QTLs across three different sets of ABs demonstrates the potential of genetic variation present in wild eggplant relatives. QTLs located on chromosome 2 for *S. dasyphyllum* and *S. elaeagnifolium*, associated with plant growth, yield and fruit size parameters, may suggest the presence of genetic linkage or a pleiotropic locus, which is consistent with results reported in previous studies (Gaccione et al., 2023; Portis et al., 2014; Rosa-Martínez et al., 2023; Wei et al., 2020a). Furthermore, QTLs detected on chromosomes 2 and 9 for fruit pedicel and calyx length, as well as fruit weight, have also been identified in different collections and eggplant populations in earlier studies (Doganlar et al., 2002; Frary et al., 2014; Mangino et al., 2020; Portis et al., 2014; Rosa-Martínez et al., 2023; Wei et al., 2020a). For traits measured with the DUALEX® optical leaf clip meter, including chlorophyll, flavonol and NBI, novel QTLs in eggplant were identified on chromosomes 1 and 2 for *S. dasyphyllum*, and chromosome 9 for *S. insanum*. Additionally, for composition traits, a novel QTL for carbon content in leaf was found on chromosome 8 in ABs of *S. elaeagnifolium*, while two novel QTLs for nitrogen content in leaf and stem were located on chromosome 9 in ABs of *S. insanum*. In comparison, Rosa-Martínez (2023) reported QTLs for carbon leaf content on chromosomes 1, 5 and 10, and for leaf nitrogen content on chromosomes 4 and 9 in *S. melongena* introgression lines (ILs) with eggplant wild relative *S. incanum* as the donor parent. Interestingly, both studies detected QTLs associated with leaf nitrogen content on chromosome 9, suggesting a possible common underlying genetic factors.

In the identified QTL regions associated with plant growth, yield, fruit size and nitrogen-related parameters in ABs of *S. insanum*, *S. dasyphyllum*, and *S. elaeagnifolium*, several potential candidate genes have been detected. On chromosomes 2 and 9, genes encoding NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (NRT1/PRT) FAMILY (NPF) proteins (SMEL_002g164340; SMEL_009g328470) were identified. These NPF proteins are involved in nitrate uptake and transport of various substrates in plants, contributing to diverse biological processes (Chen et al., 2021; Léran et al., 2014). Consequently, they may potentially influence nitrogen content and other nitrogen-related parameters in plants. Furthermore, on chromosome 2, a candidate gene (SMEL_002g164700) was identified, which encodes a PIN-FORMED (PIN) 8 auxin efflux transporter. This transporter is crucial for auxin distribution and affects a wide range of developmental processes in plants (Zhou and Luo, 2018). In the same chromosomal region, another candidate gene (SMEL_002g167520) was identified, encoding a Myb-related protein 306 (MYB306), This protein is a transcription factor (TF) involved in anthocyanin regulation (Wang et al., 2022) and has been suggested to potentially influence both anthocyanin accumulation and fruit size in eggplant (Li et al., 2023).

5. Conclusions

This study highlights the potential of wild eggplant relatives for breeding under low nitrogen conditions by evaluating three sets of advanced backcrosses (ABs) and their recurrent parental lines. The findings reveal significant differences in plant, fruit, and composition traits in response to different nitrogen levels. Furthermore, we observed notable phenotypic variation among the ABs lines under low nitrogen fertilization, revealing the potential of introgression materials for genetic improvements in eggplant. The availability of genotyped lines with genetic variation allowed for the identification of putative QTLs. These insights may contribute to the development of breeding strategies aimed at improving eggplant productivity, quality, and nitrogen use efficiency under low nitrogen conditions, supporting sustainable agriculture practices.

Data availability statement: The data presented in this study are available on request from the corresponding author.

Author contributions: Conceptualization, P.G., J.P. and S.V.; methodology, G.V., M.P. and R.-D.M.; software, G.V. and S.V.; validation, M.P., P.G., J.P. and S.V.; formal analysis, G.V. and R.-D.M.; investigation, G.V., M.P., P.G.; resources, M.P., S. V. and J.P.; data curation, G.V., P.G. and S.V.; writing—original draft, G.V.; writing—review and editing, M.P., P.G., J.P. and S.V.; visualization, G.V., R.-D.M.; and S.V.; supervision, M.P., P.G., J.P. and S.V.; project administration, M.P. and J.P.; funding acquisition, J.P. All authors have read and agreed to the published version of the manuscript.

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Supplementary Table 1. Mean values and standard error (SE) of coconut fiber substrate chemical composition used in the evaluation under low (LN) or normal (NN) of eggplant lines of *S. melongena* MEL5, MEL1 and MEL3 and its advanced backcrosses (ABs) with *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium*.

Supplementary Table 2. Chemical composition of intake irrigation water.

Supplementary Table 3. Correlation coefficients between traits evaluated and the two first principal components (PC1 and PC2) of the PCA of of advanced backcrosses (ABs) of lines of *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium* and its corresponding recurrent parents.

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Research article

Validation and identification of new QTLs for plant and fruit developmental and composition traits in eggplant under low N conditions

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Abstract

Enhancing plant adaptation to low input conditions is a fundamental goal for implementing sustainable agriculture. In the present study, two eggplant (*Solanum melongena*) accessions (MEL1 and MEL5), two introgression lines (ILs) derived from eggplant wild relatives *S. dasyphyllum* (IL-M1-D1) and *S. insanum* (IL-M5-I9), and a heterozygous version of this last IL (IL^{Het}-M5-I9), along with hybrids among them were evaluated under low N (LN) conditions. IL-M1-D1 carries an introgressed fragment of 4.9 Mb in homozygosis from *S. dasyphyllum* on chromosome 2, while IL-M5-I9 and IL^{Het}-M5-I9 carry an introgression of 21.5 Mb on chromosome 9 in homozygosis and heterozygosis, respectively, from *S. insanum*. Multiple quantitative trait loci (QTLs) for several traits of interest were associated with both introgressions under LN conditions in a previous study with segregating advanced backcrosses. Here we evaluated the performance of these materials for 22 agronomic and developmental traits under low N fertilization (LN) conditions. Hybrids with the ILs enabled the study of genetic background effects on QTLs expression. The materials evaluated showed a significant phenotypic variation, particularly within hybrids segregating for the introgression from *S. insanum* in chromosome 9. Statistical analysis revealed no significant differences among hybrids carrying or not the introgression on chromosome 2 of *S. dasyphyllum*, and only slight differences were observed between the IL-M1-D1 and its recurrent parent *S. melongena* MEL1, suggesting a limited impact of this introgression on chromosome 2 on the phenotype variation. However, the differences observed between IL-M5-I9 and its recurrent parent *S. melongena* MEL5, together with the association between genotypic and phenotypic variation in hybrids segregating for this introgression, allowed the identification of 13 QTLs on chromosome 9. These results successfully validated the previously identified QTLs for flavonol content in leaves, nitrogen balanced index, fruit mean weight, and nitrogen content in leaves and, also revealed nine new QTLs associated with the introgressed genomic region in chromosome 9. This study emphasizes the influence of environmental conditions, genotypes, and genetic backgrounds on the phenotypic expression of eggplant QTLs introgressed from wild relatives and highlights the importance of QTL validation. These findings contribute valuable insights for developing new eggplant cultivars for a more sustainable agriculture, particularly with adaptation to LN conditions.

Keywords: *Solanum melongena*; N fertilization; QTL validation; *S. dasyphyllum*; *S. insanum*; segregating hybrids.

1. Introduction

In recent years, the impact of climate change has led to increased abiotic stress conditions such as drought, salinity, extreme temperatures, and nutrient deficiencies. These factors are limiting the use of arable land globally and negatively affect crop productivity (Pascual et al., 2022). Consequently, finding new strategies to enhance sustainable crop production and resilience has become a main objective in plant breeding (Bailey-Serres et al., 2019; Zhang et al., 2022). While nitrogen (N) fertilization is commonly used to improve crop yields, its overuse and inefficiency can increase production costs and cause considerable environmental damage (Stevens, 2019).

Eggplant (*Solanum melongena* L.), also known as brinjal or aubergine, ranks as the second most important vegetable crop in global production after tomato within the Solanaceae family (FAO, 2022). Cultivated eggplant exhibits a large diversity of morpho-agronomic traits, which is highly valuable for breeding purposes (Taher et al., 2017). However, there is a limitation in its diversity within the cultivated genepool, especially in traits for adaptation to climate change (Plazas et al., 2019). Crop wild relatives (CWRs) of eggplant, comprising over 500 species of *Solanum* subgenus *Leptostemonum* (Knapp et al., 2019) across its primary, secondary and tertiary genepools, represent a valuable source of plant genetic resources adapted to diverse stressful environments (Dempewolf et al., 2017; Prohens et al., 2017). This extensive genetic diversity in eggplant wild relatives is fundamental in creating new genetic resources and developing improved varieties for a more sustainable agriculture in a climate change scenario (Gramazio et al., 2023; Plazas et al., 2020; Toppino et al., 2022). Belonging to the primary genepool (GP1), *S. insanum* L. is considered the wild progenitor of the common eggplant (*S. melongena*) (Ranil et al., 2017). *Solanum dasyphyllum* Schumach. and Thonn. is considered the wild ancestor of the African gboma eggplant (*S. macrocarpon* L.) and it belongs to the secondary genepool (GP2) of eggplant (Taher et al., 2017; Vorontsova and Knapp, 2016). Both wild species display adaptability to various environmental conditions and have been reported to display tolerance to abiotic stresses such as drought and salinity (Kouassi et al., 2020; Ortega-Albero et al., 2023; Villanueva et al., 2023b).

Recent advances in eggplant genomics have been marked by a notable increase in the development of high-quality genome assemblies (Barchi et al., 2021, 2019b; Dandan Li et al., 2021; Wei et al., 2020a). Additionally, the development of high-throughput genotyping platforms (Barchi et al., 2019a) has enabled extensive genotyping of both eggplant and its wild relatives. This has facilitated the development of interspecific hybrids, advanced backcrosses (ABs), introgression lines (ILs), and MAGIC populations in eggplant breeding (García-Fortea et al., 2019; Gramazio et al., 2017; Kouassi et al., 2016; Mangino et al., 2022; Plazas et al., 2016b; Villanueva et al., 2021). In this way, the association of phenotypic and genotypic variation in eggplant has been crucial in identifying quantitative trait loci (QTLs) and candidate genes associated with a broad range of traits (Arrones et al., 2022; Mangino et al., 2021; Portis et al., 2014; Rosa-Martínez et al., 2023; Sulli et al., 2021; Toppino et al., 2020; Villanueva et al., 2023a).

In a previous study (Villanueva et al., 2023a), QTLs associated with flavonol content in leaves, nitrogen balanced index, fruit mean weight, and nitrogen content in leaves and stem (*fv-9*, *nb-9*, *fw-9*, *nl-9* and *ns-9*) were identified on a genomic region of chromosome 9 (7.4 Mb) in advanced backcrosses (ABs) derived from *S. insanum*. Additionally, another genomic region was detected on chromosome 2 (4.8 Mb) in ABs of *S. dasyphyllum*, carrying QTLs for chlorophyll leaf content, aerial plant biomass and yield (*ch-2*, *bi-2* and *yd-2*). This current study aimed to validate these QTLs previously identified in different genetic backgrounds, discover new QTLs of interest for eggplant breeding, and assess the potential for combining the QTLs present in both introgressions. For this purpose, we evaluated two *S. melongena* accessions (MEL1 and MEL5), two introgression lines (IL-M1-D2 and IL-M5-I9) derived from eggplant wild relatives *S. dasyphyllum* and *S. insanum* carrying alternative alleles for the QTLs, and segregating hybrids resulting from crosses of both introgressions. The results will allow us to assess the impact of genetic background and environmental factors on these traits and facilitate the development of N-efficient eggplant varieties.

2. Materials and methods

2.1. Plant material and growing conditions

For the study, two accessions of *S. melongena* (MEL1 and MEL5) were assessed, along with two introgression lines (IL-M1-D2 and IL-M5-I9) derived from MEL1 and MEL5, respectively. The IL-M1-D2 line, derived from *S. melongena* (MEL1), carries an introgressed fragment on chromosome 2 (6%; 4.9 MB) from the wild relative *S. dasyphyllum* (accession DAS1) with associated QTLs for chlorophyll content in leaves, aerial plant biomass, and yield (Villanueva et al., 2023a). On the other hand, the IL-M5-I9 was derived from *S. melongena* (MEL5) with an introgressed fragment on chromosome 9 (60%; 21.5 MB) from the wild relative *S. insanum* (accession INS1), containing QTLs for flavonol content in leaves, nitrogen balanced index, fruit mean weight, and nitrogen content in leaves and stem. Additionally, a version of IL-M5-I9 heterozygous for the introgression of the genomic region of chromosome 9 of *S. insanum* (IL$^{\mathrm{Het}}$-M5-I9) was used to obtain hybrids segregating for the introgression of *S. insanum* after crossing it with MEL1 and IL-M1-D2 (MEL1 X IL^{Het}-M5-I9 and IL-M1-D2 X IL^{Het}-M5-I9). The genetic background of these hybrids was characterized by heterozygosity for polymorphic loci between MEL1 and MEL5, as well as for segregating for the introgression of chromosome 9 of *S. insanum* (Figure 1). In total, the plant material consisted of 12 plants each of MEL1, MEL5, and IL-M1-D2, 11 plants of IL-M5-I9, 19 plants of segregating hybrids for the chromosome 9 introgression of *S. insanum* from MEL1 X IL^{Het}-M5-I9, and 20 plants of segregating hybrids for the chromosome 9 introgression of *S. insanum* from IL-M1-D2 X IL^{Het}-M5-I9.

Plants were grown in an open field located in Universitat Politècnica de València (GPS coordinates: latitude, 39° 28' 55” N; longitude, 0° 20' 11” W; 7 m a.s.l.) during the summer season (July to October) of 2022. The experimental design involved the random distribution of plants in the field. Plants were grown in 17 L pots filled with coconut fiber. Irrigation and fertilization were applied using a drip irrigation system, under low nitrogen (LN) conditions.

The fertilization solutions were prepared based on the substrate composition and intake water analyses as detailed in Villanueva et al. (2023a). The LN fertilization solution was prepared by adding 1.5 mM H_3PO_4 (Antonio Tarazona SL., Valencia, Spain), 4.85 mM K_2SO_4 (Antonio Tarazona SL.), 0.58 mM $MgSO_4$ (Antonio Tarazona SL.) plus 0.025 L/m^3 of a microelements Welgro Hydroponic fertilizer (Química Massó S.A., Barcelona, Spain) containing boron (BO_3^{3-}; 0.65% p/v), copper (Cu-EDTA; 0.17% p/v), iron (Fe-DTPA; 3.00% p/v), manganese (Mn-EDTA, 1.87% p/v), molybdenum (MoO_4^{2-}; 0.15% p/v), and zinc (Zn-EDTA; 1.25% p/v). The pH of the irrigation solution was adjusted to 5.5–5.8 with 23% HCl (Julio Ortega SL., Valencia, Spain).

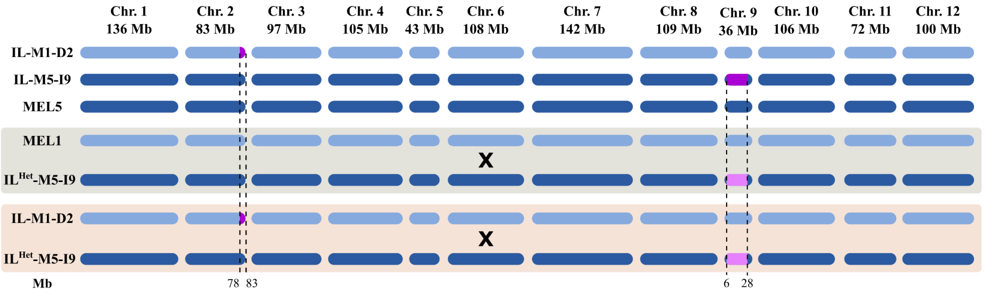

Figure 1. Graphical genotypes of introgression lines IL-M1-D2, IL-M5-I9, and MEL5, along with crosses MEL1 X IL^{Het}-M5-I9 and IL-M1-D2 X IL^{Het}-M5-I9. Columns represent the eggplant chromosomes and their respective length (Mb). Homozygous introgressions are colored in purple, heterozygous introgression in pink, and the genetic background of *S. melongena* MEL1 is colored in light blue, and *S. melongena* MEL5 in dark blue.

2.2. Genotyping

Genomic DNA extraction was performed for each individual plant using the SILEX DNA extraction method (Vilanova et al., 2020). High-throughput genotyping was conducted using the eggplant 5k probes single primer enrichment technology (SPET) platform (Barchi et al., 2019a). Single nucleotide polymorphisms (SNPs) were selected by filtering discriminant SNPs between the parental lines of introgression lines. Subsequently, a set of 824 SNPs were identified using TASSEL software (version 5.2.92; Bradbury et al., 2007).

2.3. Traits evaluation

A total of 22 plant, fruit and composition traits were evaluated. Chlorophyll, flavonol, anthocyanin contents, and nitrogen balanced index (NBI) were measured in leaves using a DUALEX® optical leaf clip meter (Force-A, Orsay, France). The dataset was obtained as the mean of 10 measurements of five leaves including upper and lower sides of each plant. At the end of the trial, aerial biomass was measured using a Sauter FK-250 dynamometer (Sauter, Balingen, Germany), and stem diameter was determined with a caliper at the base of the stem. Leaves and stems were separated and ground after drying at room temperature. The constant dry weight was measured after drying in an oven at 70ºC. Yield was determined by harvesting the total number of fruits from each plant. Nitrogen uptake efficiency (NUpE) was calculated by dividing the total N content in plant and fruit by the total N supplied with the irrigation solution per plant. Nitrogen utilization efficiency (NUtE) was calculated as total dry yield divided by the total N content in plant and fruit. Nitrogen use efficiency (NUE) was determined by multiplying NUpE with NutE (Anas et al., 2020; Han et al., 2015).

Fruit traits, including pedicel length, calyx length, fruit length, and width, were measured in fruits harvested at the commercially mature stage using a caliper. Data were calculated as the mean measurements obtained of at least three fruits per plant.

For the determination of nitrogen (N) and carbon (C) content, a minimum of five fruits per plant were harvested, frozen, and subsequently lyophilized. Analysis of N content in the dry powder from leaves, stem, and fruits was performed using the Dumas method with a TruSpec CN elemental analyzer (Leco, MI, USA). The measurement of carbon (C) content involved calculating carbon dioxide (CO2) using an infrared detector (Gazulla et al., 2012). The quantification process was performed with certified reference standards of different nitrogen and carbon concentrations with certified reference standards of different N and C concentrations.

2.4. Statistical analysis

For each trait, mean and range values were calculated for each parent, IL or segregating hybrid. To evaluate significant differences among recurrent parents, ILs and segregating hybrids, an analysis of variance (ANOVA) was performed. Significant differences were detected with the Student-Newman-Keuls multiple range test at a significance level of $P < 0.05$ using Statgraphics Centurion XIX software (Statgraphics Technologies, Inc., The Plains, VA, USA).

Principal component analysis (PCA) and partial least squares - discriminant analysis (PLS-DA) were performed for individual plant data using the package *ropls* (Thévenot et al., 2015) of the R software (R Core Team, 2021). The resulting PCA and PLS-DA plots were generated utilizing the *ggplot2* package (Wickham, 2016). PCA and PLS-DA are complementary multivariate techniques, in which PCA is a dimensionality reduction method based on maximizing the explanation of variance in the original variables, whereas PLS-DA is designed to reduce dimensionality by optimizing covariance between predictor matrix and a response matrix (Lee et al., 2018). In this approach, the PLS-DA was grounded in a predetermined group classification for each of the plant materials used, including MEL1, MEL5, IL-M1-D2, IL-M5-I9, MEL1 X IL^{Het}-M5-I9, and IL-M1-D2 X IL^{Het}-M5-I9.

2.5. Quantitative trait loci (QTL) detection

Identification of significant QTLs was performed using the single QTL model for genome-wide scanning with the R package *R/qtl* (Broman et al., 2003) of R statistical software (R Core Team, 2021). The threshold for significance in the logarithm of odds (LOD) score was established at a probability level of 0.01 for the significant QTLs. For each putative QTL detected, allelic effects were calculated by establishing differences between the means values of each genotype.

3. Results

3.1. Characterization of phenotypic traits

Significant differences among the different plant materials were found for all traits except for anthocyanin content in leaves (P-Anth), aerial plant biomass (P-Biomass), yield and NUE-related traits (NUE, NUpE and NUtE) (Table 1). No significant differences were observed between both hybrids derived from two crosses (MEL1 X IL^{Het}-M5-I9 and IL-M1-D2 X IL^{Het}-M5-I9) for any of the traits.

For plant traits, significantly lower chlorophyll content in leaves (P-Chl) and higher stem diameter (P-Diam) were observed in both hybrids in contrast to other parents and ILs, indicating negative heterosis for P-Chl and positive overdominance for P-Diam resulting from the cross between MEL1 and MEL5 genetic backgrounds. Additionally, the IL of *S. insanum* (IL-M5-I9) showed significantly lower flavonol content in leaves (P-Flav) than the rest of materials and the highest nitrogen balanced index (P-NBI) (Table 1).

In terms of fruit traits related to shape and size (F-PedLength, F-CaLength, F-Length and F-Width), the parental line *S. melongena* MEL1 displayed significantly larger fruits, characterized by longer pedicels, calyx lengths, and greater fruit length and width, in comparison to the other accessions (Table 1). Conversely, the IL-M5-I9 introgression line exhibited significantly lower values for these four traits. Both segregating hybrids showed a significantly higher fruit pedicel length (F-PedLength) together with *S. melongena* MEL1 and a significantly lower fruit length (F-Length) along with IL-M5-I9 in contrast to other accessions. Furthermore, *S. melongena* MEL1 and IL-M1-D2 presented the significantly lowest number of fruits per plant (F-Number), while IL-M5-INS9 showed the highest number of fruits. Inversely, *S. melongena* MEL1 presented the highest mean fruit weight (F-Weight), and IL-M5-I9 displayed the lowest (Table 1).

Regarding composition traits, the accession IL-M5-I9 exhibited the significantly highest content of nitrogen (N) in leaves and fruits (N-Leaf and N-Fruit). On the contrary, *S. melongena* MEL1 and IL-M1-D2 showed the significantly lowest N content in fruits. Additionally, for carbon content in leaves (C-Leaf), both segregating hybrids, along with *S. melongena* MEL5, displayed significantly lower values, and for carbon content in stems (C-Stem), the segregating hybrids, along with IL-M1-D2, demonstrated significantly lower values compared to the other accessions (Table 1). The IL-M5-I9 displayed the significant highest carbon content in stem (C-Stem).

Overall, segregating hybrids derived from the IL-M1-D2 X IL^{Het}-M5-I9 cross presented the widest ranges in most traits. Conversely, parental lines of *S. melongena* MEL1 and MEL5 displayed the narrowest ranges (Table 1).

Table 1. Mean values and range of plant, fruit and composition traits evaluated for *S. melongena* MEL1 (n=12), MEL5 (n=12), ILs of *S. dasyphyllum* IL-M1-D2 (n=12), ILs of *S. insanum* IL-M5-I9 (n=11), and segregating hybrids MEL1 X IL^{Het}-M5-I9 (n=19) and IL-M1-D2 X IL^{Het}-M5-I9 (n=20). For each trait, means with different letters are significant according to the Student–Newman–Keuls multiple range test (P<0.05).

Traits	**MEL1**	**MEL5**	**IL-M1-D2**	**IL-M5-I9**	**MEL1 X IL^{Het}-M5-I9**	**IL-M1-D2 X IL^{Het}-M5-I9**
Plant traits						
P-Chl	28.3 b (25.4-32.8)	31.9 c (28.2-36.9)	30.5 c (25.7-33.0)	32.1 c (28.1-35.9)	25.3 a (21.3-29.8)	25.6 a (21.7-33.0)
P-Flav	2.6 c (2.4-2.8)	2.4 b (2.2-2.6)	2.6 bc (1.5-2.8)	2.2 a (2.0-2.4)	2.6 bc (2.3-2.8)	2.6 bc (2.2-2.8)
P-Anth	0.3 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)	0.3 (0.3-0.4)
P-NBI	10.7 ab (9.0-12.6)	13.4 c (11.1-16.2)	11.6 b (9.6-15.6)	14.9 d (11.6-16.8)	9.9 a (7.7-12.9)	10.1 a (8.0-15.5)
P-Biomass	0.3 (0.2-0.4)	0.2 (0.2-0.3)	0.3 (0.2-0.4)	0.2 (0.1-0.3)	0.3 (0.2-0.6)	0.4 (0.2-1.3)
P-Diam	11.3 a (9.9-12.3)	11.0 a (10.1-11.9)	12.0 a (10.7-15.0)	12.2 a (11.1-13.1)	14.1 b (10.5-19.8)	14.5 b (10.5-24.4)
Yield	1160.2 (495-1709)	1086.7 (919-1355)	1182.7 (550-2072)	1095.6 (684-1410)	1188.1 (629-2269)	1256.2 (742-2126)
NUE	40.4 (16.3-62.1)	46.1 (36.9-55.5)	40.6 (19.8-73.1)	46.9 (30.6-62.3)	44.8 (24.2-86.0)	46.2 (26.1-83.2)
NUpE	1.1 (0.7-1.6)	1.3 (1.0-1.5)	1.2 (0.7-1.8)	1.4 (1.1-2.1)	1.3 (0.9-2.5)	1.5 (0.9-4.7)
NUtE	34.8 (22.8-40.8)	36.1 (31.5-39.8)	33.6 (23.1-41.0)	32.2 (27.4-36.4)	34.0 (26.9-37.7)	32.8 (13.3-37.3)
Fruit traits						
F-PedLength	41.7 c (38.6-45.3)	35.2 b (30.7-38.7)	35.9 b (29.0-40.6)	31.5 a (27.3-38.0)	40.8 c (35.9-46.7)	39.1 c (33.4-48.4)
F-CaLength	41.2 d (38.0-44.2)	26.3 a (23.1-29.3)	35.4 c (31.4-38.2)	25.8 a (22.2-27.8)	32.5 b (29.5-35.9)	32.2 b (27.5-38.1)
F-Length	86.8 b (68.8-96.9)	87.0 b (76.8-96.8)	83.9 b (70.2-97.3)	67.9 a (54.5-89.4)	64.4 a (54.7-78.1)	70.2 a (53.6-83.1)
F-Width	54.8 d (46.3-62.2)	40.5 b (37.9-45.6)	49.6 c (41.8-57.3)	36.8 a (32.7-40.8)	42.4 b (38.0-49.4)	42.1 b (35.4-46.6)
F-Number	20.3 a (9-25)	34.8 b (28-43)	25.2 a (12-34)	46.5 c (25-65)	33.8 b (18-55)	38.8 b (25-61)
F-Weight	57.6 d (42.1-68.5)	31.3 b (27.0-34.9)	46.9 c (32.6-62.8)	23.9 a (19.7-29.7)	35.0 b (28.4-41.3)	32.4 b (27.7-39.1)
Composition traits						
N-Leaf	2.13 a (1.7-3.0)	2.16 a (1.4-3.1)	2.25 a (1.8-3.2)	2.77 b (2.0-3.7)	1.85 a (1.4-2.2)	1.84 a (1.4-4.0)
C-Leaf	42.0 b (40.7-43.4)	40.1 a (38.7-43.6)	41.7 b (38.6-43.6)	41.5 b (40.0-44.5)	40.9 ab (37.9-42.8)	40.1 a (38.0-45.5)
N-Fruit	1.72 a (1.5-2.0)	1.86 b (1.7-2.0)	1.71 a (1.5-2.1)	2.07 c (1.7-2.3)	1.84 b (1.7-2.1)	1.88 b (1.7-2.1)
C-Fruit	42.1 ab (41.0-42.9)	43.5 c (42.2-44.3)	41.4 a (40.6-43.4)	44.6 d (42.1-45.8)	42.5 b (41.0-44.3)	42.8 b (41.5-43.9)
N-Stem	0.87 ab (0.8-1.0)	0.85 ab (0.7-0.9)	0.93 b (0.8-1.2)	0.93 b (0.8-1.0)	0.78 a (0.7-0.9)	0.80 a (0.6-1.4)
C-Stem	43.5 c (42.5-45.0)	41.8 b (40.8-42.4)	38.2 a (37.4-39.6)	42.3 b (36.9-44.1)	38.2 a (36.9-40.4)	38.1 a (36.8-39.5)

3.2. Principal component and partial least squares analyses

A principal component analysis (PCA) was conducted for all evaluated traits, with a principal component (PC) 1 and PC2 accounting for 27.1% and 21.0% of the total variation, respectively (Figure 2A). The distribution pattern in the PCA revealed that the PC1 was positively correlated while the PC2 was negatively correlated with nitrogen, and carbon content in fruit (N-Fruit and C-Fruit), nitrogen content in leaves (N-Leaf), chlorophyll content, and nitrogen balanced index in leaves (P-Chl and P-NBI), while. The first component was negatively correlated with leaf flavonol content (P-Flav), and traits related to fruit morphology and size (F-PedLength, F-CaLength, F-Length, F-Width and F-Weight). Regarding the individuals of the different plant materials, the IL-M5-I9 genotypes were predominantly located along the positive PC1 axis and negative PC2, while other genotypes generally displayed a wider distribution along the negative values of PC1 (Figure 2A).

The first two latent variables (LVs) of the partial least squares analysis (PLS-DA) accounted for 26.2% and 19.4% of the total variation across all traits (Figure 2B). Traits related to fruit characteristics displayed positive values along the LV1 and LV2. The first latent variable was negatively correlated with chlorophyll and nitrogen content in leaves (P-Chl and N-Leaf), and nitrogen balanced index (P-NBI). Additionally, flavonol and anthocyanin content in leaves (P-Flav and P-Anth), fruit pedicel length (F-PedLength), yield, and nitrogen utilization efficiency (NutE) had negative values for the LV1 axis and positive ones for the LV2. The distribution of the individuals in the PLS-DA plot revealed a distinct separation among the different materials. Specifically, the IL-M1-D2, along with the parental line *S. melongena* MEL1, mostly exhibited positive values along both LV1 and LV2. In contrast, IL-M5-I9 was predominantly positioned at negative values of LV1 and positive LV2 values, while *S. melongena* MEL5 was in its proximity. Hybrids resulting from two different crosses, MEL1 X IL^{Het}-M5-I9 and IL-M1-D2 X IL^{Het}-M5-I9, plotted very closely with generally positive values for the LV1 and negative ones for the LV2 (Figure 2B).

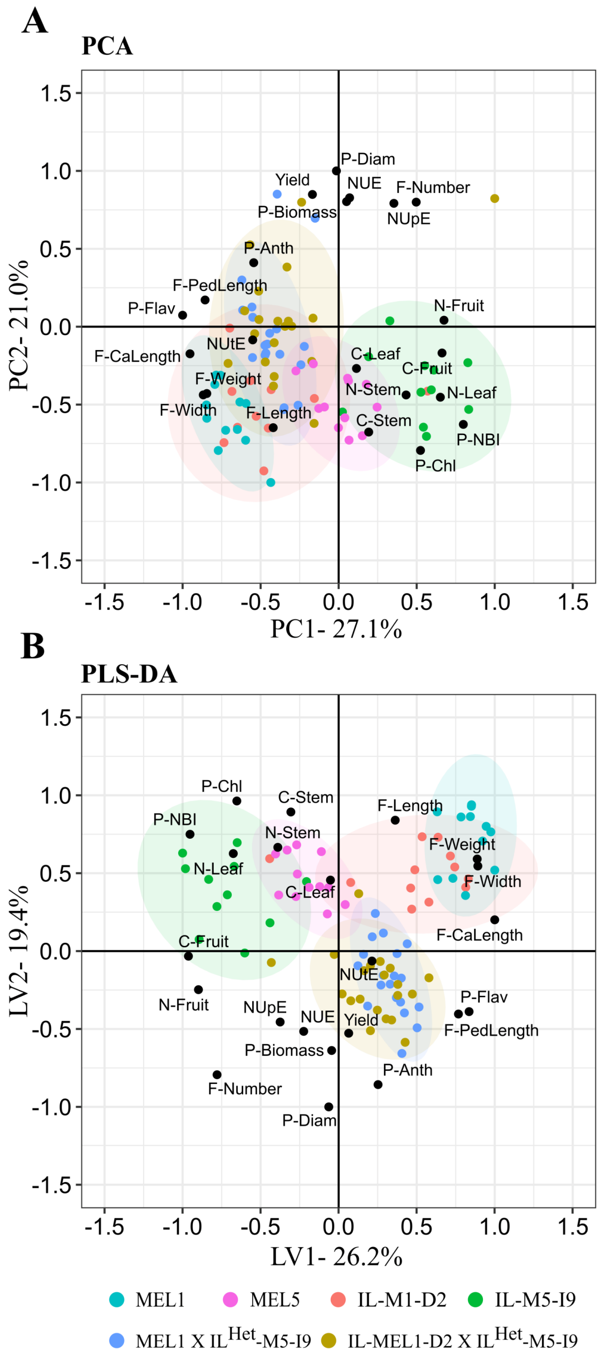

Figure 2. PCA (A) and PLS-DA (B) biplots based on the first two principal components (PC1 and PC2) for PCA, and the first two latent variables (LV1 and LV2) for PLS-DA, derived from the analysis of all traits.

3.3. Detection and effects of putative QTLs

Genotypic and phenotypic association analyses facilitated the identification of 13 putative QTLs, all located at the same position (6.43 and 27.90 Mb) on chromosome 9 (Table 2). Regarding plant traits, QTLs associated with leaf chlorophyll content (*ch-9*) and balanced nitrogen index (*nb-9*) showed a significant positive effect of the homozygous allele of *S. insanum* (10.86% and 30.27%, respectively) with a contrasting negative effect for the heterozygote (-14.97% and -17.18%, respectively). Conversely, the QTL detected for leaf flavonol content (*fv-9*) displayed a significant negative homozygous effect (-13.80%).

Regarding QTLs of fruit traits, a significant decrease in fruit pedicel and calyx length (F-PedLength and F-CaLength) was observed due to the effect of the homozygous allele from the wild species on QTLs *fp-9* and *fc*-9, respectively. Additionally, QTLs associated with fruit length (*fl-9*), width (*fd-9*) and fruit mean weight (*fw-9*), exhibited a significant negative effect for both homozygous and heterozygous alleles (Table2). The total number of fruits per plant-related QTL (*fn-9*) showed a significantly positive effect for both homozygous (56.36%) and heterozygous (26.42%) alleles, compared to the allele from the cultivated eggplant.

Nitrogen content in leaves and fruits (N-Leaf and N-Fruit), along with carbon content in fruits and stem (C-Fruit and C-Stem), were associated with QTLs *nl-9*, *nf-9*, *cf-9*, and *cs-9* respectively, demonstrating a significant positive effect of homozygous allele from the wild species (Table 2). However, QTLs *nl-9* and *cs-9* showed a negative significant heterozygous effect (-16.36% and -4.83%, respectively).

Table 2. Putative QTLs detected corresponding to an introgression between 6.43 and 27.90 Mb of chromosome 9 of *S. insanum* with QTL name, homozygous and heterozygous effects, and LOD scores.

Trait	QTL	Homozygous effect (%)	Homozygous effect (units)	Heterozygous effect (%)	Heterozygous effect (units)	LOD Score
Plant traits						
Chlorophyll leaf content (P-Chl)	*ch-9*	10,86*	3,14*	-14,97*	-4,33*	7.15
Flavonol leaf content (P-Flav)	*fv-9*	-13,80*	-0,35*	2.81	0.07	5.75
Nitrogen balanced index (P-NBI)	*nb-9*	30,27*	3,47*	-17,18*	-1,97*	9.62
Fruit traits						
Fruit pedicel length (F-PedLength)	*fp-9*	-18,42*	-7,11*	1.66	0.64	6.91
Fruit calyx length (F-CaLength)	*fc-9*	-24,1*	-8,2*	-7.52	-2.56	5.45
Fruit length (F-Length)	*fl-9*	-16.07*	-13,00*	-19.51*	-15.78*	5.72
Fruit width (F-width)	*fd-9*	-20.35*	-9.41*	-8.99*	-4.16*	5.09
Total number of fruits per plant (F-Number)	*fn-9*	56.36*	16.78*	26.42*	7.86*	3.86
Fruit mean weight (F-Weight)	*fw-9*	-42.37*	-17.58*	-20.46*	-8.49*	5.93
Composition traits						
Nitrogen content in leaf (N-Leaf)	*nl-9*	31.93*	0.67*	-16.36*	-0.34*	5.73
Nitrogen content in fruit (N-Fruit)	*nf-9*	15.37*	0.28*	3.98	0.07	5.60
Carbon content in fruit (C-Fruit)	*cf-9*	5.04*	2.14*	0.41	0.17	5.60
Carbon content in stem (C-Stem)	*cs-9*	5.45*	2.19*	-4.83*	-1.94*	4.74

* Indicates significant effects at P<0.05.

4. Discussion

The incorporation of CWR introgressions into populations with a cultivated background, such as ABs and ILs, enables the development of crop varieties with improved adaptive properties against abiotic stresses related to climate change (Prohens et al., 2017). Previous studies have made efforts to enhance understanding of N use in eggplant, aiming at developing new cultivars with improved N efficiency (Mauceri et al., 2020; Rosa-Martínez et al., 2023; Villanueva et al., 2021, 2023a). In this study, introgression lines derived from two eggplant CWRs, *S. dasyphyllum* and *S. insanum*, containing genomic regions potentially interesting for adaptation under low N conditions were evaluated together with segregating hybrids derived from them.

In a previous study, three sets of ABs of eggplant were evaluated under the same low N conditions (Villanueva et al., 2023a). The findings of that study revealed the identification of five QTLs located on a genomic region of chromosome 2 of ABs of *S. dasyphyllum* and an additional five QTLs on a genomic region of chromosome 9 of ABs of *S. insanum*. Traits associated with QTLs on chromosome 2 for ABs of *S. dasyphyllum* included chlorophyll leaf content, aerial plant biomass, yield, fruit pedicel length and fruit mean weight. For the QTLs located on chromosome 9 in *S. insanum* ABs, the associated traits include flavonol leaf content, nitrogen balanced index, fruit mean weight and nitrogen content in leaves and stems.

In our study, lines with introgressions at the same location on chromosomes 2 and 9 from *S. dasyphyllum* and *S. insanum* (IL-M1-D2 and IL-M5-I9), respectively, along with segregating hybrids derived from crosses between those lines and advanced backcrosses (ABs), cultivated under low N conditions allowed the evaluation and validation of previously detected QTLs, examining the impact of genetic background and different allele dosage. Our results revealed significant differences between the evaluated line sets, except for traits related to plant vigor and NUE parameters. Notably, both segregating hybrids resulting from two crosses (MEL1 X IL^{Het}-M5-I9 and IL-M1-D2 X IL^{Het}-M5-I9) exhibited no significant differences for any of the traits evaluated. This finding reveals that in our study the introgression from *S. dasyphyllum* on chromosome 2 did not manifest a discernible impact on the phenotypes of the segregating hybrids, revealing the importance of yearly differences in the expression of this QTL. However, within these segregating hybrids, a wider distribution ranges for all traits were observed, highlighting the potential of these materials to enhance variability in eggplant and assess the potential effects of genetic background. As occurred in other eggplant hybrids (Rehman et al., 2021), negative heterosis for specific traits was observed in segregating hybrids derived from crosses between the genetic backgrounds of *S. melongena* MEL1 and MEL5. The differences observed among materials suggest an increase in variation with respect to the MEL1 and MEL5 parents, emphasizing their potential significance for breeding.

The PCA and PLS analyses displayed a distinctive distribution pattern within the evaluated materials. Notably, a distinct grouping of individuals emerged from the IL of *S. insanum* (IL-M5-I9), differentiating it from other materials due to their association with traits related to high chlorophyll and nitrogen content. Conversely, individuals from the IL of *S. dasyphyllum* (IL-M1-D2) exhibited very slight differences with their corresponding parental line, *S. melongena* MEL1. In addition, both segregating hybrids were positioned close to each other. As stated before, these findings suggest a limited effect of the introgression of *S. dasyphyllum* on chromosome 2, with no significant differences detected.

The identification of 13 putative QTLs on a genomic region of chromosome 9 of *S. insanum* demonstrates the significant impact of this region on diverse traits. This finding allowed the validation of a set of QTLs for traits such as flavonol content in leaves, nitrogen balanced index, mean fruit weight, and leaf nitrogen content, as previously identified in Villanueva et al. (2023a) in segregating ABs. A new QTL was also identified for chlorophyll leaf content, a trait closely related to others (Cerovic et al., 2012). Also, QTLs associated with fruit size and morphology on chromosome 9 have been consistently found in various eggplant populations in different studies (Doganlar et al., 2002; Frary et al., 2014; Rosa-Martínez et al., 2023; Wei et al., 2020a). Another novel QTL was identified for the total number of fruits per plant in this region. Finally, novel QTLs were detected in chromosome 9 for nitrogen content in the fruit, as the only one detected so far was in introgression lines of *S. incanum* on chromosome 12 (Rosa-Martínez et al., 2023), and carbon content in fruit and stem. Our results provide further evidence that environmental conditions, their interaction with genotypes, and genetic background can profoundly influence the phenotypic expression and stability of eggplant QTLs (Mangino et al., 2020; Mistry et al., 2016). In this way, the validation of detected QTLs in multiple locations and populations becomes crucial, considering the importance of the conditions established in the study (Diouf et al., 2018; Frary et al., 2014).

5. Conclusions

The evaluation of introgression and segregating hybrids revealed significant phenotypic variations in the materials evaluated, suggesting the potential of these materials for eggplant breeding for more sustainable agriculture practices. Notably, we confirmed that a genomic region from chromosome 9 of *S. insanum* may have a large impact on the adaptation of eggplant to low N conditions, as it contains a large number of QTLs, some of which are novel and that can be of interest for eggplant breeding. In contrast, we could not validate a genomic region from chromosome 2 of *S. dasyphyllum* that had been associated with chlorophyll content in leaves, aerial plant biomass and yield. The findings highlight the importance of validating eggplant QTLs across diverse conditions and populations, employing techniques like evaluating the genomic regions under different backgrounds, and considering the complex interactions influencing quantitative trait expression, including environmental factors, genotypes, and genetic backgrounds.

Data availability statement: The data presented in this study are available on request from the corresponding author.

Author contributions: Conceptualization, S.V. and J.P.; methodology, P.G. and J.P.; software, G.V.; validation, S.V.; formal analysis, G.V. and S.V.; investigation, G.V., S.V., M.P., P.G. and J.P.; resources, J.P.; data curation, G.V.; writing—original draft, G.V.; writing—review and editing, G.V., S.V., M.P., P.G. and J.P.; visualization, S.V. and J.P.; supervision, S.V., M.P. and J.P.; project administration, M.P. and J.P.; funding acquisition, J.P. All authors have read and agreed to the published version of the manuscript.

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Chapter II:

Drought response mechanisms in eggplant and a wild relative

Research article

Transcriptome profiles of eggplant (*Solanum melongena*) and its wild relative *S. dasyphyllum* under different levels of osmotic stress provide insights into response mechanisms to drought

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G.V. had a main role in the following activities: formal analysis, investigation, data curation, drafting manuscript, manuscript review and editing.

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Abstract

Defence mechanisms to abiotic stresses, like drought, are very broad and RNA sequencing (RNA-Seq) can help in understanding the complex responses triggered. In this study, we performed RNA-Seq of the transcriptomes of eggplant (*Solanum melongena*) and its related wild species (*S. dasyphyllum*) under two PEG concentrations (20% and 30%), two different times (after 0.5 h and 2 h of osmotic stress) and at two plant phenological stages (three and five true fully developed leaves). *Solanum dasyphyllum* was more tolerant to osmotic stress, and a differential expression pattern of drought-related genes was identified between the two species. Plants subjected to a higher osmotic potential, at a more adult stage and at a higher stress exposure time displayed a higher number of DEGs (differential expressed genes). Gene ontology (GO) enrichment analysis revealed that, compared to *S. melongena*, *S. dasyphyllum* triggered the regulation of a wide range of transcription factors (*AP2/ERF*, DREB, bZIP, WRKY and bHLH). In both species, the abscisic acid (ABA) signaling response pathway played a crucial role leading to stomatal closure. Other important pathways involved in abiotic stresses tolerance including flavonoid, carotenoid and phenylpropanoid biosynthesis, chlorophyll metabolism and photosynthesis pathway among others were found to have a relevant role under both moderate and severe osmotic stresses. Our results reveal that *S. dasyphyllum* is a potential source of genes for breeding resilient eggplant varieties.

Keywords: transcriptome; RNA Seq; osmotic stress; drought; *Solanum melongena*; *S. dasyphyllum*.

1. Introduction

Drought spells occur naturally in many areas of the world, but climate change has accelerated and intensified them, with dramatic consequences on agriculture (Mukherjee et al., 2018). Projections indicate that the risk and severity of drought episodes will increase across the subtropics and mid-latitudes in both hemispheres as a consequence of global warming and decreased regional precipitation (Cook et al., 2018; IPCC, 2022). Drought stress triggers morphological, physiological, biochemical, cellular and molecular response mechanisms in plants with a potentially severe reduction in plant growth and crop production as a major consequence. Therefore, determining plant response and tolerance mechanisms against drought stress is fundamental to mitigating its effects (Hossain et al., 2016; Ilyas et al., 2021).

The development of new molecular and bioinformatics tools has allowed the expansion of applied knowledge in breeding programs. In this way, transcriptomics has provided new potential resources for studying the molecular response of abiotic stress in crops (Zhuang et al., 2014), being RNA sequencing (RNA-Seq) the general method of choice. This method allows a broad coverage of the transcriptome, providing a significant characterization of mRNA transcripts of specific tissue and time and, in addition, is a quantitative method that yields a digital gene expression atlas at a genomic scale (Kido et al., 2016).

Drought tolerance is a complex trait involving different components at the physiological, biochemical and genetic levels (Samarah, 2016). Osmotic stress, resulting in an increased difficulty for water uptake by the roots, is one of the most important factors in drought (Haswell and Verslues, 2015). To unravel the effects of water deficit in genetic networks, the use of a solution containing polyethylene glycol (PEG) in hydroponic culture is a common practice to induce osmotic stress and reduce the water potential of tissues in plants (Claeys and Inzé, 2013; Osmolovskaya et al., 2018). In this way, the transcriptome of PEG-treated plants provides information regarding drought-related genes, which can be primarily classified in protective and regulatory genes (Kido et al., 2016). Regarding the former, these are genes that encode LEA proteins, chaperones, osmoprotectants, water channels, ion exchangers, and enzymes involved in the osmolyte biosynthesis and the reactive oxygen species (ROS), among others (Chen et al., 2019; Riyazuddin et al., 2021). On the other hand, genes encoding regulatory proteins act on the expression of stress-responsive, including transcription factors, protein kinases and phosphatases, enzymes involved in phospholipid metabolism and abscisic acid (ABA) biosynthesis and epigenetic-related genes (Shinozaki and Yamaguchi-Shinozaki, 2007; Wang et al., 2021).

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expression of stress-responsive, including transcription factors, protein kinases and phosphatases, enzymes involved in phospholipid metabolism and abscisic acid (ABA) biosynthesis and epigenetic-related genes (Shinozaki and Yamaguchi-Shinozaki, 2007; Wang et al., 2021).

Crop wild relatives (CWRs) are an increasingly fundamental resource for plant breeding to improve the adaptative capacity of agricultural systems to climate change-related stresses (Prohens et al., 2017). Among vegetable crops, eggplant (*Solanum melongena* L.) can be highly benefited by introgression breeding, as many eggplant CWRs thrive in areas affected by moderate to severe drought (Vorontsova and Knapp, 2016). Eggplant is an important crop, being the eighth vegetable crop in terms of cultivated area in the world, being widely grown in Asia, Africa and Europe (FAO, 2021). It has been described as a relatively drought-tolerant crop and different degrees of drought tolerance have been observed in cultivated accessions and CRWs (Díaz-Pérez and Eaton, 2015; Kouassi et al., 2020; Plazas et al., 2019). Among these CWRs, *S. dasyphyllum* Schumach. and Thonn. grows naturally in areas where drought spells are frequent and it has been reported to exhibit significant drought tolerance both under field and experimental conditions (Kouassi et al., 2020; Plazas et al., 2022). It is considered the wild ancestor of the gboma eggplant (*S. macrocarpon* L.) (Bukenya and Carasco, 1994; Plazas et al., 2014a) and is classified in the Anguivi clade, which includes several African and Southeast Asian "prickly" species (Vorontsova et al., 2013; Vorontsova and Knapp, 2016). *Solanum dasyphyllum* is a member of the secondary genepool of eggplant (Syfert et al., 2016), and interspecific hybrids and advanced backcross materials of *S. dasyphyllum* with *S. melongena* have been obtained (Kouassi et al., 2016; Plazas et al., 2016b).

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In the present study, we analyzed the transcriptomes of a cultivated *S. melongena* and a drought-tolerant *S. dasyphyllum* accessions under PEG-induced osmotic stress in two different plant phenological stages and at two times for each phenological stage. By evaluating its physiological responses in conjunction with the analysis of the gene expression we aimed at a better comprehensive understanding of the different response mechanisms against osmotic stress in these materials. The results are of great interest for a better understanding of drought tolerance and to foster introgression breeding of drought-tolerant resilient cultivars in eggplant.

2. Materials and methods

2.1. Plant material and growth conditions

Solanum melongena MEL1 and *S. dasyphyllum* DAS1 accessions were used for the present study. Seeds were germinated according to Ranil et al. (2015) protocol for uniform eggplant CWRs germination and plants were grown in hydroponic culture according to Renau-Morata et al. (2016) with Hoagland solution (Hoagland and Arnon, 1950) in a growth chamber with a 16/8 h light/dark photoperiod, 25ºC temperature and 60-65% of humidity. The nutrient solution was resupplied every four days and an air compressor was used to supply aeration.

2.2. PEG-induced osmotic stress

To evaluate the effect of the plant phenological stage and the stress response, two osmotic stress experiments were conducted using PEG 6000 (Bio Basic Inc., Ontario, Canada). One experiment was performed with 20% PEG at a phenological stage of three fully developed true leaves (Ex_1), while the other with 30% PEG at the five fully developed true leaves stage (Ex_2). In each experiment, leaves of three biological replicates (i.e., three different plants uniformly developed, each one constituting a replicate and for each one a library was developed) were taken for each species at three times: 0 h (control; T0), 0.5 h (T0.5) and 2 h (T2) after initiation of the stress treatment. Immediately, leaf samples were frozen with liquid nitrogen and stored at -80ºC for RNA extraction. Plant symptoms were registered at different times of the treatments.

2.3. *RNA extraction, sequencing and data processing*

Total RNA was extracted from leaves samples of each biological replicate using TRIzol^{TM} Reagent (Invitrogen, Carlsbad, CA, USA). For each of the 36 replicates, the RNA library was performed by Novogene Co., LTD (Beijing, China) and sequenced on an Illumina NovaSeq 6000 (paired-end 150 bp). Raw data in FASTQ format were filtered by removing reads with adaptor contamination, reads containing N > 10% and low-quality reads (Qscore of over 50% bases below 5). Error rate (%), Q20 (%), Q30 (%) and GC content (%) were calculated for data quality control of clean data. Gene expression levels were estimated by calculating fragments per kilobase of transcript sequence per million of base pairs sequenced (FPKM). All raw data were deposited in the Short Read Archive (SRA) of the National Library of Medicine repository and the accession number is PRJNA939933 (https://www.ncbi.nlm.nih.gov/sra/PRJNA939933).

2.4. *Transcriptomic analysis*

Differentially expressed genes (DEGs) analysis was performed using the DEseq2 R package (Anders and Huber, 2010), and the resulting *p*-values were adjusted using Benjamini and Hochberg's correction for controlling the false discovery rate (FDR) (Benjamini and Hochberg, 1995). Genes with adjusted *p*-value < 0.05 and $|\log_2(\text{fold change})| > 1$ were considered as differentially expressed. DEGs were annotated based on the functional annotation information of genes of the eggplant reference genome "67/3" V3 (Barchi et al., 2019b). Venn diagrams of DEGs were displayed using jvenn, a plug-in for the jQuery JavaScript library (Bardou et al., 2014).

Hierarchical clustering analysis was carried out of $\log_2$ (FPKM+1) of union differential expression genes, within all comparison groups. Heatmaps were performed selecting drought-related DEGs, based on the scientific literature, which were classified according to their function into four groups: osmoprotectants, phytohormones, protein kinases and transcription factors using the web tool ClustVis (Metsalu and Vilo, 2015).

Gene ontology (GO, http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) enrichment analyses of the DEGs were performed. The tomato (*S. lycopersicum* L.) KEGG pathways annotated database was used for the analysis, being the closest species with more comprehensive and reliable information. GO and KEGG terms with an adjusted *p*-value < 0.05 were considered significantly enriched for the DEGs.

3. Results

3.1. Physiological responses to osmotic stress

As a general trend, in both experiments, *S. dasyphyllum* (DAS) displayed a better water stress tolerance than *S. melongena* (MEL). In Ex_1, DAS presented visual symptoms only at T2 while MEL started to show symptoms of stress at T0.5 (Figure 1). In Ex_2, manifestations of water stress in plants were observed at T0.5 and T2 in both species in a faster way with more severe symptoms compared with Ex_1, although DAS, again, exhibit more tolerance, with fewer symptoms of wilting (Figure 1).

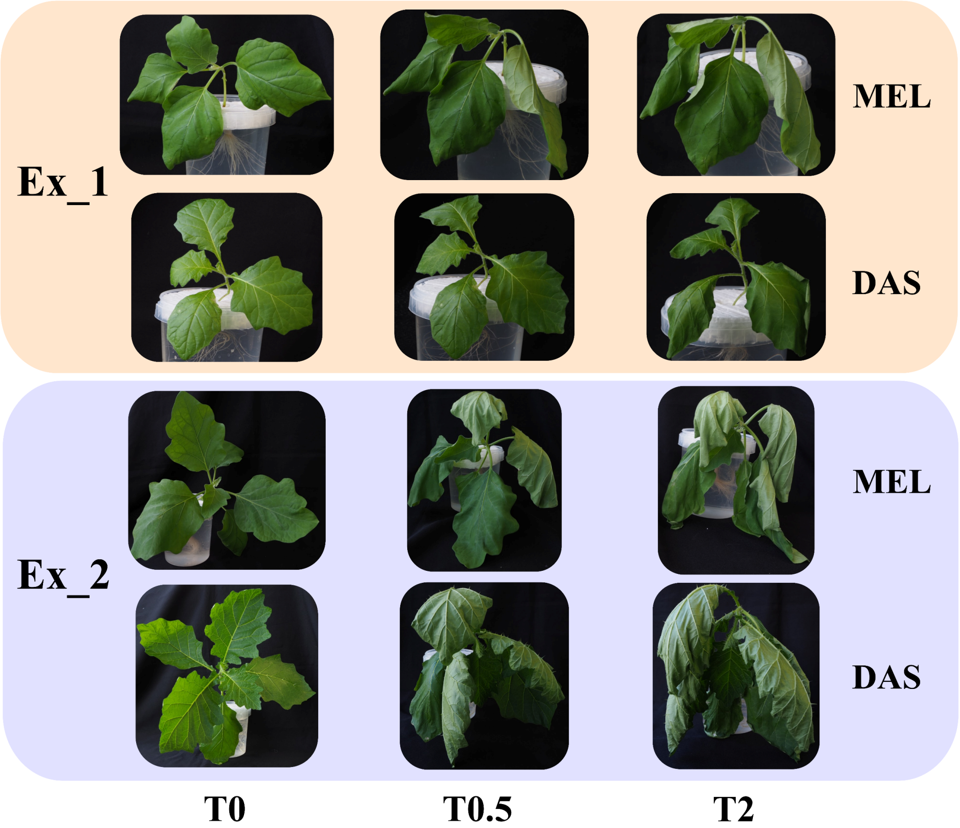

Figure 1. Representative phenotypes of *S. melongena* (MEL) and *S. dasyphyllum* (DAS) after 0, 0.5 and 2h of PEG stress in hydroponic culture in both experiments (Ex_1 and Ex_2).

3.2. *Differential gene expression over time in response to PEG treatment*

After filtering raw sequencing data, clean reads showed an error rate between 0.02% and 0.03%, an average Q30 of 93.85% and GC content of 43.17% (Table S1). For each experiment, DEGs with an adjusted *p*-value < 0.05 and a $|\log_2$ (fold change)$|$ > 1 were selected by performing pairwise comparisons at each time of PEG treatment (T0.5 and T2) with the non-stressed control (T0).

In Ex_1 (20% PEG and three fully developed true leaves stage), a total of 894 and 433 DEGs were detected for DAS and MEL, respectively. For DAS a total of 114 (74 up-regulated [UR], 40 down-regulated [DR] and 33 related to drought stress) and 840 DEGs (475 UR, 365 DR and 171 related to drought stress) were detected at T0.5 and T2, respectively (Table 1). For MEL, a total of 327 (273 UR, 54 DR and 89 related to drought stress) and 117 DEGs (76 UR, 41 DR and 24 related to drought stress) were detected at T0.5 and T2, respectively (Table 1). Venn diagram analysis showed that in DAS 52 DEGs were commonly regulated at T0.5 and T2 while 49 and 707 DEGs were specific at 0.5 and T2, respectively (Figure 2A). In MEL, seven DEGs were commonly regulated after both times of treatment, 273 and 67 DEGs at T0.5 and T2 respectively (Figure 2A).

In Ex_2 (30% PEG and five fully developed true leaves) a total of 2,037 and 4,375 DEGs were detected for DAS and MEL, respectively. For DAS, a total of 147 (109 UR 38 DR and 53 related to drought stress) and 1,999 DEGs (1,040 UR, 959 DR and 363 related to drought stress) were detected at T0.5 and T2, respectively (Table 1). For MEL, a total of 198 (134 UR, 64 DR and 62 related to drought stress) and 4,360 DEGs (2,252 UR, 2,108 DR and 774 related to drought stress) were detected at T0.5 and T2, respectively (Table 1). Venn diagram analysis showed that 31 and 80 DEGs were commonly regulated at T0.5 and T2 exclusively in DAS and MEL respectively (Figure 2B). A total of 20 and 1,065 DEGs were detected only in DAS at T0.5 and T2 respectively. In MEL, 11 and 3,363 DEGs were detected exclusively at T0.5 and T2 respectively. A total of 38 common DEGs were detected for both times and both accessions. (Figure 2B).

Table 1. Differentially expressed genes that were up-regulated or down-regulated after 0.5 h (T0.5) and 2 h (T2) of PEG stress in *S. dasyphyllum* (DAS) and *S. melongena* (MEL) in experiments 1 and 2 (Ex_1 and Ex_2).

Experiment	**Time comparison**	**Number of DEGs**		**Up-Regulated DEGs**		**Down-Regulated DEGs**		**Number of DEGs related to drought**	
		DAS	**MEL**	**DAS**	**MEL**	**DAS**	**MEL**	**DAS**	**MEL**
Ex_1	T0 vs T0.5	114	327	74	273	40	54	33	89
	T0 vs T2	840	117	475	76	365	41	171	24
Ex_2	T0 vs T0.5	147	198	109	134	38	64	53	62
	T0 vs T2	1999	4360	1040	2252	959	2108	363	774

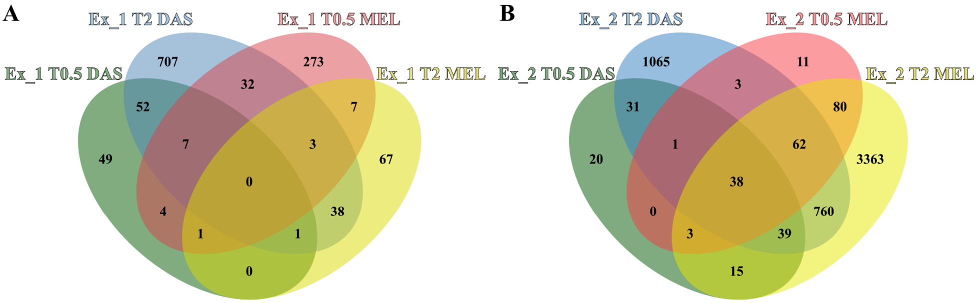

Figure 2. Venn diagram of DEGs under 0.5 and 2 h of PEG stress of *S. dasyphyllum* (DAS) and *S. melongena* (MEL) in experiment 1 (Ex_1; **A**) and experiment 2 (Ex_2; **B**).

Drought-responsive DEGs were classified according to their function into four groups: osmoprotectants, phytohormones, protein kinases and transcription factors related to the drought stress response. A total of 264 DEGs related to drought were observed in Ex_1, of which 38 of them were genes related to osmoprotectants, 46 were related to the synthesis of phytohormones, 67 were protein kinases genes and 113 were transcription factors. In Ex_2 a total of 953 DEGs were detected, of which 150 were genes that encode for proteins related to osmoprotectants, 180 were related to phytohormones, 296 for protein kinases and 327 transcription factors genes (Figure 3).

In both experiments, in general, the expression pattern of drought-responsive genes changed over time for both accessions, allowing clear differentiation between accessions and time of exposure to stress. In all cases, up-regulated and down-regulated genes from the different groups of the classification were observed (Figure 3).

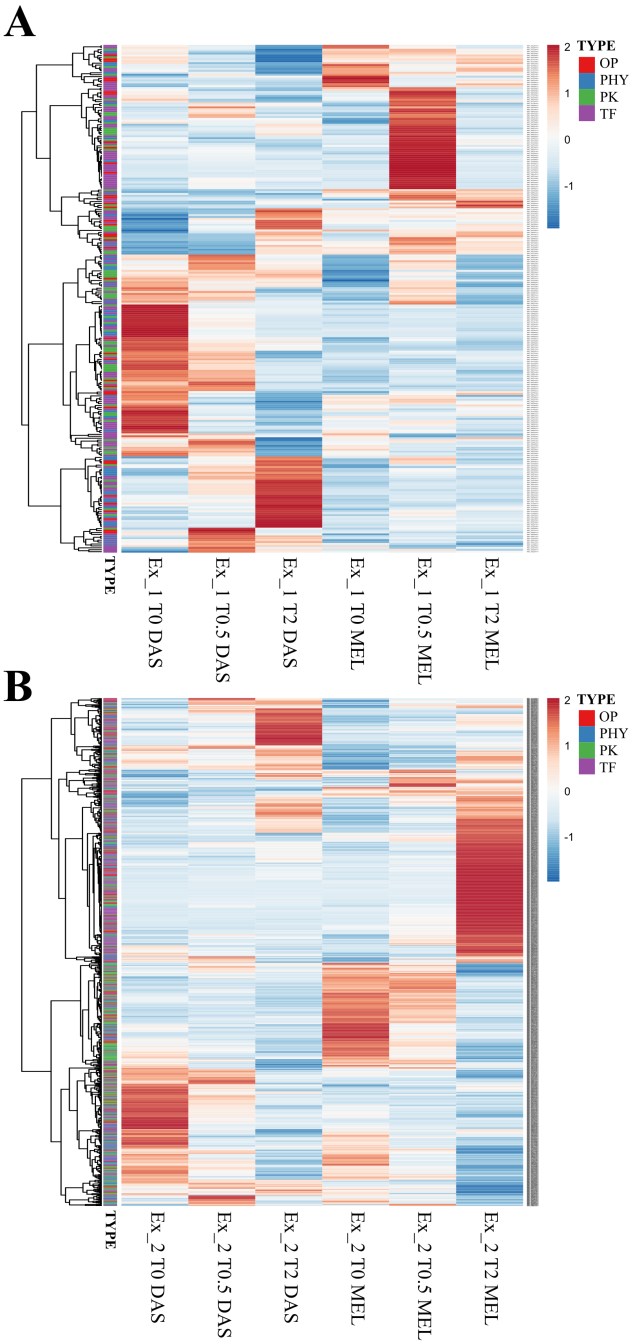

Figure 3. Heatmap of DEGs related to drought stress, osmoprotectants (OP), phytohormones (PHY), protein kinases (PK) and transcription factor (TF) after 0, 0.5 and 2 h of PEG stress of *S. dasyphyllum* (DAS) and *S. melongena* (MEL) in experiments 1 (Ex_1; **A**) and 2 (Ex_2; **B**).

3.3. *GO and KEGG enrichment in DEGs according to phenological stage and stress conditions*

A gene ontology (GO) analysis was performed with DEGs being annotated as a biological process (BP), cellular components (CC) and molecular function (MF). In Ex_1, for DAS at T0.5, 37 DEGs were annotated as MF, 13 of them as DNA-binding transcription factor activity (10 UR and three DR), three as xyloglucan:xyloglucosyl transferase activity (UR), six as sequence-specific DNA binding (UR), eight as transferase activity (transferring glycosyl groups; six UR and two DR), seven as transferase activity (transferring hexosyl groups; five UR and two DR). Regarding CC, three were annotated as apoplast and cell wall (Figure 4A). After 2 h of osmotic stress (T2), in DAS, all significant DEGs were annotated as MF, 45 as DNA-binding transcription factor activity (24 UR and 21 DR), 25 as sequence-specific DNA binding (16 UR and nine DR) and eight as terpene synthase activity (DR; Figure 4B). For MEL, at T0.5, a total of 45 DEGs were annotated as MF, 22 of them as DNA-binding transcription factor activity (19 UR and three DR), nine as calcium ion binding (UR) and 10 as sequence-specific DNA binding (seven UR and three DR) and four as G protein-coupled receptor signaling pathway as biological process (UR; Figure 4C). In MEL at T2, three CC DEGs were annotated as apoplast and cell wall (DR) and three BP DEGs as phosphorelay signal transduction system (two UR and one DR; Figure 4D).

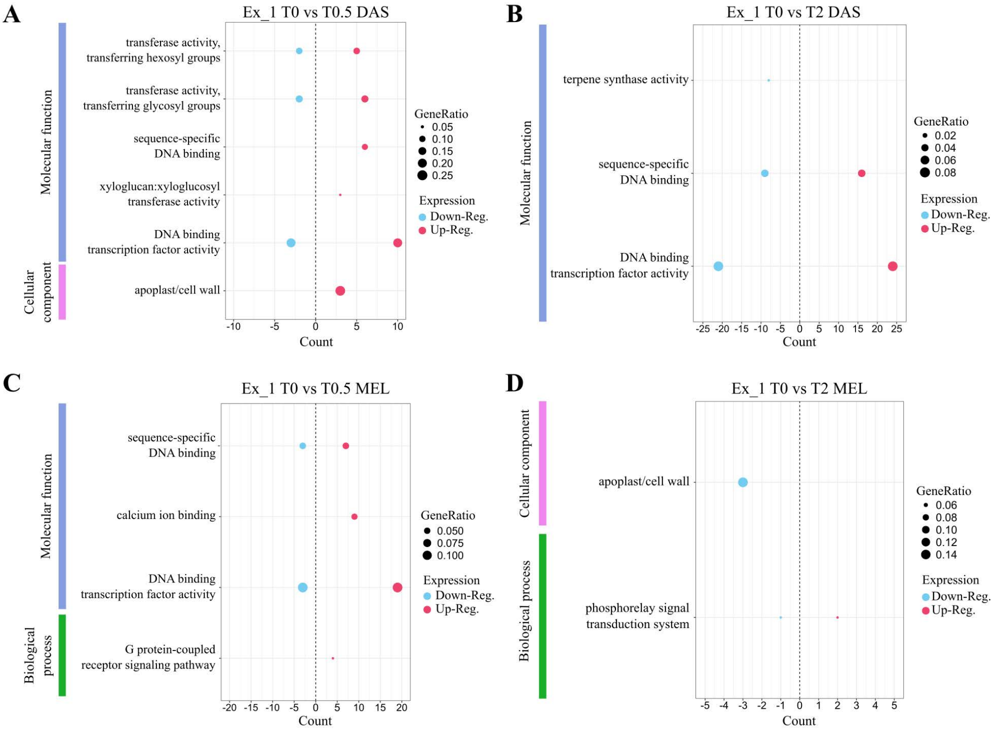

Figure 4. Gene ontology (GO) terms enrichment scatter plot in DEGs of *S. dasyphyllum* (DAS) after 0.5 (T0.5) **(A)** and 2 h (T2) **(B)** versus 0 h of PEG stress and *S. melongena* (MEL) after 0.5 (T0.5) **(C)** and 2 h (T2) **(D)** compared with 0 h of PEG stress in experiment 1 (Ex_1).

In Ex_2 for DAS at T0.5, a total of 44 DEGs were annotated, 17 of them as BP, three as CC and 24 as MF. Within the biological process category, seven DEGs were annotated as a response to chemical (one UR and six DR), six as response to auxin (DR) and four as cellular glucan metabolic process (UR), while as CC, three as apoplast and cell wall (UR). As molecular function, 11 were annotated as DNA-binding transcription factor activity (10 UR and one DR), three as xyloglucan:xyloglucosyl transferase activity (UR), four as glucosyltransferase activity (UR) and six as sequence-specific DNA binding (UR; Figure 5A). At T2, in DAS, a total of 82 DEGs were annotated as BP, 37 as response to chemical (10 UR and 27 DR), 24 as response to hormone (one UR and 23 DR) and 21 as response to auxin (DR). As MF, 49 DEGs as protein dimerization activity (29 UR and 20 DR) and 57 as DNA-binding transcription factor activity (35 UR and 22 DR) (Figure 5B). For MEL, significant GO terms annotated for BP at T0.5 were seven to response to hormone (one UR and six DR), six to response to auxin (DR) and eight to response to chemical (two UR and six DR). Also, 16 DEGs were annotated as DNA-binding transcription factor activity (15 UR and one DR) in MF classification (Figure 5C).

At T2, 161 DEGs were classified as BP, 51 as response to hormone (12 UR and 39 DR), 44 as response to auxin (10 UR and 34 DR) and 66 as response to chemical (24 UR and 42 DR). As molecular function, 125 were annotated as DNA-binding transcription factor activity (102 UR and 23 DR), 72 as sequence-specific DNA binding (52 UR and 15 DR), 41 as enzyme inhibitor activity (27 UR and 14 DR), 52 as transferase activity (transferring acyl groups other than amino-acyl groups; 31 UR and 21 DR), 62 as transferase activity (transferring acyl groups; 37 UR and 25 DR), 11 oxidoreductase activity (acting on the aldehyde or oxo group of donors as; five UR and six DR), seven as chitinase activity (six UR and one DR), seven as calcium-dependent phospholipid binding (UR) and 19 as endopeptidase inhibitor activity (17 UR and two DR) (Figure 5D). Enriched genes annotated in each GO term classification were included in Table S2.

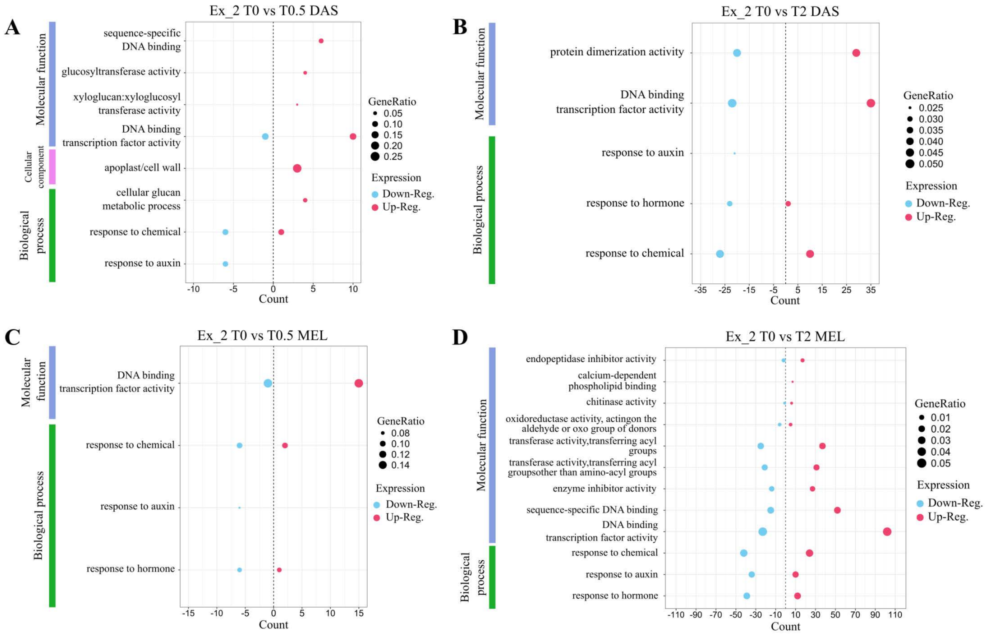

Figure 5. Gene ontology (GO) terms enrichment scatter plot in DEGs of *S. dasyphyllum* (D) after 0.5 h (T0.5) **(A)** and 2 h (T2) **(B)** versus 0 h of PEG stress and *S. melongena* (M) after 0.5 h (T0.5) **(C)** and 2 h (T2) **(D)** compared with 0h of PEG stress experiment 2 (Ex_2).

A pathway enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed to identify significant (padj < 0.05) enriched metabolic or signal transduction pathways associated with differentially expressed genes (DEGs) comparing the whole genome background. In Ex_1 more DEGs were assigned to KEGG pathways in DAS than in MEL. For DAS, at T0.5 and T2, plant hormone signal transduction and MAPK (mitogen activated protein kinase) signaling pathway were identified as enriched pathway (five UR and five DR DEGs). For T2 were also determined circadian rhythm (seven UR and two DR DEGs), sesquiterpenoid and triterpenoid biosynthesis (seven DR DEGs), galactose metabolism (seven UR DEGs) and zeatin biosynthesis (one UR and eight DR) as enriched pathways. For MEL at T0.5, plant-pathogen interaction (10 UR DEGs) and, at T2, circadian rhythm (eight UR and two DR DEGs) were enriched pathways detected (Table 2). In Ex_2, more expressed genes were assigned to metabolic pathways in MEL than for DAS. For DAS at T0.5, DEGs were assigned to plant hormone signal transduction (six UR and eight DR) and also to MAPK signaling pathway (three UR and four DR). At T2, plant hormone signal transduction (16 UR and 29 DR DEGs) and the phenylpropanoid biosynthesis (17 UR and 11 DR) were determined as enriched pathways. For MEL at T0.5, plant hormone signal transduction (nine UR and six DR DEGs), MAPK signaling pathway (three UR and four DR), fatty acid elongation (three DR), and carotenoid biosynthesis (three UR) were found to be enriched pathways. At T2, DEGs were linked to porphyrin and chlorophyll metabolism (seven UR and 21 DR), plant hormone signal transduction (58 UR and 37 DR), photosynthesis and antenna proteins (10 DR), α-linolenic acid metabolism (24 UR and two DR MAPK signaling pathway (53 UR and 16 DR), flavonoid biosynthesis (nine UR and nine DR and glutathione metabolism (21 UR and seven DR) (Table 2). Enriched genes annotated in each KEGG pathway classification were included in Table S3.

Table 2. Significant Kyoto Encyclopedia of Genes and Genomes (KEGG) enriched pathways and its ID of tomato database in *S. dasyphyllum* (DAS) and *S. melongena* (MEL) after 0.5 h (T0.5) and 2 h (T2) of PEG stress in experiments 1 (Ex_1) and 2 (Ex_2).

Experiment	Time comparison	Accession	Pathway Terms	ID	Count	Up-Regulated	Down-Regulated
Ex_1	T0 vs T0.5	DAS	Plant hormone signal transduction	sly04075	10	5	5
			MAPK signaling pathway - plant	sly04016	8	3	5
		MEL	Plant-pathogen interaction	sly04626	10	10	0
	T0 vs T2	DAS	Plant hormone signal transduction	sly04075	32	12	20
			MAPK signaling pathway - plant	sly04016	27	10	17
			Circadian rhythm - plant	sly04712	9	7	2
			Sesquiterpenoid and triterpenoid biosynthesis	sly00909	7	0	7
			Galactose metabolism	sly00052	7	7	0
			Zeatin biosynthesis	sly00908	8	1	7
		MEL	Circadian rhythm - plant	sly04712	10	8	2
Ex_2	T0 vs T0.5	DAS	Plant hormone signal transduction	sly04075	14	6	8
			MAPK signaling pathway - plant	sly04016	7	3	4
		MEL	Plant hormone signal transduction	sly04075	15	9	6
			MAPK signaling pathway - plant	sly04016	10	7	3
			Fatty acid elongation	sly00062	3	0	3
			Carotenoid biosynthesis	sly00906	3	3	0
	T0 vs T2	DAS	Plant hormone signal transduction	sly04075	45	16	29
			Phenylpropanoid biosynthesis	sly00940	28	17	11

Experiment	Time comparison	Accession	Pathway Terms	ID	Count	Up-Regulated	Down-Regulated
		MEL	Porphyrin and chlorophyll metabolism	sly00860	28	7	21
			Plant hormone signal transduction	sly04075	95	58	37
			Photosynthesis - antenna proteins	sly00196	10	0	10
			α-Linolenic acid metabolism	sly00592	26	24	2
			MAPK signaling pathway - plant	sly04016	69	53	16
			Flavonoid biosynthesis	sly00941	18	9	9
			Glutathione metabolism	sly00480	28	21	7

4. Discussion

Eggplant has been considered a relatively drought-tolerant crop since a long time ago (Behboudian, 1977) and several studies to evaluate the physiological and biochemical responses to water stress of different eggplant cultivars and wild relatives have been performed (Delfin et al., 2021; Fu et al., 2013; Plazas et al., 2019). However, detailed molecular mechanisms in response to drought stress in eggplant are not well known and, to our knowledge, transcriptional analysis by RNA-Seq method has not been reported so far. In the current study, we evaluated plants of the cultivated eggplant *S. melongena* and its wild relative *S. dasyphyllum* under two concentrations of PEG (20% and 30%) at two different phenological stages (three and five fully developed true leaves) in hydroponic conditions in order to obtain a general overview of their response to osmotic stress and get insight in the gene expression involved in response and tolerance to drought (Figure 1). *Solanum dasyphyllum* displayed a better water deficit tolerance than *S. melongena*, confirming its already recently reported drought tolerance in field and experimental conditions (Delfin et al., 2021; Fu et al., 2013; Plazas et al., 2019). PEG concentration had a visually significant effect in physiological response, with more symptoms in Ex_2, in which plants were subjected to a higher PEG concentration, resulting in a higher osmotic potential (Michel and Kaufmann, 1973).

RNA sequencing is a tool for transcriptome analysis that has allowed a better understanding of the functions of the genome (Stark et al., 2019). The robustness of modern RNA-seq methods results in a high correlation between gene expression detected by RNA-seq and qRT-PCR (Everaert et al., 2017) and RNA-Seq has demonstrated high reliability in capturing gene expression patterns and trends, something that in the past required validation by complementary methods such as qRT-PCR (Coenye, 2021; Mantione et al., 2014; Marguerat and Bähler, 2010). In this research, the analysis of differential gene expression has enabled the study of the response to osmotic stress in both species at the genomic level. One of the most important components of drought stress is osmotic stress and it has been widely used to study drought tolerance in many species (Haswell and Verslues, 2015). In our study, in general, osmotic stress treatments mainly triggered an activation response, as more significantly up-regulated than down-regulated DEGs were observed (Table 1). The number of DEGs increased as PEG concentration was higher and longer in time, as was previously reported in potato (*Solanum tuberosum* L.) (Moon et al., 2018; Yang et al., 2019). The expression pattern of drought-responsive genes displayed large differences between *S. dasyphyllum* and *S. melongena*, revealing very divergent response mechanisms under an osmotic stress according to plant physiological observations (Figure 3).

This study has disclosed the main functions and pathways expressed of two related species with large differences in osmotic stress response. GO enrichment of the identified DEGs has allowed establishing the biological functions associated to those genes (Figure 4, 5). *Solanum dasyphyllum* expressed genes were involved in diverse functions related to osmotic stress response. On one side, genes involved in the modification of cell wall and apoplast structure, such as xyloglucan:xyloglucosyl transferases (Stratilová et al., 2020; Tenhaken, 2015), were enriched in the wild species. Other genes up-regulated in *S. dasyphyllum* under osmotic stress belong to DNA-binding transcription factor activity and sequence-specific DNA binding GO terms. This includes a wide range of transcription factors (TFs) with crucial roles in responding to different abiotic stress, such as *AP2/ERF* (APETALA2/Ethylene Response Factor) family (Dalu Li et al., 2021) and its major subfamilies, such as dehydration-responsive element binding proteins (DREBs) and ethylene-responsive element (ERE) binding factors (Table S2) (Islam and Wang, 2009; Sun et al., 2008). The same occurs with TFs, from homeobox-leucine zipper family (Gao et al., 2015; Jiao et al., 2022; Perotti et al., 2017), basic leucine zipper (bZIP) (Zhu et al., 2018) and WRKY family (Chen et al., 2017). Meanwhile, in the case of *S. melongena*, the expression of *AP2/ERF*, WRKY and bZIP TFs was also observed, however, in general, the number of differential genes expressed under the stress treatments was fewer. When plants were subjected to the higher osmotic potential, the overall gene expression was also higher and included DEGs classified in response to chemical and hormones and also down-regulated genes related to auxin response. Auxins are involved in the regulation of plant growth and development and auxin response factors (ARFs) gene family play an essential role in the regulation of auxin-relative genes in abiotic stress responses in tomato (*S. lycopersicum*) (Bouzroud et al., 2018). Basic helix-loop-helix (bHLH) transcription factors were overexpressed in *S. dasyphyllum*, which they have been reported to be involved in the response to abiotic stresses in potato (*S. tuberosum*) (Wang et al., 2018) and pepper (*Capsicum annuum* L.) (Zhang et al., 2020). For *S. melongena*, exposure to a higher osmotic stress resulted in the differential expression of genes related to enzyme inhibitor activity, transferases, chitinase and oxidoreductase activities, among others. The overall response observed was very broad, with the wild species (*S. dasyphyllum*) showing a greater and more diverse expression of genes involved in drought response, which could be related to its increased tolerance.

KEGG analysis revealed significant enriched pathways related to osmotic stress such as plant hormone signal transduction and MAPK signaling. In these pathways, genes encoding for the three main components of the core Abscisic Acid (ABA) signaling response were up-regulated, a pathway that has been widely reported as a key drought stress response (Table 2) (Daszkowska-Golec, 2016). Among those genes, protein phosphatases type-2C (PP2Cs), ABA receptors PYRPYR/PYL/RCAR (PYRABACTIN-RESISTANCE 1/PYRABACTIN RESISTANCE

LIKE/REGULATORY COMPONENT OF ABA RECEPTOR) and SNF1-Related Protein Kinases type 2 (SnRK2s) were identified as DEGs (Table S3) (de Zelicourt et al., 2016). Although PP2Cs are negative regulators of ABA signaling, an increased relative expression under drought stress conditions has been reported in other similar studies (Gong et al., 2015; He et al., 2019; Yang et al., 2018), suggesting that these apparent contrasting effects need to be further investigated. AREB/ABF transcription factors and MAPKKs (mitogen activated protein kinase kinase) were also activated as a response to ABA signaling, which leads to stomatal closure, one of the most important drought responses (de Zelicourt et al., 2016; Wu et al., 2014). *Solanum dasyphyllum* displayed a wide variety of response mechanisms along with the ABA pathway. These included galactinol synthase and transferases related genes, which have been reported to improve drought tolerance (Selvaraj et al., 2017). Also, zeatin biosynthesis was down-regulated, in particular the cytokinin signaling repressors A-type ARABIDOPSIS RESPONSE REGULATORS (ARRs), which have been reported to negatively regulate by drought stress, promoting cell division in meristems (Le et al., 2012; S. M. Li et al., 2021; Nishiyama et al., 2011). In addition, GIGANTEA (GI) protein synthesis was activated, which is a regulator in the circadian rhythm plant pathway and improves drought tolerance (Baek et al., 2020). Finally, phenylpropanoid biosynthesis pathway was detected, which exhibits different important roles in the regulation under abiotic stress conditions (Sharma et al., 2019). On the other hand, *S. melongena* showed different drought response pathways, including the carotenoid biosynthesis, which has been reported to have a similar regulation in *S. tuberosum* (Gong et al., 2015), the inactivation of porphyrin, chlorophyll metabolism and photosynthesis pathways as a consequence of the osmotic stress (Reddy et al., 2004). Furthermore, the regulation of flavonoid biosynthesis, which has an important role in coping with environmental stress (Sharma et al., 2019), the expression of plant glutathione transferases (GSTs), which has been reported to be involved in responses to biotic and abiotic stress (Dixon et al., 2010), and the synthesis of the stress signaling molecule, such as jasmonic acid (JA) by the metabolism of α-Linolenic acid (Zi et al., 2022) were linked to osmotic stress. When the plants are more adult and under a more intense osmotic stress, ABA signaling response leads to stomatal closure and to the down regulation of small auxin up-regulated RNA (SAUR) genes, which induce plant growth (Stortenbeker and Bemer, 2019). In our study, a common response as stress adaptation has been observed, including ABA signaling response and inhibition of plant growth.

5. Conclusions

The present work provides an overview of the osmotic stress response at the transcriptomic level of cultivated eggplant (*S. melongena*) and its drought-tolerant wild relative *S. dasyphyllum*. We have found that osmotic potential and plant phenological stage play a crucial role in the response, which is increased when the exposure time was longer and osmotic stress was more intense. Our data showed that response mechanisms at the gene expression level were very wide-ranging, including transcription factors, phytohormones, osmoprotectants and protein kinases, being ABA response signaling an important pathway. Clear differences observed between the two species in the response to osmotic stress and overall gene expression pattern confirmed that *S. dasyphyllum* is a potential source for breeding to drought tolerance in eggplant. Overall, our work provided insights into the gene expression mechanisms of tolerance to osmotic stress in eggplant and its wild relative *S. dasyphyllum*, which is of great relevance in the improvement of drought tolerance of cultivated eggplant.

Data availability statement: The data presented in the study are deposited in the NCBI SRA repository, IDs PRJNA939933, (https://www.ncbi.nlm.nih.gov/sra/PRJNA939933). The data presented in this study are available on request from the corresponding author.

Author contributions: Conceptualization, J.P., P.G. and S.V.; methodology, G.V. and M.P.; software, G.V., P.G. and S.V.; validation, S.V.; formal analysis, G.V. and S.V.; investigation, G.V., J.P., P.G and S.V.; resources, J.P.; data curation, G.V. and M.P.; writing—original draft, G.V.; writing—review and editing, G.V., M.P., J.P., P.G. and S.V.; supervision, M.P., J.P., P.G. and S.V.; funding acquisition, J.P. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table 1. Data quality summary of samples of experiment 1 and 2, after 0, 0.5 and 2h of PEG stress of *S. dasyphyllum* (DAS) and *S. melongena* (MEL).

Supplementary Table 2. Gene ontology (GO) terms enrichment and regulation, ID, description and transcription factor family of DEGs in *S. dasyphyllum* after 0.5 (T0.5) (A) and 2h (T2) versus 0h of PEG stress and *S. melongena* (M) after 0.5 (T0.5) and 2h (T2) compared with 0h of PEG stress experiment 2 (Ex_2).

Supplementary Table 3. Significant Kyoto Encyclopedia of Genes and Genomes (KEGG) enriched pathways, its ID of tomato database, and regulation, ID, description and transcription factor family of DEGs in *S. dasyphyllum* (D) and *S. melongena* (M) after 0.5h (T0.5) and 2h (T2) of PEG stress in experiments 1 (Ex_1) and 2 (Ex_2).

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General discussion

The increasing impact of climate change highlights the urgent need to select and develop plant materials capable of adapting to diverse abiotic stress conditions, a crucial step towards more sustainable agriculture (Pourkheirandish et al., 2020). Crop wild relatives (CWR), which thrive in a wide range of environments and exhibit valuable traits for plant breeding, have largely untapped potential due to their limited direct use in agriculture. However, the incorporation of CWR introgressions into populations with cultivated backgrounds, such as advanced backcrosses (ABs) and introgression lines (ILs), is a fundamental strategy. This approach allows the study and utilization of genetic variation in CWR and facilitates the development of crop varieties with improved adaptive properties in the face of abiotic stresses related to climate change (Prohens et al., 2017). Furthermore, understanding the molecular mechanisms through which CWRs respond to stress conditions like drought is essential to reveal adaptive strategies different from those utilized by cultivated species.

The objective of this doctoral thesis consists in advance the understanding of abiotic stress tolerance in eggplant. To this end, we evaluated introgression breeding materials derived from wild relatives in cultivated eggplant under different nitrogen conditions, focusing to select plant materials with improved nitrogen use efficiency (NUE) and identifying and validating QTLs for traits related to plant, fruit, and composition (Chapter I). Additionally, we have elucidated the molecular response mechanisms to drought stress in both cultivated eggplant and its wild relative *S. dasyphyllum* (Chapter II).

The findings presented in this doctoral thesis provide valuable knowledge about potential eggplant materials derived from CWR under low nitrogen conditions, highlighting the benefits of integrating genetic diversity from CWR into cultivated species. Additionally, the detection and analysis of QTLs have shed light on the genetics related to traits of interest, providing new tools necessary for developing new varieties of eggplant better suited to the challenges of modern agriculture and environmental stressors. Furthermore, this doctoral thesis offers insights into the molecular response mechanisms that wild species utilize under drought stress, contrasting with the responses of less tolerant cultivated species, thereby deepening the understanding of their adaptive strategies.

Evaluation of advanced backcrosses of eggplant and wild relatives for nitrogen use efficiency

Efficient nitrogen management as a fertilizer is critical for reducing environmental impacts and enhancing the economic sustainability of agriculture, while maintaining high yields. Several strategies have been developed to improve nitrogen use efficiency (NUE) across different field crops, particularly cereals (Omara et al., 2019). However, advancements in NUE for Solanaceae crops such as potatoes, tomatoes, and eggplants have been relatively recent (Aci et al., 2021; Liang et al., 2019; Mauceri et al., 2020; Rosa-Martínez et al., 2023b; Tiwari et al., 2018).

In this doctoral thesis, the first comprehensive evaluation of the entire genome of the wild relative species *S. elaeagnifolium* from the tertiary genepool, represented in advanced backcrosses (ABs), was conducted. Alongside, *S. melongena* and *S. elaeagnifolium*, utilized as parentals in the development of these ABs, were examined under low N cultivation conditions in the soil and irrigation water, reflecting the poor environments where this wild relative grows (Roberts and Florentine, 2022). The fertilization irrigation solution did not contain any added N fertilizer, resulting in a 96% reduction of N compared to the standard irrigations used solutions in soilless eggplant cultivation (Baixauli and Aguilar, 2002). Despite these constraints, all plant individuals, including those *S. melongena* plants, developed adequately.

The considerable phylogenetic distance between the parental species (Knapp et al., 2017) correlated with significant differences in all evaluated plant, fruit, and composition traits. High phenotypic diversity was observed in the ABs, with much higher ranges of variation than the parents for all traits. Notably, transgressive individuals with many favorable traits, including higher yields and NUE than the cultivated parent, were identified, potentially contributing to the development of improved materials adapted to low N conditions. Additionally, *S. elaeagnifolium* was found to have a higher total phenolics content (TPC) and a distinct phenolic acid chromatogram pattern when compared to the cultivated eggplant, supporting findings from previous studies (Kaushik et al., 2017; Meyer et al., 2015). Some ABs not only had higher TPC than the recurrent parent, *S. melongena*, but also exhibited similar phenolic acid profiles to the wild parent, indicating potential for developing varieties with enhanced bioactive properties.

In addition to the study previously mentioned, this doctoral thesis also involved the evaluation of three sets of advanced backcrosses (ABs) of eggplant wild relatives from different genepools, namely *S. insanum* (GP1), *S. dasyphyllum* (GP2) and *S. elaeagnifolium* (GP3), under the same low nitrogen conditions for the first time. The inclusion of eggplant ABs with introgressions from different chromosomes provided an overview of the potential these wild species hold for breeding under low nitrogen input abiotic stress conditions. Furthermore, to explore the impact of nitrogen on cultivated eggplant, recurrent parental lines of *S. melongena* (MEL5, MEL1 and MEL3) were tested under low and normal N input, providing valuable insights into how nitrogen availability influences different traits in cultivated eggplants.

The utilization of pots with an automated N fertilization system for this study, provides a more controlled condition that allowed for precise assessments of the impact of low nitrogen input on plant traits. This approach differs from other studies that may use hydroponic culture (Mauceri et al., 2022, 2021; Singh et al., 2019) or soil cultivation (Aminifard et al., 2010; Rosa-Martínez et al., 2023b; Zhou et al., 2023).

Different N treatments significantly influenced the traits of *S. melongena* recurrent parents. Generally, normal N conditions increased chlorophyll content but decreased flavonol and anthocyanin levels, traits that correlate with leaf nitrogen content and are measurable using proximal optical sensors (Padilla et al., 2018). These normal N conditions also promoted enhanced aerial biomass, stem diameter, yield, total fruit number per plant, and increased nitrogen and carbon content in both plants and fruits, aligning with prior studies on nitrogen fertilization effects on eggplant (Mauceri et al., 2020; Rosa-Martínez et al., 2023b; Ulas et al., 2018; Zhou et al., 2023). In contrast, low N treatment significantly improved NUE, NupE and NUtE compared to normal N conditions, contradicting the previous results of Mauceri et al. (2020), and aligning with Rosa-Martinez et al. (2023). These results suggest that carefully managed fertigation with reduced nitrogen inputs can enhance NUE and yield per unit of nitrogen applied, presenting a more sustainable approach to cultivating eggplants.

The previously observed high phenotypic diversity and wider distribution ranges in the ABs of *S. elaeagnifolium* relative to their parents, are similarly manifested across the traits of the three sets of ABs. Moreover, no significant differences were found between each set of ABs and their respective recurrent parents under low N conditions. Although the ABs used in this study do not cover the entire genome of wild eggplant relatives, the presence of transgressive individuals in ABs from *S. insanum*, *S. dasyphyllum*, and *S. elaeagnifolium* may reaffirm and expand the interest in utilizing CWRS to enhance the resilience and genetic diversity of eggplants under abiotic stress conditions.

The PCA analyses from both studies revealed a relatively wide distribution among the different sets of ABs. A general trend was observed where ABs with lower recovery percentages of the recurrent parent genome tend to be distributed separately from the recurrent parents, and conversely, those with higher recovery percentages are generally closer. However, there are notable exceptions to this trend, which are particularly valuable for breeding programs, as they are suitable for selection and useful for genomic studies of specific traits.

The study of correlations among traits can aid breeding programs by enabling the prediction of specific phenotypes with fewer trait evaluations. Consistent associations across each set of ABs have been identified between traits related to plant vigor, such as plant biomass and stem diameter, and those related to yield, NUE, and the number of fruits per plant. Correlations related to fruit morphology, such as fruit pedicel length, fruit calyx length, fruit length, fruit width, and mean fruit weight, have also been established. These correlations are consistent with finding from several previous studies (Frary et al., 2014; Mangino et al., 2021; Patel et al., 2017; Portis et al., 2015; Rosa-Martínez et al., 2023b).

Additionally, each set of ABs, showed unique correlations, providing valuable insights for selective breeding strategies. Particularly, in the ABs of *S. elaeagnifolium*, specific correlations were observed withing composition traits. Notably, correlations between total phenolics content (TPC), chlorogenic acid content (CGA), and total phenolic acid peaks area (TP-Area) were have found, aligning with previous studies (Kaushik et al., 2017; Plazas et al., 2013). Additionally, significant negative correlations between some fruit size traits and both TPC and CGA content suggest a potential balancing effect between the size of the fruits and their phenolic content in these ABs (Hanson et al., 2006; Plazas et al., 2013; Villanueva et al., 2024). Furthermore, negative correlations were noted between nitrogen content and both flavonol and anthocyanin content in leaves along with a positive correlation between nitrogen content and stem across the three sets of ABs from *S. insanum*, *S. dasyphyllum*, and *S. elaeagnifolium*. Variations in the correlations between NUE, NUpE, NUtE, and other traits such as fruit calyx length or those associated with yield were also observed across different sets of ABs.

Identification and validation of QTLs for plant, fruit and composition traits in eggplant

The development of advanced genotyping tools, specifically the high-throughput 5k probes SPET (single primer enrichment technology) platform for eggplant (Barchi et al., 2019), together with the availability of phenotyping, have allowed the identification of QTLs and candidate genes associated with traits of interest.

First, nine QTLs were identified across the set of ABs of *S. elaeagnifolium* (BC2 and BC3), which covered the entire genome, under low N soil cultivation. Notably, QTLs located on chromosome 6 at the same position were associated with the presence of prickles in leaves, stems and fruit calyx. These findings are in agreement with recent studies that identified the gene responsible for the formation of prickles in eggplant (Li et al., 2024; Qian et al., 2021; Satterlee et al., 2024). Additionally, a novel QTL for stem diameter was detected on chromosome 4, whereas previous reports located a similar QTL on chromosome 2 in ILs populations of *S. incanum* (Mangino et al., 2020; Rosa-Martínez et al., 2023b). Regarding fruit traits, a QTL for fruit width was detected on chromosome 7, a chromosome where similar QTLs have been noted at different loci in both interspecific and intraspecific F_2 populations of eggplant (Portis et al., 2014; Wei et al., 2020). The compositional analysis of fruit flesh led to the identification of QTLs for phenolic acid and total phenolics at different locations in the genome of eggplant (Rosa-Martínez et al., 2023a). Specifically, a QTL for chlorogenic acid content was found on chromosome 5, coinciding with the chromosome identified in *S. melongena* ILs with eggplant wild relative *S. incanum* as the donor parent (Rosa-Martínez et al., 2023b). For traits related to total phenolic acids, a QTL on chromosome 6 associated with peak area involves two putative genes: one described as similar to CCR1, cinnamoyl-CoA reductase 1 (SMEL_006g261420.1.01), involved in lignin biosynthesis pathway, a response mechanism to biotic and abiotic stresses (Park et al., 2017; Sattler et al., 2017), and another gene described as similar to 4CL2, 4-coumarate-CoA ligase 2 (SMEL_006g261630.1.01), involved in the CGA synthesis pathway in eggplant (Gramazio et al., 2014). Moreover, for the closely correlated traits of chlorogenic acid peak area and phenolic acids pattern, a previously unreported QTL was detected at the same position on chromosome 1.

The analysis of three sets of ABs from *S. insanum*, *S. dasyphyllum*, and *S. elaeagnifolium* resulted in the identification of 16 QTLs. Specifically, in the ABs of *S. elaeagnifolium*, a novel QTL for carbon content in leaves was found on chromosome 8. For ABs of *S. dasyphyllum*, new QTLs related to flavonol leaf content, nitrogen balanced index (NBI), and stem diameter, were identified on chromosomes 1. Additionally, QTLs located on chromosome 2 are associated with plant growth traits such us chlorophyll leaf content, plant biomass and yield in *S. dasyphyllum*, as well as fruit size parameters including fruit pedicel and calyx length, and fruit mean weight in both ABs *S. elaeagnifolium* and *S. dasyphyllum*. This may indicate the presence of genetic linkage or a pleiotropic locus, supporting similar findings in previous studies (Gaccione et al., 2023; Portis et al., 2014; Rosa-Martínez et al., 2023b). For ABs of *S. insanum*, QTLs detected at the same location on chromosome 9 include those for flavonol leaf content, NBI, fruit mean weight, and nitrogen content in leaves and stems, coinciding with earlier findings where QTLs for leaf nitrogen content were also identified on chromosomes 4 and 9 in ILs with *S. incanum* (Rosa-Martínez et al., 2023b). In the QTL regions associated with plant growth, yield, fruit size, and nitrogen traits in ABs potential candidate genes were detected on chromosomes 2 and 9. These include genes for NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (NRT1/PRT) FAMILY proteins (SMEL_002g164340; SMEL_009g328470), which are crucial for nitrate uptake and substrate transport (Akbudak et al., 2022; Dechorgnat et al., 2011; Léran et al., 2014). Additionally, on chromosome 2 a gene encoding the PIN-FORMED (PIN) 8 auxin efflux transporter (SMEL_002g164700), which plays a significant role in auxin distribution affecting various developmental processes (Zhou and Luo, 2018), and another candidate gene (SMEL_002g167520) for Myb-related protein 306 (MYB306), a transcription factor (TF) that potentially influences anthocyanin regulation and fruit size in eggplant (Li et al., 2023).

Building on the identification of QTLs on chromosomes 2 and 9 in ABs from *S. dasyphyllum* and *S. insanum*, respectively, a study was conducted to evaluated and validate these findings. This research utilized specific introgression lines, IL-M1-D2 and IL-M5-I9, with introgressions from these respective chromosomes, alongside segregating hybrids from crosses between these lines and advanced backcrosses, as well as parental *S. melongena* lines, all under low nitrogen conditions. This approach allowed for an integrated evaluation of the impact of genetic background and allele dosage on the traits of interest.

The analysis of the results revealed significant differences between the evaluated line sets, except for traits related to plant vigor and NUE parameters. The two segregating hybrids resulting from two crosses (MEL1 X IL^{Het}-M5-I9 and IL-M1-D2 X IL^{Het}-M5-I9) showed no significant differences, clustering closely together in both PCA and PLS analyses. These findings indicates that the introgression of *S. dasyphyllum* on chromosome 2 had a limited effect on the phenotypes of the segregant hybrids, as evidenced by only slight differences from their corresponding parental line, *S. melongena* MEL1, in the PCA and PLS plots. The segregating hybrids exhibited a wider range trait distribution, demonstrating their ability to enhance variability in eggplant and to reveal the effects of genetic background, with observed differences suggesting a significant increase in variation compared to the cultivated parents, thereby underscoring their potential value for breeding. Furthermore, in the same PCA and PLS analyses, individuals from the IL of *S. insanum* (IL-M5-I9) formed a distinct group, noteworthy for their association with traits related to high chlorophyll and nitrogen content, setting them apart from other materials.

The identification of 13 putative QTLs within the corresponding genomic region of chromosome 9 of *S. insanum* was consistent with these results. Validation was achieved for a subset of QTLs associated with various traits, including flavonol content in leaves, nitrogen balanced index, mean fruit weight, and leaf nitrogen content, as previously identified in AB sets from *S. dasyphyllum* and *S. insanum*. Additionally, a novel QTL was detected for chlorophyll leaf content, a trait closely related to other measurements obtained with an optical leaf-clip meter (Cerovic et al., 2012). Furthermore, QTLs associated with fruit weight and morphological traits have been found at the same location on chromosome 9. Similar findings have been reported on the same chromosome in studies of different eggplant populations (Frary et al., 2014; Portis et al., 2015; Rosa-Martínez et al., 2023b; Wei et al., 2020). Despite the negative effect of *S. insanum* introgression on fruit size, a QTL with significant positive effect detected, both in homozygous and heterozygous, on the total number of fruits per plant. Also, novel QTLs were detected on chromosome 9 for carbon content in fruit and stem and showing a positive effect of introgression on fruit nitrogen content, which correlates with leaf nitrogen content.

These findings showed the significant impact of annual environmental fluctuations, their interaction with genotypes, and genetic background on the phenotypic expression and stability of QTLs (Mangino et al., 2020; Priyadarshan, 2019). Consequently, validating detected QTLs across diverse locations and populations is crucial for understanding how these genetic regions contribute to trait variation and adaptation, thus advancing sustainable agricultural development (Frary et al., 2014; Sulli et al., 2021).

The overall results obtained in this doctoral thesis revealed the potential of wild eggplant relatives for breeding tolerance to abiotic stresses. The development of advanced backcrosses derived from wild relatives from different genepools, including *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium,* expanded the genetic variation readily available to breeders. Our findings demonstrated significant effect of different nitrogen doses on plant, fruit, and composition traits. The transgressive individuals identified within these introgression materials showed the potential for developing new, improved varieties with better agronomic characteristics and adaptation to low nitrogen conditions. Furthermore, the detection and validation of QTLs have provided valuable insights into the genetics related to eggplant productivity, nitrogen use efficiency (NUE), and overall crop performance, contributing to a more sustainable agriculture. Advances in understanding the molecular mechanisms of drought response resulting from our studies offer valuable knowledge about the adaptive strategies of wild species, particularly *S. dasyphyllum,* under osmotic stress, which is of great relevance for the development of more resilient eggplant cultivars. This doctoral thesis has made relevant contributions to the development of more resilient and sustainable eggplant varieties, highlighting the potential of wild relatives to address the challenges of modern horticulture.

General conclusions

1. Advanced backcrosses with genome-wide introgressions of *S. elaeagnifolium*, an American wild species of the tertiary genepool, were evaluated for the first time using low nitrogen fertilization conditions. These introgression materials represent a highly valuable resource for eggplant improvement, as they expand the genetic diversity of the crop with previously unexploded exotic variations.

2. Transgressive advanced backcrosses with *S. elaeagnifolium* were identified for many traits, including significantly higher yield, nitrogen use efficiency (NUE), and phenolics content compared to cultivated eggplant. The results demonstrated that introgressions from *S. elaeagnifolium* may have potential for significant agricultural advancements and leading to the development of improved eggplant varieties for a more sustainable agriculture.

3. The integration of the genomic information obtained in the *S. elaeagnifolium* advanced backcrosses enabled the detection of nine putative QTLs associated with various traits, including plant vigor, fruit size, the presence of prickles, and composition traits related to total phenolic acids and chlorogenic acid content. Through selection and further backcrosses, eggplant introgression lines with enhanced agronomic characteristics and composition can be developed, facilitating their integration into breeding programs.

4. The evaluation of three sets of advanced backcrosses of eggplant with wild relatives from *S. insanum*, *S. dasyphyllum* and *S. elaeagnifolium* under low nitrogen fertilization conditions revealed phenotypic variation with the presence of valuable advanced backcross for genetic improvement in eggplant breeding programs. Plant, fruit, and composition traits varied according to the nitrogen levels applied.

5. The identification of 16 putative QTLs in the three sets of advanced backcrosses, particularly those related to plant growth, yield, fruit size, chlorophyll, flavonol, nitrogen balance index (NBI), and composition content on chromosomes 1, 2, 8, and 9, demonstrates the significant genetic variation in these wild eggplant relatives and their potential for agronomic improvement. These insights may contribute to developing eggplant varieties with enhanced productivity, quality, and nitrogen use efficiency, supporting more sustainable agricultural practices.

6. The phenotypic variations observed in the introgressions and segregant hybrids evaluated in the three sets of advanced backcrosses suggested the potential of these materials for eggplant breeding for more sustainable agricultural practices. Given the complex interactions influencing the expression of quantitative traits, such as environmental factors, genotypes, and genetic backgrounds, it becomes

important to validate eggplant QTLs under diverse conditions and populations by evaluating genomic regions in different genetic backgrounds.

7. The evaluation of introgression materials and segregating hybrids validated a genomic region from chromosome 9 of *S. insanum* that may hold significant potential for adapting eggplant to low N conditions, as it contains numerous QTLs, including several novel ones valuable for breeding. Conversely, the genomic region on chromosome 2 from *S. dasyphyllum* that was initially associated with chlorophyll content in leaves, aerial plant biomass and yield could not be confirmed.

8. Transcriptomic analysis of the osmotic stress response in cultivated eggplant (*S. melongena*) and its drought-tolerant wild relative *S. dasyphyllum* revealed that osmotic potential level and plant phenological stage play a crucial role. The intensity of the response increased with longer exposure times and more severe osmotic stress.

9. Osmotic stress response triggered wide-ranging differential gene expression, including transcription factors, phytohormones, osmoprotectants and protein kinases, being ABA response signaling an important pathway. The clear differences observed between *S. melongena* and *S. dasyphyllum* in their response and gene expression pattern confirmed the potential of *S. dasyphyllum* as a valuable source for breeding to drought tolerance in eggplant, emphasizing the importance of these findings for future breeding programs.

10. As a general conclusion we found that wild eggplant relatives offer valuable genetic resources for improving tolerance to abiotic stresses. Incorporating these resources into breeding programs is facilitating the development of more resilient eggplant varieties for a more sustainable agriculture.

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