Erasmus Mundus Master´s degree in Plant Breeding

DISSECTING THE STAY GREEN PHENOTYPE IN COMMON BEAN (Phaseolus vulgaris) USING NATURAL VARIATION

Diana Laura Labastida Jaimes

Tutors: Dr. Prof. Pär K. Ingvarsson
Dr. Prof. Ana María Fita Hernández
Dr. Martha Rosalía Rendón Anaya

Co-funded by the Erasmus+ Programme of the European Union

Acknowledgments

Uppsala, 2020

Two years ago, a new adventure began in my academic life. I would never have imagined what this trip would bring with it. It has been two intense years in my life in many aspects. I have learned a lot academically and personally. There have been many adventures and experiences and, even a pandemic is attacking the world. Today that I am about to finish my master's degree, after so many hours of study, classes and, exams, I know that it was worth it.

First, I would like to express my gratitude to Pär Ingvarsson, director of this thesis, for opening the doors of his group and showing me his confidence to carry out this project. My warmest thanks to Martha Rendón for supervising this thesis, for her enormous patience and dedication throughout this project. Overall, for offering me her friendship and sharing her knowledge with me. Thanks also to the entire lab group for being always nice to me.

Many thanks to the EMplant consortium for admitting me to this program. Thanks to Alicia and Aude for coordinating this program, for their enormous help, and for always answering our questions, I know it was not easy. I also want to thank Anders Kvarnheden and Ana Fita for their effort at making easier our arrival in Sweden and Spain. Specially thanks to Ana Fita for supervising this thesis from the distance and attend my questions.

To my colleagues from EMplant and my friends for the adventures, laughs, and difficult moments that we have shared together, I could not have done it alone.

Finally, I want to thank my family for their effort so that I could get here. To my parents that have always supported me and especially to my mother, to whom I dedicate this thesis, I know she would be happy for me.

Resumen

El frijol común, *Phaseolus vulgaris,* es la leguminosa más importante para el consumo humano, contribuyendo con el 30% de la ingesta diaria total de proteínas en países subdesarrollados, lo que lo convierte una de las principales fuentes de nutrientes. La principal limitación de este cultivo es la sequía, que causa más del 60% de las pérdidas anuales. La regulación genética de la tolerancia a sequía está controlada por varios QTL de pequeño efecto en combinación con interacciones ambientales lo que dificulta su investigación. Una estrategia importante para hacer frente a la sequía es el retraso de la senescencia, que conlleva al denominado fenotipo "*stay-green*". A pesar de su importancia en la resistencia a sequía, hay poca información sobre la base genética de la senescencia en *P. vulgaris*.

Este estudio tiene como objetivo buscar cultivares de frijol que presenten el fenotipo stay-green (SG), evaluar su rendimiento ante estrés por sequía extrema y conocer la base génica de dicho fenotipo. Para ello utilizamos un conjunto de 71 accesiones de frijol común provenientes de los principales pools genéticoss, seleccionados para abarcar el rango de variación natural. Bajo condiciones controladas en cámaras de crecimiento e invernadero, se encontró que 6 de las accesiones mostraron fenotipo SG, mientras que otras mostraron estrategias diferentes como escape o recuperación tras el riego post-sequía. Adicionalmente, se realizó un estudio de asociación genética con los datos fenotípicos medidos. De esta forma, encontramos genes candidatos detrás de la tolerancia a sequía implicados en diversos mecanismos como el almacenamiento de carbohidratos y la estabilización de proteínas.

Resum

El fesol comú, *Phaseolus vulgaris*, és la lleguminosa més important per al consum humà, contribuint amb el 30% de la ingesta diària total de proteïnes en països subdesenvolupats, el que el converteix una de les principals fonts de nutrients. La principal limitació d'aquest cultiu és la sequera, que causa més de l'60% de les pérdues anuals. La regulació genètica de la tolerància a sequera està controlada per diversos QTL de xicotet efecte en combinació amb interaccions ambientals, el que dificulta la seva investigació. Una estratègia important per fer front a la sequera és el retard de la senescència, que comporta a l'anomenat fenotip "stay-green". Malgrat la seva importància en la resistència a sequera, hi ha poca informació sobre la base genètica de la senescència en *P. vulgaris*.

Aquest estudi té com a objectiu buscar cultivars de fesol que tinguen el fenotip stay-green (SG), avaluar el seu rendiment davant estrès per sequera extrema i conèixer la base gènica d'aquest fenotip. Per a això utilitzem un conjunt de 71 accessions de fesol comú provinents de les principals pools genètics, seleccionats per abastar el rang de variació natural. Baix condicions controlades en cambres de creixement i hivernacle, es va trobar que 6 de les accessions van mostrar fenotip SG, mentre que altres van mostrar estratègies diferents com escapament o recuperació després del reg post-sequera. Addicionalment, es va realitzar un estudi d'associació genètica amb les dades fenotípiques mesurades. D'aquesta manera, trobem gens candidats darrere de la tolerància a sequera implicats en diversos mecanismes com l'emmagatzematge de carbohidrats i l'estabilització de proteïnes.

Abstract

The common bean *Phaseolus vulgaris* is the most important legume for human consumption, contributing 30% of the total daily protein intake in developing countries. The main limitation for its cultivation is drought, which causes more than 60% of the annual losses. The genetic regulation of drought tolerance is controlled by several small-effect QTLs in combination with environmental interactions, which makes identifying the underlying genetic basis difficult and complicates breeding. An important strategy for coping with drought is delayed senescence or the "*stay-green*" phenotype. Despite its importance in drought resistance, there is little information on the genetic basis of senescence in *P. vulgaris*.

This study aims at identifying common bean cultivars displaying the stay-green (SG) phenotype, evaluate their performance under drought stress, and dissect the genetic basis of this phenotype. For this purpose, we used a set of 71 common bean accessions belonging to the three most important gene-pools, selected to cover as much of the natural variation of the species as possible. Experiments in climatic chambers and under greenhouse-controlled conditions identified six cultivars with a clear SG phenotype, while other cultivars could either escape or recover successfully after the drought stress. In addition, we ran genomic association studies that allowed us to identify candidate genes behind the SG phenotype, and identified genes involved in different biological processes, such as storage of protein stabilization.

Contents

1. Background

Legumes play a fundamental role in food security in developing countries. Within this group, the common bean, *Phaseolus vulgaris*, is especially important for human consumption in terms of nutrients provided, since it is a good source of proteins, iron, folic acid, and complex carbohydrates. It contributes to about 15% of the total daily calorie intake and 30% of the protein intake in many parts of America and Africa. (Bitochi *et al*., 2012; Wu *et al*., 2020).

The current scenario for climate change has emphasized problems such as drought, which in turn has aggravated the fluctuation of crop production, especially in arid and semi-arid areas (Jha *et al.,* 2019). The main constraint facing common bean cultivation nowadays is drought stress and drought is the main cause of yield losses. This is especially true for developing countries where it is often cultivated by small farmers and hence depend on natural rainfall (Mukeshimana *et al*., 2014). Unfortunately, efforts to alleviate yield loss in grain legumes have been limited due to the complex genetic basis of drought tolerance controlled by various small-effect QTLs in combination with environmental interactions (*Jha et al*., 2019). Thanks to new large-scale genotyping methods, as next-generation sequencing (NGS) techniques or single nucleotide polymorphism (SNP) arrays, it has become possible to correlate such data with phenotypic characteristics in germplasm collections to identify genes or genomic regions involved in controlling different traits.

It has been seen that early senescence has adverse effects on annual plants in terms of yield, while late senescence, also known as ”stay-green” (SG) phenotype, infringes both positive and negative effects on traits such as the amount of nutrients and tolerance to abiotic stresses (Woo *et al*., 2013). The SG phenotype in plants is defined as the ability to maintain green coloration in the leaves for a longer time compared to normal phenotypes; this characteristic is classified as cosmetic or functional (Myers *et al*., 2018, Thomas & Ougham, 2014). The functional SG phenotype is especially important since it represents a possible solution for the improvement of crops against abiotic stress conditions such as drought, due to the retention capacity of water and nutrients, a character that has been extensively explored in cereals (Sivasakthi*, et al*., 2019).

Despite the importance of senescence and the SG phenotype, there is currently little information on the genetic basis of senescence in common bean. Therefore, the dissection of the SG phenotype at the molecular level is essential for implementing efficient breeding strategies, aiming at developing new varieties capable of adapting to adverse environmental conditions.

1.1 *Phaseolus vulgaris*

1.1.1 Description and biology

The genus *Phaseolus* belongs to the Fabaceae family, within *Phaseolus vulgaris*, better known as common bean, is the most widely used species for human consumption. This family comprises an extensive diversity of forms, which includes trees, shrubs, and herbs (OECD, 2016); its taxonomic classification is shown below (ITIS, 2020).

Common bean (*Phaseolus vulgaris*)

Order Fabales

Family Fabaceae

Genus *Phaseolus* L.

Species *Phaseolus vulgaris L.*

Phaseolus vulgaris is a diploid species 2n=22, with a genome size of 520 Mbp (Schmutz, *et al.,* 2014). It can grow as both an annual and a short-lived perennials, depending on the climate. Annual forms are common in temperate zones while short-lived perennial forms are found under tropical conditions. The days to seed maturity is very variable, ranging from 50 to more than 250 days, depending on the cultivar, the photoperiod response and environmental conditions (OECD, 2016).

Common beans have different growth habits: they can develop as determinate and indeterminate plants (Fig.1) and there are also twining or climbing cultivars. Plants are termed as determinate when the branches and stem end in a cluster, while in indeterminate plants the branches and stem end with a vegetative meristem (Durán, 2016).

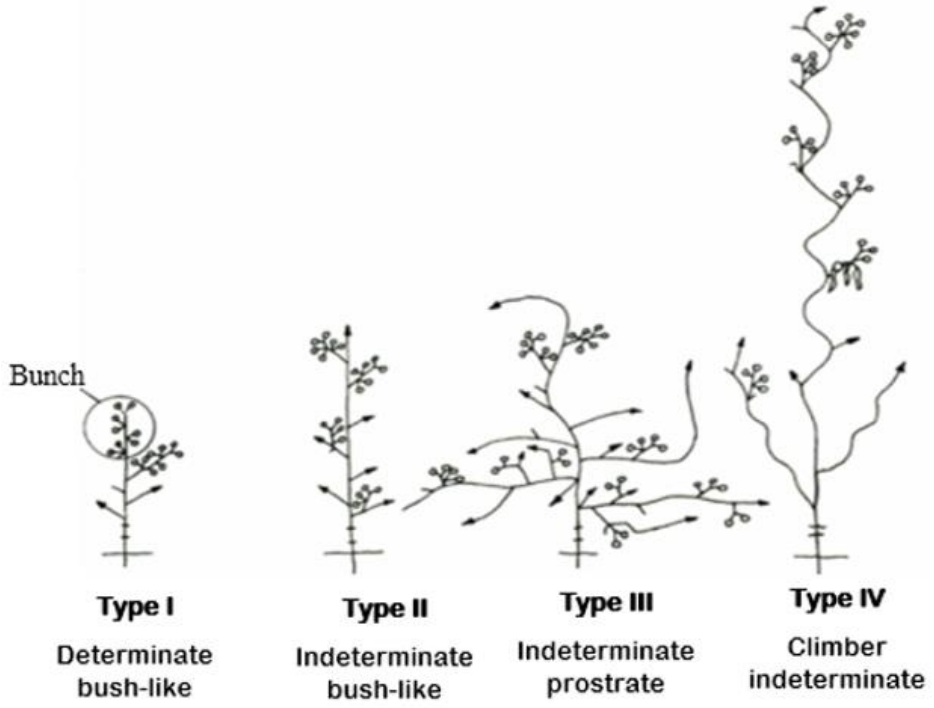

Figure 1. *Phaseolus vulgaris* growth habit. Modified from*:* Debouck & Hidalgo, 1984).

Shrub varieties can grow of up to 60 cm in height, while the indeterminate ones can reach 3 m. In both cases, the leaves are compound pinnately trifoliate and are arranged alternately on the stem. The flowers vary in colors from white to pink to purple, while the pods turn into wider range of colors, from green and yellow to purple and blue. Pods range in size from 8 to 20 cm in length. The optimum growth temperature of this species is between 20°C and 25°C and they require slightly acidic soils (pH 6.0-6.5) (Rubatzky and Yamaguchi, 1997).

1.1.2 Developmental stages

The biological cycle of *P. vulgaris* is divided into the vegetative and reproductive stages (fig. 2). The vegetative phase begins when conditions are appropriate for seed germination to be induced and lasts until the first flower bud appears, giving way to the start of the reproductive stage. In plants with determinate growth, the reproductive stage begins with the appearance of the first flowering bud, while in plants with an indeterminate growth habit it starts with the first raceme. In both forms, this stage lasts until the harvest maturity (Fernández *et al*., 1986).

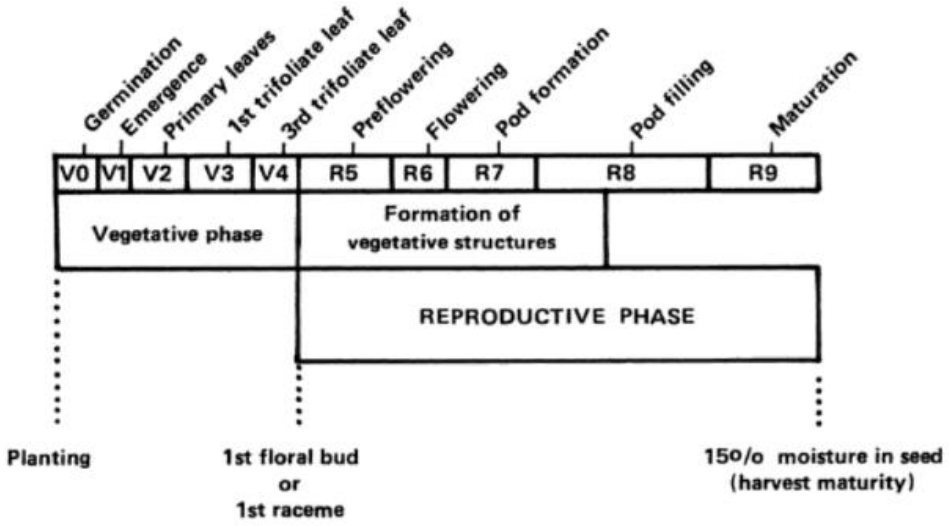

Figure 2. Developmental stages of common bean. Each stage is represented with a code formed by a letter followed by a number. Letter V is used for vegetative and R for reproductive. The number ranges from 1 to 9 and corresponds to the moment in which each stage is found (Durán, 2016).

1.1.3 Origin and distribution

It is well accepted that the origin of *Phaseolus vulgaris* is located in Mesoamerica, although it is now a worldwide crop. The species was introduced to Europe first to the Iberian Peninsula and subsequently to England until it spread throughout the whole continent (Gepts & Bliss 1988).

Nowadays the wild distribution range of common bean extends from northern Mexico to northwestern Argentina (Bitochi *et al*., 2012). Thanks to various genetic analyses, it has been determined that the species comprises two well-differentiated ecogeographic genetic pools, the Mesoamerican (mainly Mexico) and the Andean (southern Peru, Bolivia, and northern Argentina) gene pools. The two gene pools are differentiated by partial reproductive isolation, leading to morphological and genetic divergence (Galván *et al*. 2006; Li & Olsen, 2016; Rendón *et al.,* 2017; Raggi *et al*., 2019).

Although the species has its origin in the New World, several secondary centers of diversification, including Europe, Brazil, central and southern Africa as well as China, have been proposed due to the high level of genetic diversity of found in these areas (Bitochi *et al.,* 2017).

1.1.4 Economic importance

Common bean is the most important legume grain in terms of direct human consumption, with a production of around 23 million metric tons (MT) (Singh, 2007). It is of particular importance in Latin America and Africa, where seven of the 23 million MT are produced, almost twice as high as chickpea production (Broughton *et al*., 2003).

Beans are the most consumed legume around the world; this crop provides around 15% of daily calorie intake and up to 36% of total daily protein in many Latin-American and African countries. Almost 200 million people in sub-Saharan Africa consume common beans as a primary staple (Schmutz *et al*., 2014). The economic importance of beans extends not only to undeveloped countries but also to countries with high economic development as the United States where this

crop is considered a commodity valued in around 1 billion dollars per year. In the US, areas of the highest production include North Dakota and Michigan. After dried peas, common bean is also the biggest dried leguminous vegetable imported into Europe (fig. 3; Hoyos-Villegas *et al.*, 2017).

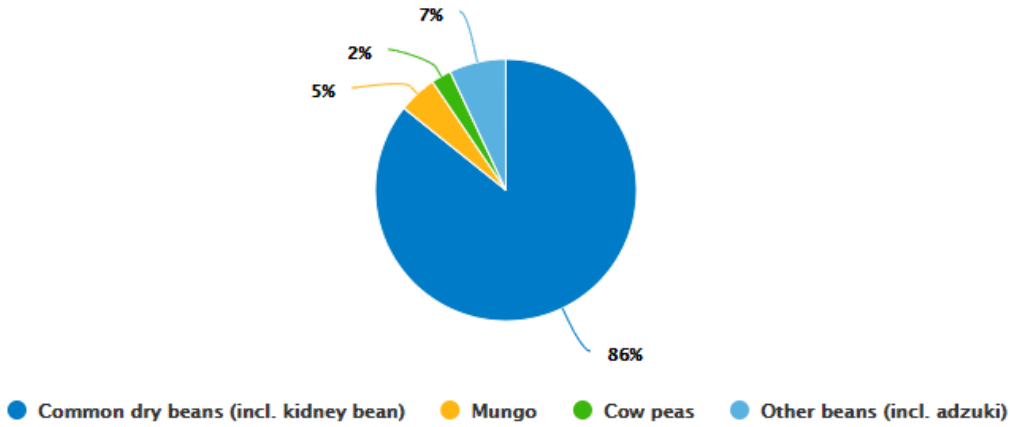

Figure 3. European import of dry beans in 2018 in 1000 tones. Source: Eurostat / Market Access Database, 2019.

Bean cultivation is not limited to Latin America or to developing countries; the United States leads the list in the production of common beans. Furthermore, about half of the US bean acreage is grown under rainfed conditions, making it susceptible to intermittent drought. On the other hand, maintaining the crop under external sources of irrigation involves a higher cost (Trapp, 2015).

In view of the accelerated increase of the human population and the upward trend in bean consumption, a greater demand for common bean is expected in the future. To achieve this and ensure food security in the face of the challenge presented by climate change, immediate breeding objectives include an improvement of productivity, nutritional quality as well as resistance to biotic and abiotic factors.

1.2 Drought overview

Drought stress is one of the abiotic factors that are most limiting to bean production, affecting up to 60% of worldwide production, and it is the second cause of loss of yield after diseases (Villordo-Pineda *et al.*, 2015).

Drought can be divided into three different types based on when it occurs (fig. 4). When there is a water shortage during the first two weeks after planting, it can be defined as early drought. When there are short periods of drought within the entire phenological cycle, it is called intermittent drought. Finally, in terminal drought, there is a long period of water scarcity that affects the stages of flowering and grain filling (Durán, 2016).

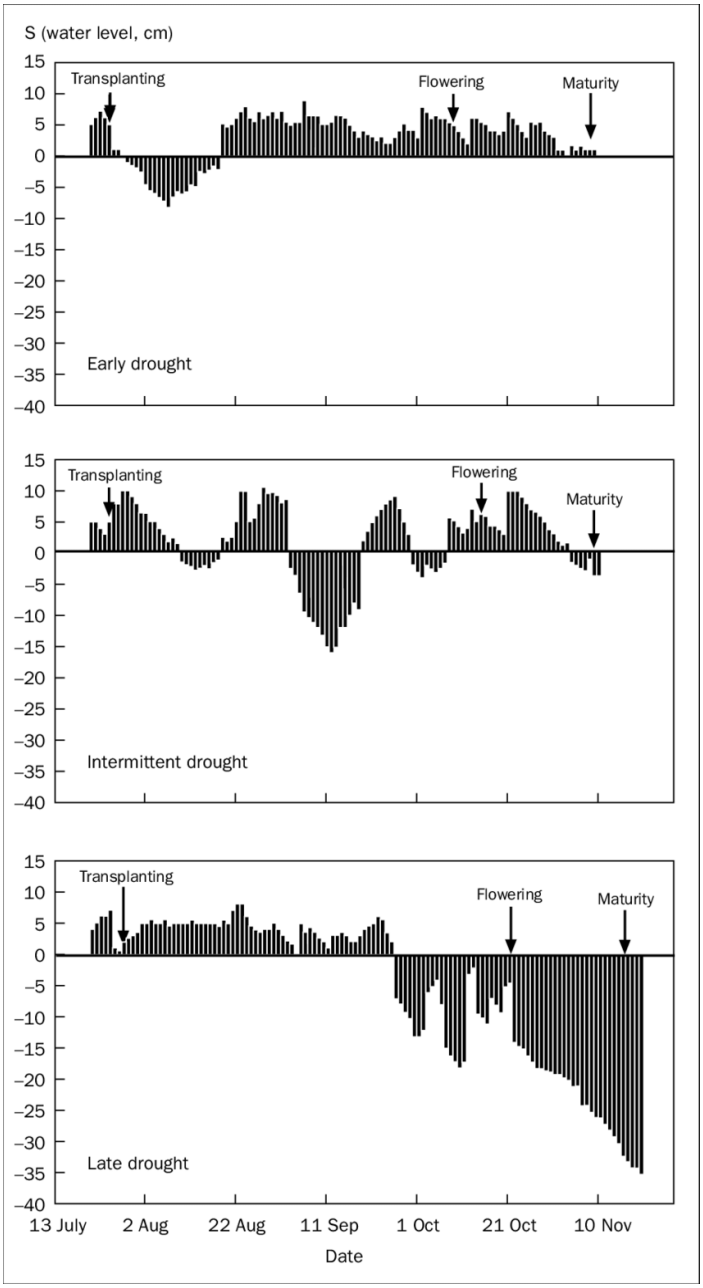

Fig 4. Different types of drought events according to the pattern of the level of free water. Source: Lafitte *et al.,* 2013.

Drought episodes can be produced by various environmental factors, such as periods of little or no rain, and they depend on the type of soil, its capacity to retain water, as well as the evapotranspiration rate. Drought stress can occur even if the water is not scarce, for example in saline environments and in soils with temperatures between 0-15°C. (Rosales-Serna *et al.,* 2014).

1.2.1 Strategies to cope with drought stress

Crop phenotype relies on the genotype (G), in combination with the surrounding environment (E) and the interaction between the two (GxE). Predicting phenotype from environmental and genetic information would be of great value for plant breeders because it could facilitate the development of cultivars adapted to a specific range of environments.

The mechanisms behind drought resistance are not easy to elucidate as they are made up of various components. For example, early maturation and accelerated seed development allow plants to complete their life cycle before the onset of a harsh period of drought. Some plants instead have evolved special morphological, physiological, or anatomical characteristics to maintain high water potential in the face of water scarcity or while others have evolved mechanisms that allow them to survive with a low water potential (Sedlar *et al.,* 2019). These strategies have been categorized in different ways, classically divided into escape, avoidance, tolerance and, recovery, although the different strategies are not mutually exclusive. The strategies are described in more detail below (Rosales-Serna *et al.,* 2014).

- *Drought escape* relies on rapid reproduction before drought strikes. A successful reproduction involves a better partition of assimilates towards the seeds and fruits and the plant must have therefore have the capacity to store reserves efficiently in organs, such as stems and

roots, and be able to relocate them for the production of fruits. This strategy is been widely seen in annuals and especially in ephemeral plants in desert environments (Bacelar *et al.,* 2012).

- *Drought avoidance* refers to the ability of the plant to maintain high water content in the tissues despite the lack of water (Basu *et al.,* 2006). This strategy is commonly found in plants with tissues sensitive to dehydration and which therefore needs to avoid a water deficit. This has been seen in both annual and perennial plants and is characterized by the development of various adaptive traits (Bacelar *et al.,* 2012), such as a highly branched and deep root system, which allows a more effective water absorption; the reduction of the foliar area and the closing of stomata to limit the loss of water by perspiration (Sedlar *et al.,* 2019).

- *Drought tolerance* involves a series of adaptations that allow a plant to withstand arid or drought conditions without affecting performance. These adaptations involve mechanisms to maintain turgor pressure through osmotic adjustment that includes an increase in the concentration of solutes, such as sugars, organic acids and ions. Increased cellular elasticity and decreased cell size due to protoplasmic resistance are also mechanisms contributing to drought tolerance (Bacelar *et al.,* 2012; Azhar & Rehman, 2018).

- *Drought recovery* can be defined as the ability of the plant to recover after a period of drought. The mechanisms behind this strategy have not yet been elucidated. However, studies in crops, such as *Medicago truncatula*, suggest that the nutritional status of the plant could be an important part of its post-stress recovery, highlighting the ability of nodulated plants to recover after drought which could be explained by the source of N nutrition. However, the knowledge of antioxidant processes and the dynamics of osmolytes during recovery from drought is limited (Couchoud *et al.,* 2020; Abid *et al.,* 2018).

1.2.2 Stay-green Phenotype

Senescence is an important process in plants for the recycling of resources, such as nitrogen and carbon, from old organs to sink organs or to those under development. This process contributes to the fitness of the plant. Indeed, studies in corn have revealed that a large amount of the dry matter accumulated in the grains is fixed during the filling of the grain, while the remobilization towards the organs developed before flowering is very low (Sekhon *et al.,* 2019).

In stay-green (SG) genotypes, also called evergreen genotypes, there is a delay in senescence caused by the degradation of chlorophyll, contrary to what occurs in normal genotypes. This characteristic represents a very important trait in agronomic terms, as the ability to keep the leaves photosynthetically active can positively influence the subsequent filling of the grain even under stress conditions. There are two types of SG genotypes, functional and cosmetic (fig. 5). A functional SG genotype occurs when the plant performs photosynthesis normally for a prolonged period of time. Two variants of functional SG can be seen, type A, where the onset of senescence is delayed, while in type B, senescence begins normally, but the process is slower (Khamal., *et al.,* 2019). In the cosmetic SG phenotype, the plant retains chlorophyll but its photosynthetic capacity is lost (Thomas & Ougham, 2014). The characteristic of remaining green associated with the prolongation of photosynthesis is possibly related to the strengthening of

the plant throughout its development. Common bean plants that possess this characteristic have been found to have greater resistance to lodging, however, the studies that address the possible advantages and disadvantages of this trait in beans are few (Schmit *et al.,* 2019).

Given the importance of senescence in final crop yield, the development of new varieties with late senescence or stay-green (SG) with delayed physiological maturity is an important key in crop improvement.

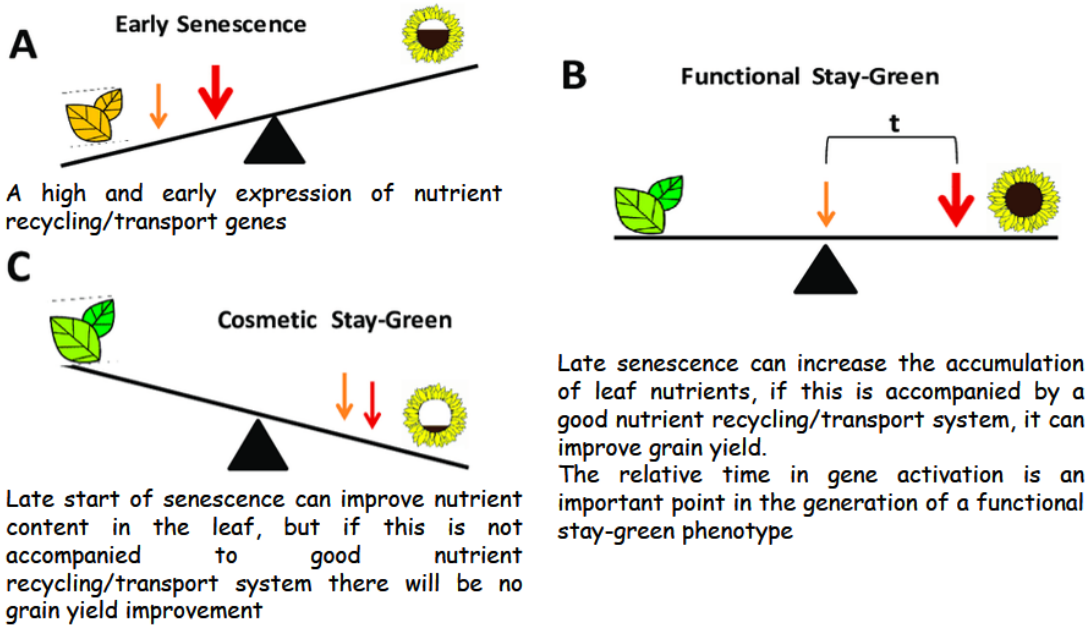

Figure 5. Stay-green phenotype vs. recycling/transport nutrient process. Orange arrows represent upregulation of genes that contribute to the onset of the senescence process. Red arrows represent upregulation of genes related to the catabolic leaf process. Modified from Bengoa *et al.*, 2019.

1.3 Molecular approaches to cope drought

The estimation of genetic differentiation play a central role in population genetics, have broad applications in mapping associations and allows for the identification of genomic regions that have been targeted by natural selection (Holsinger & Weir, 2015).

Wright's index of genetic differentiation (Fst, Wright 1951), is the summary of statistic for variation in allele frequencies among populations and provides a measure of the degree of similarity between individuals within populations. If F_{ST} is small, it means that the allele frequencies within each population are similar; if it is large, it means that the allele frequencies are different. Therefore, it is a very useful tool in genome scans comparing populations (Holsinger & Weir, 2015). Additionally, genome-wide association studies have become an important tool in genetic studies. A GWAS includes the scanning of markers in entire DNA sets, or genomes, in order to find genetic variations associated with a particular trait. In the world of plant breeding, once new genetic associations are identified, researchers can use the information to develop better strategies for the improvement and development of new varieties (NHI, 2020).

Studies of genetic markers associated with drought tolerance in common beans are limited. Mukeshimana *et al*. (2014) reported QTLs for days to flowering and maturity located on chromosome 1 in plants subjected to drought stress. Recent work in a recombinant population of common beans found QTLs for pod harvest index, yield under drought stress conditions, highlighting its importance in the remobilization of photosynthates (Berny Mier y Teran *et al.,* 2020). Asfaw *et al*., (2012) found QTLs for traits related to drought tolerance, suggesting that the fraction of photosynthates remobilized from pods to seed is related to plant performance both under stress and non-stress conditions. Other works have used SNP-type molecular markers in recombinant inbred populations for the construction of linkage maps where they found 14 regions of the genome associated with characteristics that may be related to drought tolerance (Durán, 2016).

Although studies on the SG phenotype and its relationship to drought tolerance are not abundant, candidate genes have been reported in species such as corn (Bengoa *et al*., 2019) and sorghum (Johnson *et al*., 2015; Rama *et al*., 2014). In legumes, sequencing data have allowed advances in this field, for instances studies in chickpea identified a cognate stay-green gene located on chromosome 8 (Sivasakthi *et al.,* 2019).

Hoyos-Villegas *et al.,* (2017) performed a GWAS analysis on a panel of various bean genotypes native of Central America that were selected based on their previously described tolerance to drought. They found several associations in a number of traits related to biomass, seed weight, and wilting that may be involved in drought resistance. At the transcriptional level, Pereira *et al*. (2020) analyzed the response to drought in common bean roots and leaves, contrasting the genotypes BAT477 and Pérola which are resistant and susceptible to drought, respectively.

Despite the efforts made so far, information on the genetic basis of the SG phenotype and its relation with drought resistance remains limited, especially in legumes. For that, the dissection of the SG phenotype is essential to improve bean-breeding strategies, aiming at developing new varieties capable of adapting to adverse environmental conditions.

1.4 Objectives of the study

Identify different strategies against drought in a species-wide collection of *Phaseolus vulgaris.*

Evaluate the response of plants with the stay-green phenotype to drought.

Dissect the genetic basis of the stay-green phenotype.

2. Materials and methods

2.1 Plant material

We used a set of 71 *Phaseolus vulgaris* accessions selected to span the range of natural variation, that were provided by the International Center of Tropical Agriculture (CIAT), the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) and NordGene seed banks. This collection includes accessions from three genetic pools the European, the Mesoamerican and the Andean (table 1). These plants were evaluated in order to identify accessions with 'stay green' and drought tolerance phenotypes.

Table 1. List of the *P. vulgaris* accessions examined in this study. MA Mesoamerican, MW Mesoamerican wild, EU European, A Andean, AW Andean wild.

Accession	Genepool	Accession	Genepool	Accession	Genepool
G11015	MA	NGB23936	EU	PHA6066	EU
G1282	EU	NGB24038	EU	PHA6155	EU
G12865	MW	NGB9300	EU	PHA6254	EU
G12947	MW	PHA13609	EU	PHA6389	EU
G13094	MA	PHA13666	EU	PHA6437	EU
G14629	EU	PHA13736	EU	PHA7150	EU
G19898	AW	PHA13928	EU	PHA725	EU
G21201	AW	PHA13960	EU	PHA 1076	EU
G23426	AW	PHA14278	EU	PHA 99	EU
G23455	AW	PHA167	EU	PHA1022	EU
G23556	MW	PHA1753	EU	PHA1077	EU
G23578A	MA	PHA1772	EU	PHA1086	EU
G3296	MA	PHA2682	EU	PHA1137	EU
G4383	MA	PHA366	EU	PHA1138	EU
G5340	EU	PHA3673	EU	PHA1139	EU
G7930	A	PHA4008	EU	PHA1142	EU
G8658	EU	PHA419	EU	PHA12934	EU
NGB 18415	EU	PHA4534	EU	PHA13035	EU
NGB13468	EU	PHA4620	EU	PHA13099	EU
NGB17826	EU	PHA49	EU	PHA13228	EU
NGB20124	EU	PHA5866	EU	PHA7309	EU
NGB23857	EU	PHA5934	EU	PHA7313	EU
NGB23858	EU	PHA5989	EU	PHA7686	EU
NGB23934	EU	PHA6011	EU		

2.2 Phenotypic data and growth conditions

A first screening was carried out in phytotron climatic chambers. Conditions were kept at 10hr light/14hr darkness, 50% humidity and 18°C darkness 20°C light temperature. We started with a panel of 23 accessions from the three main genepools, Mesoamerican, Andean and European. The opportunity to regulate light and temperature conditions in the chambers allowed us to screen photoperiod sensitive accessions, such as wild genotypes from the Americas. To obtain an accurate assessment of the senescence and drought response across the panel, two replicates of every genotype were planted in small pots and allowed to grow under well-watered (WW) conditions until they reached the pre-flowering stage (R5). At that point, the dry-down was initiated and kept for two weeks (water-stressed, WS). Conversely, the plants under non-stress conditions were watered on a regular schedule. After two weeks of drought treatment the irrigation was reestablished and kept for two weeks. We measured the number of flowers and pods during the treatment and after recovery. This first experiment allowed us to identify different strategies used by the plants to cope drought stress, including the SG phenotype.

In a second stage, we performed a wider greenhouse (GH) experiment that allowed us to screen more accessions and their response to drought. We used a panel of 71 accessions from the three main gene pools, excluding cultivars that were photoperiod sensitive but including wild accessions. The conditions in the GH were: temperature ranging between 25-28° C, 50% humidity and a photoperiod of 16hr light/8hr darkness. The plants were sown in medium pots (10 cm of diameter) with 750 gr of soil and organized in two experimental blocks, WW and WS conditions (fig. 6). The day before the dry-down was initiated all pots were abundantly watered to reach the saturation point, that refers to when all spaces in the soil are filled with water and allowed to drain overnight. Later, we measured the humidity with a kit W.E.T. Sensor HH2 Moisture Meter Delta-T® ensuring it was at least 35% in the starting point. The drought treatment started at the reproductive stage of the plant that begins when the first flower opens (R6 growth stage) (Fernández *et al*., 1986). After two weeks of drought treatment, the plants were re-watered, and the phenotypic traits were measured weekly.

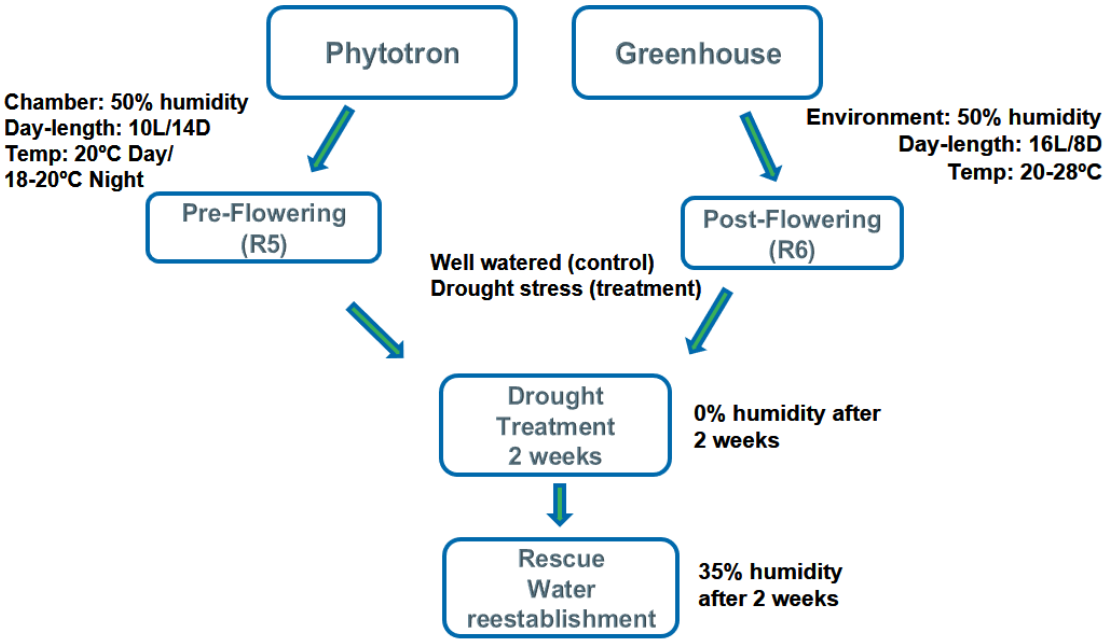

Figure 6. Experimental design to evaluate plant performance under drought stress

The phenotypic data considered in this study include the number of flowers, number of pods per plant, total number of seeds, and number of seeds per pod; the data was collected weekly when possible. Regarding the classification of strategies, in addition to the parameters mentioned above, the development of the plant was closely followed. We took the days to flowering into account to assess the beginning of R5 or R6 and thus, the start of the treatment. The days to flowering correspond to the number of days that the genotype takes from the day of sowing to flower in at least 50% of the sown plants.

The percentage of yield loss or gain was measured in terms of the number of seeds produced in comparison with the control. We used the formula as it follows:

$$\text{Yield loss} = \frac{(control - treatment)}{Control} x100$$

Plant responses to drought were classified according to their performance during the treatment. Stay-green accessions were identified based on the maintenance of greenness in both stems and leaves throughout the treatment. Escape was identified if accessions increased the production of pods in response to stress and had a yield loss less than 75%. Recovery was assigned to accessions that recovered greenness, produced new trifoliate leaves and/or restarted the reproductive stage once irrigation was reestablished.

2.3 Population structure

Using SNP data already available in our group, we performed population structure and GWAS analyses in 85 selected accessions (71 screened + wild accessions included for balancing the number of individuals per gene pool). First, using 126,111 pruned sites along the 11 chromosomes (un-linked sites, with minor allele frequency, MAF >5%), we produced a PCA (plink –pca; v1.90b4.9)

2.4 F_{ST}: Differentiation index

Pairwise F_{ST} between batches of accessions grouped according to their drought response was calculated on each chromosome in the *Phaseolus vulgaris* genome in 50kb, non-overlapping genomic windows, using the python popgen pipeline developed by Simon Martin's group, available at https://github.com/simonhmartin/genomics_general. For this screening, we used all SNPs on each chromosome that passed the following criteria: min/max sequencing depth of 8 and 25 respectively and that were present in at least 70% of the accessions (vcftools --min-meanDP 8 --max-meanDP 25 --max-missing 0.7).

2.5 Genome-wide association studies

An association analysis using minor allele frequency pruned SNPs (MAF>0.05) was used to identify associations between individual SNP markers and the different drought responses. The association analyses was performed using plink –association, where .fam files were generated as follows: individuals belonging to the phenotype to be evaluated were assigned the code "2"; control individuals, i.e. drought intolerant or displaying other responses, were tagged "1"; other individuals included in the vcf file were set as "-9", which means, unknown phenotype. Association values were obtained for each SNP, and the p-values were corrected using FDR adjustment (plink –assoc -adjust). SNPs displaying an FDR<0.05 were binned in 10KB windows, and the gene content in each of those bins was later analyzed for functional terms. Manhattan plots were constructed using the R module qqman (v.0.1.4).

3. Results

3.1 Drought stress responses

Once germinated, the plants took between 25 and 30 days to reach the flowering stage. After two weeks of severe drought stress, we were able to identify cultivars displaying at least one of the drought tolerance strategies described above. We observed six SG accessions, 16 escaping drought and 17 that recovered after irrigation re-started (Table 2). It was noteworthy that the results from the phytotron and greenhouse were consistent for those accessions that were evaluated under both conditions, even when drought stress was initiated at different points in the development of the plants, pre-flowering (R5) and flowering (R6), respectively. For example, PHA6155 and PHA2682 cultivars displayed SG phenotype under both conditions. Additionally, under GH conditions, thanks to the increase in number of accessions, it was possible to identify four more SG cultivars (Fig. 7, Supplementary Fig. 18, 19 & 20).

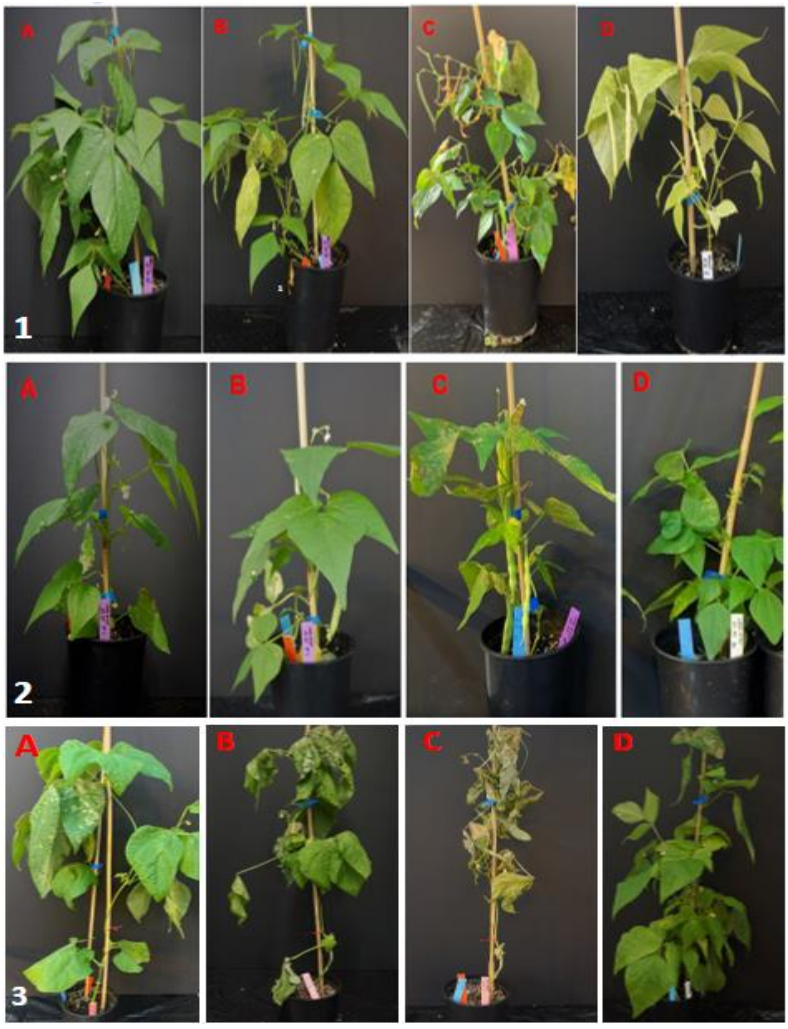

Figure 7. Accession PHA6155 (1), NGB 18415 (2) during the weeks of treatment (WOT). It can be seen the delay in senescence. Accession PHA 13609 (3) sensible to drought A: 1 WOT, B: 2 WOT, C: 1 WAR. D: Control. WOT=weeks of treatment, WAR=Weeks after re-watering

As the first response to deal with drought, we observed that a large number of plants began to accelerate their phenological process, increasing the number of pods produced especially during the first week of stress (fig. 8). However, we observed a high percentage of pod abortion; due to this, those accessions that followed this behavior but managed to complete their cycle and had a yield loss no larger than 75% were considered as “escaped”. Examples of the escape strategy are highlighted in figure 8 (red boxes), such as PHA1142, G11015 and PHA6254.

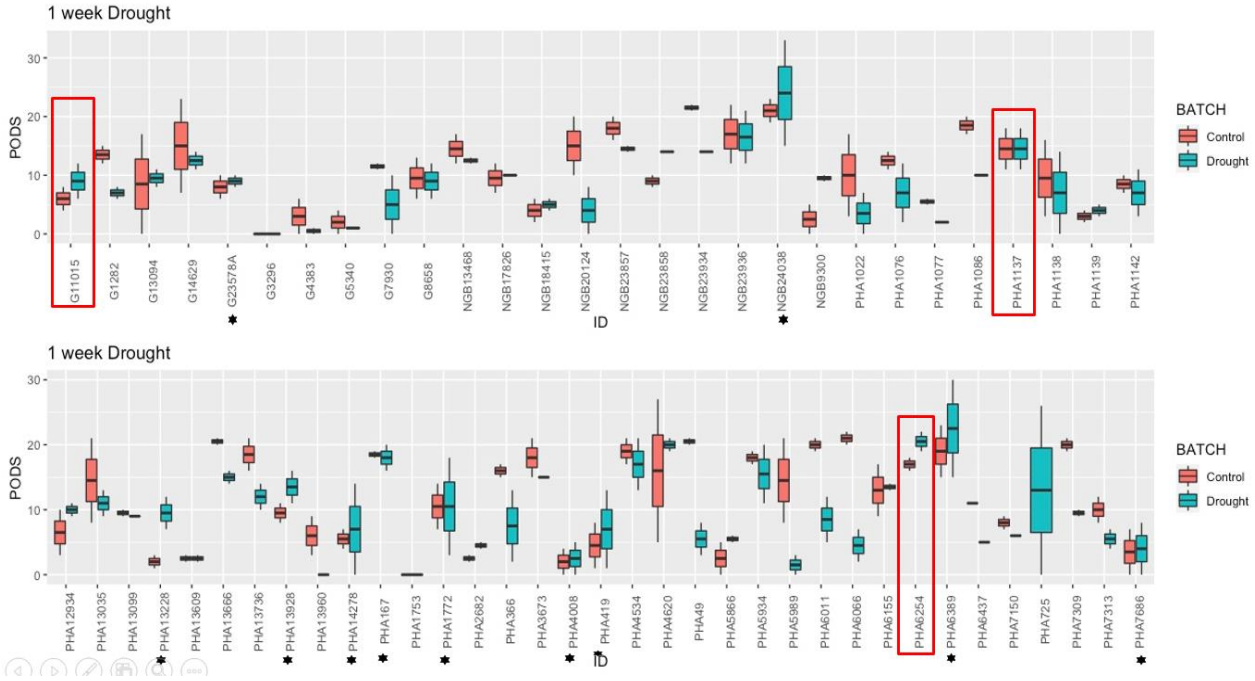

Figure 8. Number of pods produced after 1 week of treatment (WOT). Control correspond to the pink boxes vs Treatment. Red square represents accessions that escape to drought. Accessions marked with * accelerate production of pods

Once the drought treatment finalized, the plants were re-watered until the soil reached ~35% moisture. Following this, we found that 17 cultivars recovered successfully regardless of their performance during the experiment. They recuperated their greenness and produced new trifoliate leaves (fig. 9). In some of them even reproduction was restarted.

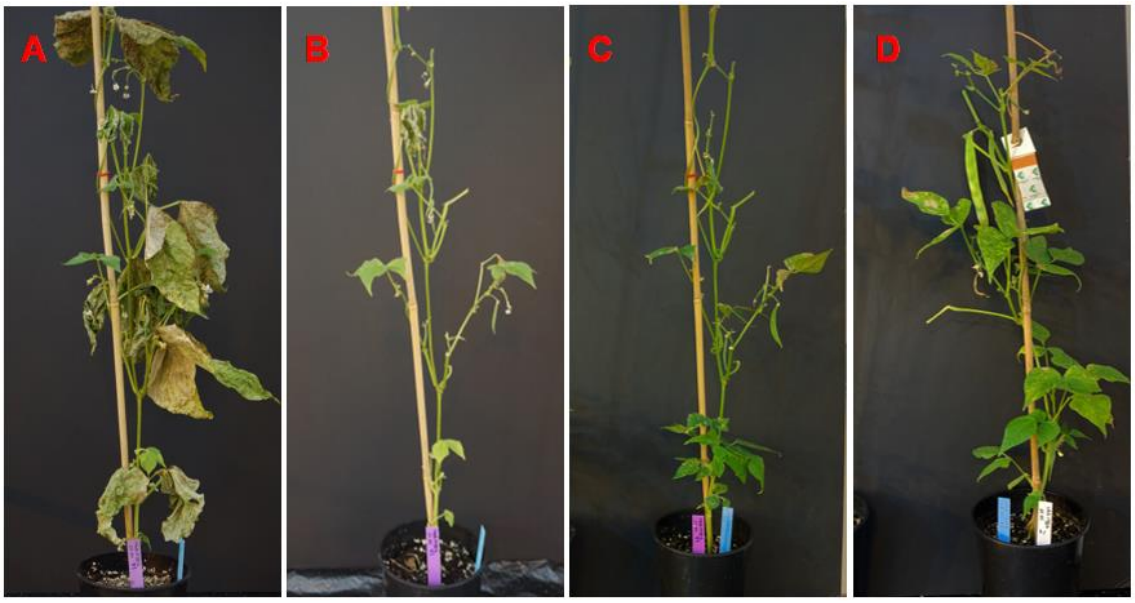

Figure 9. Accession G7930 during the weeks of treatment (WOT). A: 1 WOT, B: 2 WOT, C: 1 WAR. D: Control. WOT=weeks of treatment, WAR=Weeks after re-watering

In total, 22 accessions were considered susceptible (Table 2), since they suffered yield loss exceeding 75% (Fig. 11) or they were dead after the two-week treatment. For 14 of the accessions, it was not possible to evaluate their performance to drought under GH conditions since they did not reach the flowering stage. However, with the data previously obtained in phytotron chambers we identify some of these strategies in photoperiod sensitive accessions G3296 (MA) that followed escape, and G23458 (AW) and G12875 (MW) recovered after the stress.

Table 2. Strategy followed by the different accessions. E= escape, R=recovery, S=sensible, EG=Evergreen and NA=No data

Accession ID	Strategy	Accession ID	Strategy	Accession ID	Strategy
G11015	E	G16843	NA	PHA6437	R
G14629	E	G19898	NA	PHA7309	R
G8658	E	G21043	NA	PHA7313	R
NGB23936	E	G21201	NA	G13094	S
PHA1076	E	G23426	NA	G23556	S
PHA1137	E	G23455	NA	G4383	S
PHA1138	E	G24323	NA	NGB13468	S
PHA1142	E	PHA1753	NA	NGB23857	S
PHA13666	E	PHA725	NA	NGB23934	S
PHA13928	E	PHA7686	NA	NGB24038	S
PHA4534	E	PHA99	NA	PHA1086	S
PHA4620	E	G1282	R	PHA12934	S
PHA49	E	G23578A	R	PHA13228	S

PHA5934	E	G3296	R	PHA13609	S
PHA6066	E	G5340	R	PHA13736	S
PHA6254	E	G7930	R	PHA13960	S
NGB18415	EG	NGB17826	R	PHA14278	S
NGB9300	EG	NGB20124	R	PHA1772	S
PHA1077	EG	NGB23858	R	PHA3673	S
PHA2682	EG	PHA1022	R	PHA4008	S
PHA366	EG	PHA1139	R	PHA419	S
PHA6155	EG	PHA13035	R	PHA5866	S
G12865	NA	PHA13099	R	PHA6011	S
G12947	NA	PHA167	R	PHA6389	S
G13955	NA	PHA5989	R	PHA7150	S

3.2 Yield

When evaluating the performance in terms of seeds per plant (fig. 11), only six accessions under drought stress were capable of producing more seeds than their controls in the final harvest (Fig.10), interestingly three of these accessions (NGB18415, PHA6155, and PHA2682) displayed the SG phenotype. The other three accessions followed the escape strategy.

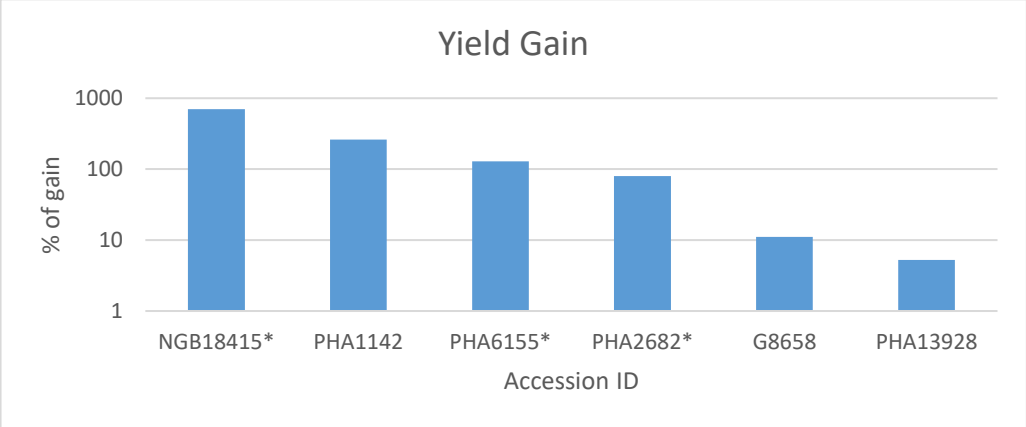

Figure 10. Gain yield in percentage of seeds in comparison with the control. Accessions marked with * denotate stay-green phenotype.

Grain yield was calculated in terms of seeds per plant at the final harvest. As expected, based on previous observations in common bean, most genotypes suffered substantial yield loss under drought conditions (Fig. 11). However, accessions G1282, PHA13099, PHA167 (consider as R), PHA4534, PHA1137 and PHA13666 (consider as E) had a loss of less than 35% and although these accessions do not show a stay-green phenotype, they might be a good alternative for breeding programs aimed at breeding for drought tolerance. On the other hand, accessions PHA4008, PHA1086, PHA419, PHA12934, PHA13609, PHA14278, and PHA3673 were the least

productive, with yield losses exceeding 85% and hence were consider as highly susceptible to drought.

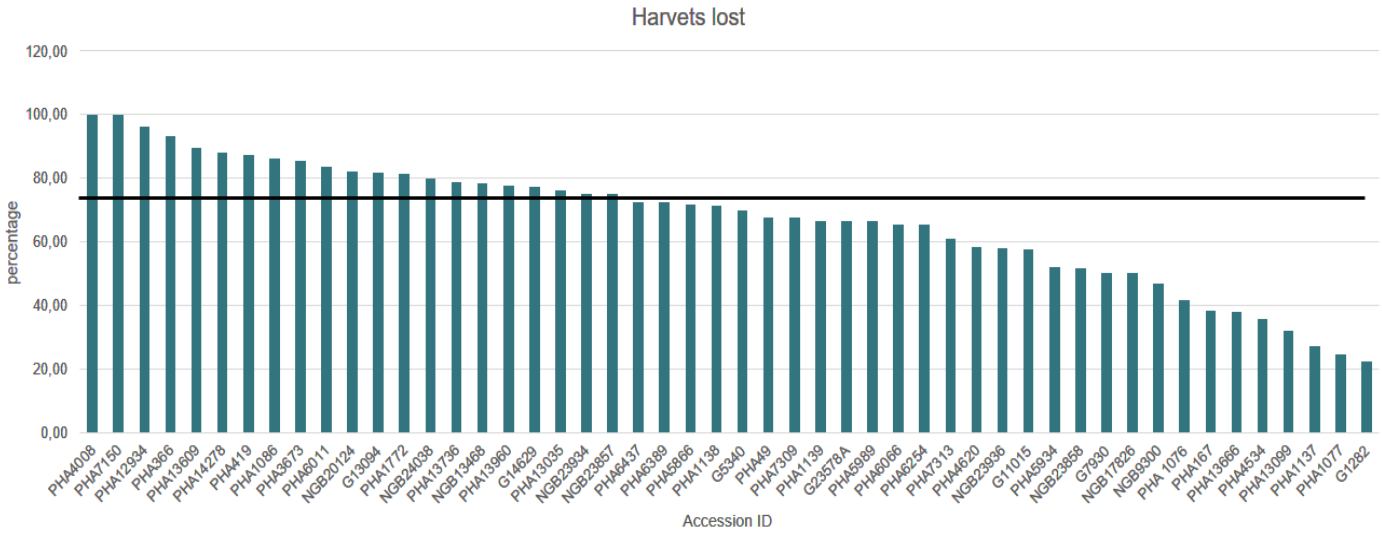

Figure 11. Final yield in number of seeds of the accessions in terms of percentage lost with respect to the control. Accessions under the black line a yield loss of less than 75%.

3.3 Population structure

A SNP-based principal component analysis was carried out (Fig. 12) in order to understand the population structure in common bean, as well as to assess the distribution of the drought responses across the gene pools. Consistent with the sites of collection, the accessions were grouped according to their Mesoamerican (MA), Andean (A) or European (EU) origin, the latter represented by a large cluster that comprises apparent hybrid individuals between MA and A, whereas others display a clear, almost intact MA or A genetic background (Fig. 12, left panel). We identify 15 accessions as escape, 14 as recovery and 6 as stay-green. Although most of the accessions that showed some type of drought resistance have an European origin, (due to a bias in the number of EU samples considered from the start point), drought tolerance is present in all three gene pools, as can be seen in the right panel of Figure 12, where the accessions are colored according to their drought response. Interestingly, all accessions identified as SG belong to the EU gene pool.

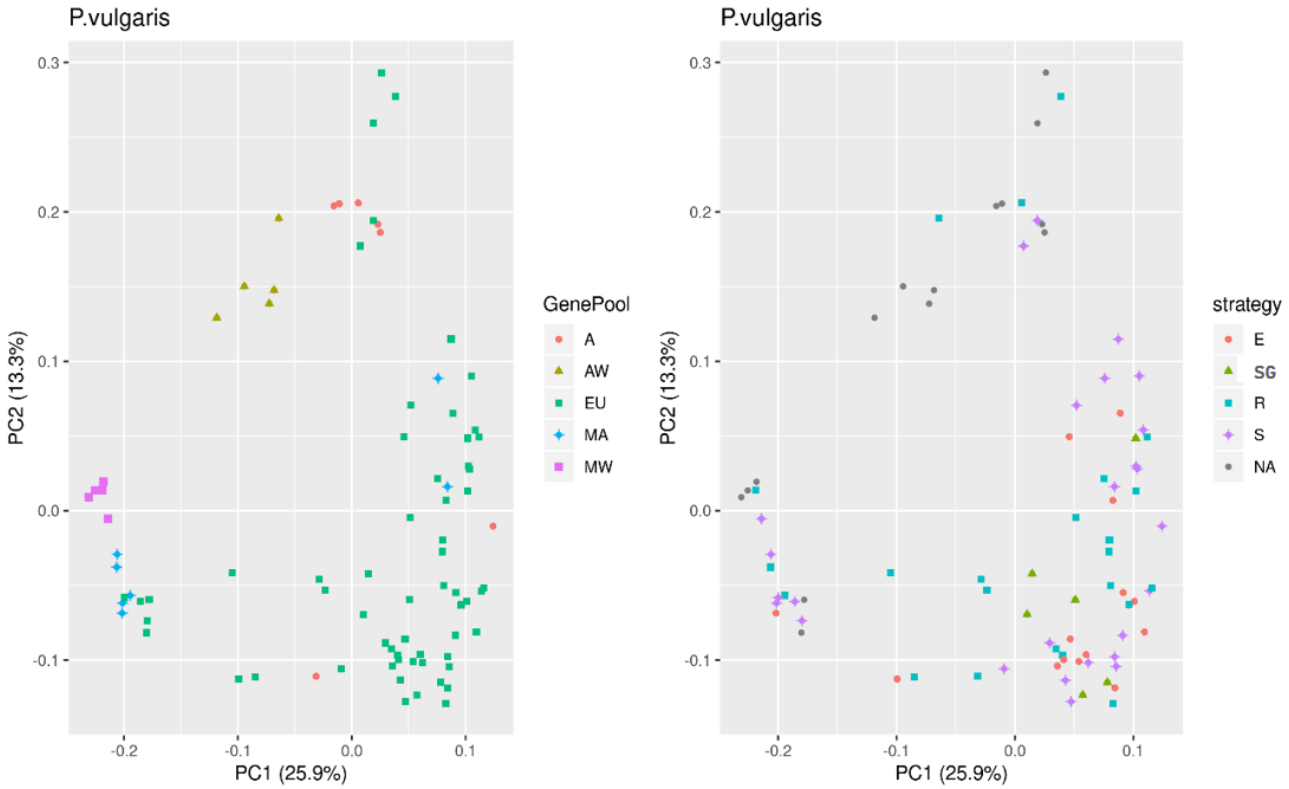

Figure 12. Principal component analysis demarcating the accessions belonging to each gene pool. Left side colored by gene pool and right side colored by strategy. Genepool: A=Andean, AW=Andean wild, EU= European, MA=Mesoamerican, MW=Mesoamerican wild. Strategy: E=escape, SG=stay-green, R=recovery and S= susceptible**.**

3.4 F_{ST}: Differentiation index

We calculated pairwise F_{ST} between the subpopulations obtained after classifying the bean accessions according to their response to drought, stay-green (SG), escape (E), recovery (R) and susceptible (S). We obtained F_{ST} outliers in chromosomes 01, 05 and 10 that reached values above 0.2 while the genome-wide average between strategies were all well below 0.02: $\bar{X}_{(SG\ vs\ E)}$=0.0125, $\bar{X}_{(SG\ vs\ R)}$= 0.013 and , $\bar{X}_{(SG\ vs\ S)}$= 0.009.

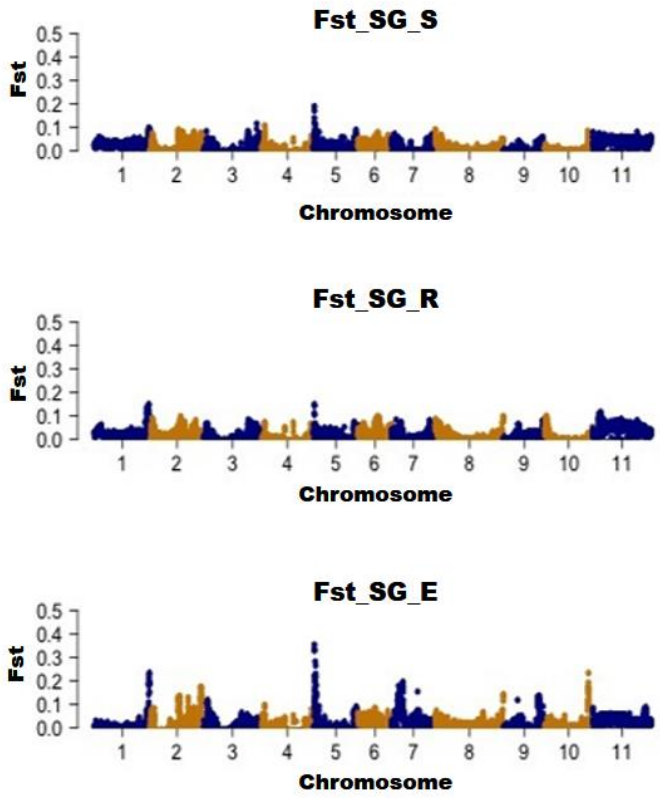

Figure 13. Fst analysis showing differentiate regions in EG the across the chromosomes in comparison with the other strategies found.

The most strongly differentiated region on chromosome Pv01 spans from 50.45-50.90 Mb. In chromosome Pv05 we identified four regions, located at 0.55-0.75 Mb, 1.0-1.05 Mb 1.40-1.50Mb and 1.80-1.90. Remarkably, it is in this chromosome where the highest differentiation occurs, $F_{st\ SGvsE}$>0.35. Chromosome 10 has the most relevant Fst outlier located at 40.25 to 40.30 Mb.

The functional description of the protein-coding gene encoded in these regions will be described below.

3.5 Genome-Wide association analysis

GWAS analysis showed multiple associations with the SG trait based on 126,111 SNPs (fig.14). The resulting p-values were corrected and at a FDR cutoff of 0.05, we identified 52 significant SNPs on chromosomes 01, 05, and 10. Consistent with our previous analysis, the regions were these SNPs were located match the F_{ST} outliers.

In chromosome Pv01, the region harboring the majority of associated SNPs (14 SNPs) goes from 50.83Mb-50.845Mb. Within these coordinates, we identified the gene model *Phvul.001G260300* (from 50838135-50893093pb). Four significant SNPs fall within this gene while the three SNPs more are located 2kb upstream of the gene, which could indicate that they play a regulatory role in the expression of the gene. It also coincides with the most strongly differentiated region in our F_{ST} analysis. In chromosome Pv05, 13 SNPs were identified in a region spanning 48000pb to 610000pb and this region matches the most differentiated window in the F_{ST} analysis (>0.35).

The strongest association (i.e., the lowest p-value) was observed on chromosome Pv10 where we identified 21 significant markers located across two regions, from 40.46-40.50 Mb and 42.29-

42.63Mb. Seven SNPs fall within the gen model *Phvul.010G144600*, remarkably, the two most strongly associated SNPs located at 42 612 777pb and 42 612 787pb.

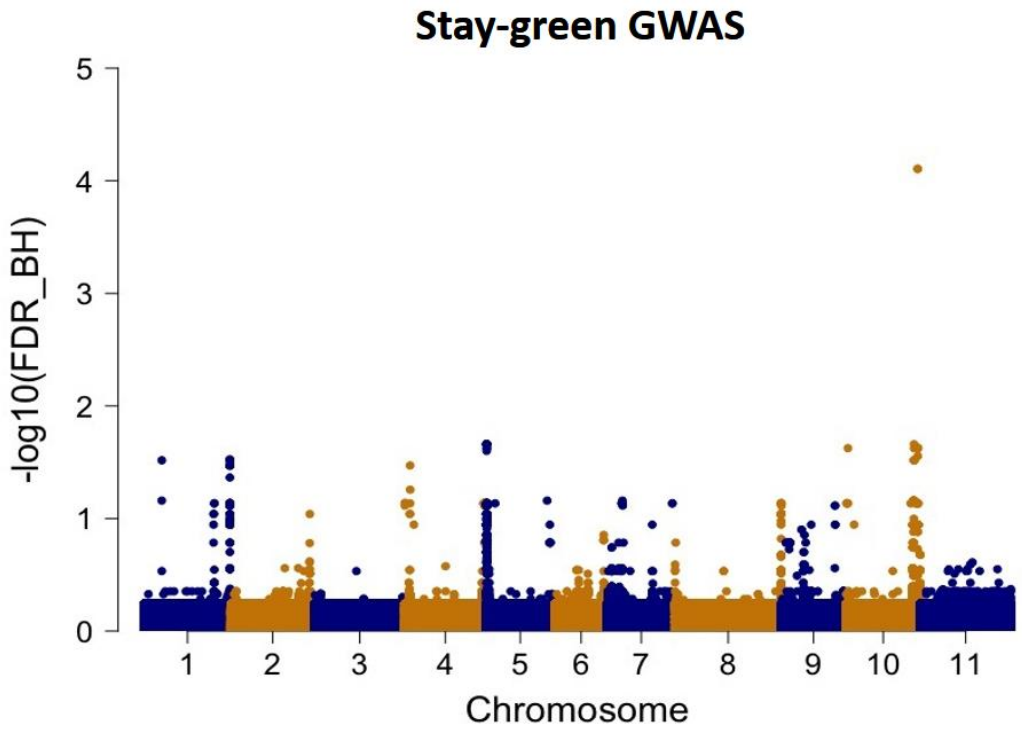

Figure 14. Manhattan graph from an association study of the evergreen phenotype across the genome.

3.6 Putative candidate genes

Once the genomic regions associated to the SG phenotype were defined, we searched for candidate genes located in these regions and assessed their functional annotations. We observed 118 genes with different annotations that are summarized in Sup. Table 3 and 4. Among these, three genes called our attention as they have been directly associated with the senescence processes and stress response in other species. On chromosome Pv01, the gene model *Phvul.001G260300* (50838135-50893093 bp) encodes a ARM repeat superfamily protein whose orthologue in *Arabisopsis thaliana* encodes the RING-type E3 ubiquitin ligase *PUB*6. On chromosome Pv05 we identified the gene *Phvul.005G008300* (719 827pb-725 719 pb) that was located in the most strongly differentiated region according to the F_{ST} analysis (F_{ST}>0.30) and ~10kb upstream from significant GWAS SNPs. This gene encodes a trehalose-6-gene – phosphate-phosphatase (TPP6).

The third region with high number of SNPs is localized in chromosome Pv10. Interestingly the most significant SNPs (with the the best FDR; fig. 14) are located within a gene model, *Phvul.010G144600* (42 612 137-42 614 661 bp), annotated as a HAD superfamily, subfamily IIIB acid phosphatase.

4. Discussion

One of the main constraints around the world for crop productivity is drought. To cope with this abiotic stress, it is necessary to understand the response mechanisms of plants that face scarce water conditions in order to improve yield (Huang *et al.,* 2008). Common bean is highly sensitive to variations in temperature, humidity and amount of nutrients (Schmit *et al.,* 2018), hence unraveling the mechanisms behind drought tolerance is of utmost importance for its production.

4.1 Drought stress responses

Plants use various strategies to cope with drought, generally grouped into escape, avoidance (mostly in CAM plants), tolerance and recovery (Rosales-Serna *et al*., 2014). Although it is not strictly considered as part of the basic strategies, delayed senescence seems to play an important role in drought tolerance as well (Sekhon *et al*., 2019). Drought affects more or less severely depending on the developmental stage of the crop when it occurs. In the emergence and vegetative growth stages, the numbers and biomass are reduced. However, in general, dry beans are more sensitive to drought during the pre-flowering and flowering stages, causing an excessive abortion of flowers, young pods and seeds (Singh, 2007). Because of this, we decided to evaluate the drought strategies at both stages, pre-flowering and flowering, in our study and in climatic chambers and under greenhouse conditions, respectively.

In this project, we were able to observe and describe different phenotypic responses to drought stress in common bean (Table 2). Not only were escape and recovery observed, but we also identified accessions with a clear stay green (SG) phenotype in our collection. Our classification of responses was based on the final yield loss, greenness kept during the experiment as well as the ability of the plants to produce new trifoliate leaves and even re-start the reproductive stage once irrigation started again.

As expected, several accessions could not tolerate the severe lack of water and died or had a yield loss of >75% and these were considered susceptible to drought. We identified 15 accessions as escaped, 14 as recovered and 6 as stay-green. Although photoperiod sensitivity did not allow us to evaluate wild accessions under greenhouse conditions, we could identify three drought tolerant accessions, G3296 (MA), G12875 (MW) and G23458 (AW), in the climatic chambers which gives us an indication that resistance is not associated with a particular gene pool.

Six of our screened accessions showed an important yield gain, as depicted in Figure 10; remarkably, three of them, PHA6155, NGB18415 and PHA2682 belong to the EU gene pool and were also classified as SG plants. Based only on the greenness during the experiment, which evidently encompasses many physiological processes beyond late senescence (Pinto *et al.,* 2016), a total of six accessions were classified as SG, despite the differences in yield gain or loss they displayed. For instance, PHA366, had 93% of yield loss although it recovered quickly after irrigation was re-established. Furthermore, the SG behavior was consistent under phytotron and greenhouse conditions in those accessions that were evaluated both times (PHA6155 and PHA2682), which means that the SG phenotype persists independently of the start point of the drought stress.

The onset of foliar senescence depends mainly on the ontogeny of the plant. However, this process can be induced prematurely to accelerate the remobilization of nutrients in response to environmental changes, such as biotic or abiotic stress conditions. This process provides enough energy to start the reproductive stage, especially important in annual species, in order to complete their life cycle and generate offspring (Luoni *et al.,* 2019). This was observed in the majority of the screened accessions (Fig. 9) that tried to accelerate their reproductive process by increasing pod production, especially during the first week of treatment, although in many cases the pods were aborted or not filled with seeds. The opposite was observed in the SG genotypes, in which development was not interrupted, just slowed down while water was scarce. The fact that these plants could be harvested, even with differences in yield loss, suggests a functional SG phenotype, probably type A. In this type of SG response, the onset of senescence is delayed compared to susceptible plants as the duration of photosynthetic activity of plants is prolonged (Kusaba *et al.,* 2013). It is evident in that senescence is closely related to important agronomic traits such as biomass and harvest index and in fact, genetic association studies found candidate genes that control senescence and nutrient recycling (Moghaddam *et al.,* 2016). This reinforces that the SG trait could be favorable for yield and performance of plants that face abiotic stresses such as drought. Fixing such a phenotype in breeding programs would represent a major advance for coping with dry climatic conditions.

4.2 Genetic basis of stay-green phenotype

The SG strategy has been identified in various crops as a key component in breeding to increase yield and stress tolerance to drought and salinity (Luche *et al.,* 2015). The advantages provided by delayed senescence have been previously reported in model species such as *Arabidopsis thaliana* (Wingler *et al*., 2012) and in some cereals (Fahad *et al*., 2017), where a greater capacity to tolerate abiotic stress as high temperatures and drought in green genotypes was identified. This increased tolerance comes as a result of the protection of photosynthetic structures against reactive oxygen species, such as superoxide and peroxide (Luche *et al*., 2015). Also, the relationship between senescence and stress caused by drought in plants became evident when studies on multi-parent advanced generation inter-cross (MAGIC) wheat lines indicated that, in general, in all lines the onset of senescence can be predicted from the plant water consumption (Camargo *et al.,* 2019). In common beans, drought tolerance has been studied on several occasions (Asfaw *et al.,* 2012; Durán 2016; Hoyos-Villegas *et al.,* 2017); however, few studies address the relationship between delayed senescence and its role in drought tolerance.

In this study, combination of F_{ST} outlier and GWAS analyses made it possible to detect genomic regions and SNPs on three chromosomes: Pv01, Pv05, and Pv10, significantly associated with the SG phenotype. The functional annotation of the genes encoded in these genomic windows revealed candidate genes that could play important roles in the control of drought tolerance (Table 3).

First, we identified a relevant drought response candidate gene on Chr01, *Phvul.001G260300* (located between 50838135 and 50893093 bp; F_{ST}>0.2; four associated SNPs, FDR <0.05), which encodes a RING-type E3 ubiquitin ligase. It is well known that the ubiquitin-proteasome system is highly conserved in eukaryotes. It involves an intricate collection of enzyme complexes, which conjugate ubiquitin for specific targets and hence facilitate the degradation of ubiquitinated proteins. Target proteins are ubiquitinated through a cascade of ATP-dependent reactions that

involve the serial action of three enzymes: ubiquitin activating enzyme E1, ubiquitin conjugating enzyme E2, and ubiquitin ligase E3. The resulting polyubiquitinated proteins are degraded through the 26S proteasome (Hershko & Ciechanover, 1992). In this way, it is not surprising that E3 ubiquitin ligases also play an important role in mediating cellular responses to drought stress. For example, in *Arabidopsis thaliana* the 26S proteasome mediates the degradation of the *DEHYDRATING ELEMENT BINDING PROTEIN* (*DREB2A*), through the negative regulation of drought-sensitive gene expression DREB2A-INTERACTIVE PROTEIN1 (*DRIP*1) *and DRIP*2, hence avoiding dehydration. Additionally, it was reported in *Arabidopsis thaliana* that the action of *PLANT U-BOX*22 (*PUB*22) and *PUB*23, both E3 U-box type ubiquitin ligases that jointly regulate a drought signaling pathway by the ubiquitination of the cytosolic *REGULATORY PARTICLE NON-ATPASE12A* and the drought stress-induced *Rma*1*H*1 (Zhang *et al*. , 2017).

Additionally, several reports have demonstrated that single E3 ligases are involved in ABA-dependent or independent pathways in response to drought and salt stress. In fact, in *Arabidopsis thaliana* it has been shown that the *SDIR*1-*ATP*1/*SDIRI*P1 complex plays an important role in ABA signaling through the ubiquitination pathway. The RING finger E3 ligase, *SALT-AND DROUGHT-INDUCED RING FINGER*1 (*SDIR*1) acts as a positive regulator of stress signal transductions involving abscisic acid (Fig. 15). It regulates abscisic acid (ABA) and salt stress responses by destabilizing *ATP*1/*SDIRIP*1complex (Le & Kim, 2011).

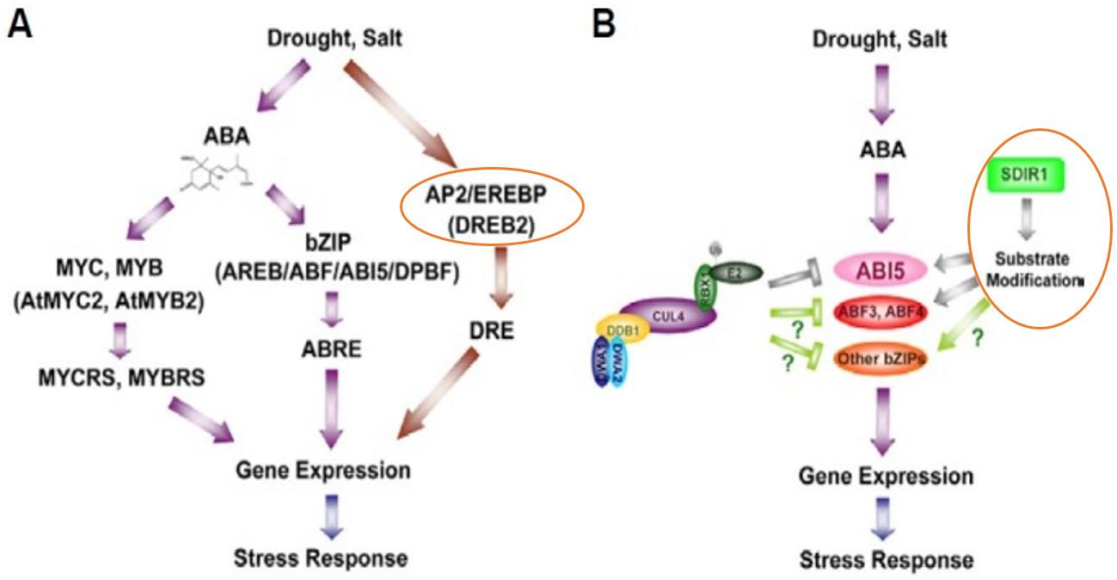

Figure 15. Rol of E3 ubiquitin ligase in drought response. (A) represents the involvement of E3 ligases in ABA-mediated drought signaling via AREB/ABF/ ABI5/DPBF bZIP subfamily (B). 'Substrate modification' indicates either negative regulation by polyubiquitination or positive regulation by monou-biquitination. Modified from: Le & Kim, 2011.

Studies in hot pepper report the activity of *Capsicum annuum RING type E3 Ligase* 1 gene (*CaREL*1), which encodes a RING type E3 ligase, a negative regulator of drought stress via

inhibiting ABA sensitivity. Adititionally *CaREL* expression results in lower acummulation of the stress-responsive genes *DREBA*, *RAB*18, *RD*20, RD29*B*, *RD*29*A*, and *KIN*2 (Lim *et al.,* 2017).

Another drought response candidate gene is located on Chr05, *Phvul.005G008300* altought no significant SNP was found within the gene it is located in the most strongly differentiated region according to the F_{ST} analysis (F_{ST} >0.3). The orthologue of this gene in *A. thaliana, AT5G51460*, is annotated as a trehalose-phosphate phosphatase A (*AtTPPA*) that removes the phosphate from trehalose 6-phosphate to produce free trehalose (Ponnu *et al.,* 2011). The accumulation of this free sugar improves abiotic stress tolerance, as it has been reported to have a function in stabilizing proteins against denaturation (Suárez *et al.,* 2008). Given that, the concentration of trehalose in the cell is very low (approximately three orders of magnitude lower than sucrose), small changes in its concentration can lead to large changes in stress tolerance compared to other sugars (Lin *et al*., 2019). In fact, trehalose-6-phosphate acts as a marker molecule for detecting the concentration of saccharose; this is especially important since carbohydrate and sugar storage and remobilization are major process in the greenness maintenance (Fig. 16) (Jagadish *et al.,* 2015).

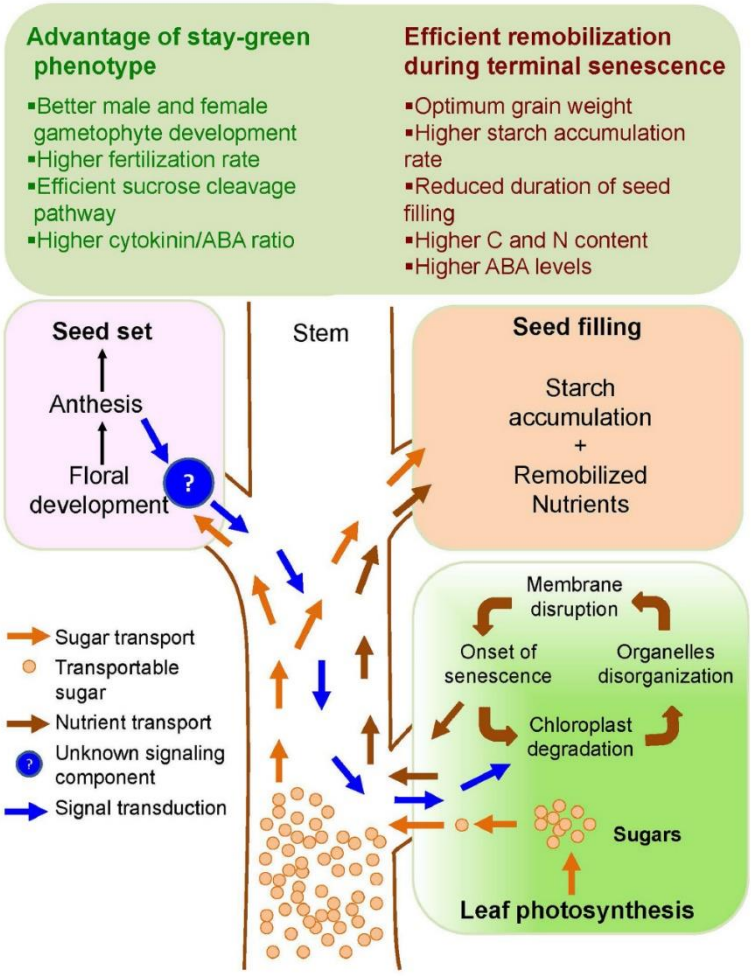

Figure 16. Schematic diagram showing integrative effect of stay-green and terminal senescence traits in plants. Source: Jagadish et al., 2015).

Trehalose-6-phosphate synthase (*TPS*) and *trehalose-6-phosphate phosphatase* (*TPP*) are the catalysts in the synthesis of trehalose. As reviewed by Oladosu *et al*, 2019, the expression of a

fusion *TPP*/*TPS* gene from *E. coli* in rice, resulted in a higher concentration of trehalose and better resistance to drought and less photooxidation to salt stress. Similarly, in *A. thaliana*, the loss-of-function mutation of a *trehalose-6-phosphate phosphatase* (*TPPF*), resulted in a drought-sensitive phenotype, while overexpression of the gene triggered a significantly increased drought tolerance and trehalose accumulation (Lin *et al*. 2019).

The effects of trehalose were found to be suppressed by autophagy inhibitors, so trehalose concentrations have been associated with autophagy and not with proteasome-mediated pathways (Williams *et al.,* 2015). As mentioned above, protein degradation via proteasome is important in resistance to abiotic stress (Wang & Schippers, 2019), however autophagy could also play an important role since autophagy triggered by trehalose does not involve reactive oxygen species. Therefore, it has been proposed that trehalose metabolism could induce and maintain autophagy pathways that prevent senescence and programmed cell death (Williams *et al.,* 2015).

Regarding the regions with high differentiation detected in Pv10, the two most significant SNPs (FDR<0.0002) at 40,612,777pb and 42614,661 pb, are located within *Phvul.010G144600*. It encodes a HAD superfamily, subfamily IIIB acid phosphatase. Interestingly, a homology search of the encoded protein on the non-redundant protein database (blastp, NCBI) revealed a putative conserved domain, corresponding to a vegetative storage protein (HAD_VPS; fig. 17). In soybean, the main role of vegetative storage proteins (VSPs) is to function as a reserve of nutrients and they are induced by the removal of pods and by the excess of available nitrogen. First, VSPs accumulate in developing vegetative sink tissues and are then degraded (DeWald *et al*., 1992; Leelapon *et al.,* 2004). The increase in the concentration of these storage proteins could present an interesting key for the SG phenotype, since the immediate availability of nutrients allows the plant to continue physiological processes in a normal way. Indeed, in 1990 Masson & Muller observed that in soybeans the VSP mRNA increased in the internodes of the mature stem when plants were subjected to drought while in the young stems a significant increase was not observed. Interestingly, the irrigation of the dried plants caused the almost complete recovery of the water potential in the leaf, which resulted in the decrease of VSP mRNA. Based on these results, it seems that the concentration of VSPs and its mRNAs could be modulated by the relative activities of the source sink tissues, and therefore disturbed by water deficit.

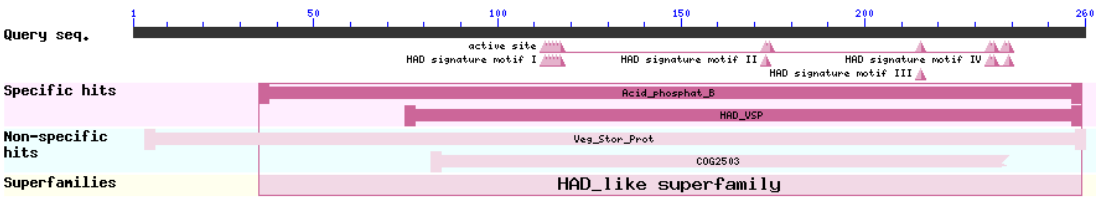

Figure 17. HAD like protein scheme. VSP putative conserved protein domains can be apreciated. Soure: NCBI. https://blast.ncbi.nlm.nih.gov/Blast.cgi

Although this study identified six accessions with the SG phenotype, not all had the same performance during the treatment. The accessions NGB18415, PHA6155 and PHA2682 experienced increased performance in addition to maintaining their greenness. On the other hand, accessions PHA366 and NGB9300 remained green during treatment (supplementary fig. 17), but experienced a decrease in the number of seeds due to the abortion of the pods that had been produced up to moment when the drought stress was applied. However, they recovered favorably after watering was returned, resulting in a restart of their reproductive stage. This may be similar to a specific phenomenon, "Zhengqing", reported in soybean. This syndrome is characterized by delayed senescence in leaves, but with a large number of aborted pods and dead seeds. It was observed that depodding and seed damage result in delayed leaf senescence and plants in a vegetative or green state (Zhang *et al.,* 2016). Hence, the elimination of the pods could exert an important influence on the progress of foliar senescence, although the molecular mechanisms behind this have not been widely studied (Zhang *et al.,* 2016).

The performance of crops under water deficit or heat stress is largely mediated by the remobilization of photosynthesis, which are subsequently stored in the stem. An alternative assimilation source to reduced photosynthesis under drought stress consists of carbohydrates (sugar, starch and fructans) accumulated during pre-anthesis. These reserves can be used during grain filling, especially if current photosynthesis is reduced due to drought. However, this mechanism is effective only during seed filling, but not necessarily advantageous during other stages involving gametogenesis, anthesis or fertilization (Jagadish *et al.,* 2015). Hence the importance of plant strategies (staying green versus remobilizing) for an efficient seed filling under terminal drought conditions depends on the plasticity of the genotype and its ability to cope with the severity of stress. The candidate genes found in this study have important roles in different metabolic pathways of the plant, however, their participation in some way in the storage and transport of sugars and carbohydrates, as well as the maintenance of the integrity of reproductive structures, seems concurrent.

Conclusions

Six *P. vulgaris* accessions with stay-green phenotype were identified. Additional cultivars that escaped drought or that recovered successfully after irrigation reestablishment were identified.

The analysis of common bean population structure revealed that the drought strategies are not associated with a particular gene pool.

Strong genetic associations were found between genetic variants and the stay green phenotype on three *P. vulgaris* chromosomes, Pv01, Pv05 and Pv10. These regions harbor genes encoding a RING-type E3 ubiquitin ligase, a trehalose-6-phosphate phosphatase and a vegetative storage protein. These three genes represents the strongest candidate genes associated with the stay-green phenotype.

The mechanisms behind the different strategies that plants adopt in the face of drought stress are varied and has a complex genetic regulation. However, the remobilization and storage of nutrients seems to be a key process underlying the stay-green phenotype.

Maintaining a stay-green phenotype has advantages in the optimization of photoassimilates and enhancing yield stability. The results of this research are an important step towards understanding the genetic control of senescence in common beans

Perspectives

Further examination of the additional strategies found in this study including wild accessions is suggested, since due to the limitation of photoperiod sensitivity it was not possible to analyze them in detail.

The information obtained from this work can be of useful for breeding programs to develop new varieties with functional stay-green phenotype that are better adapted to changing climatic conditions by the exploitation of the genes identified in this study.

Expand the search to more detail for associations within noncoding regions with a focus on noncoding RNAs.

Perform expression studies of genes of importance in plants under water stress.

5. References

Abid, M., Ali, S., Qi, L. K., Zahoor, R., Tian, Z., Jiang, D., Snider, J. L., & Dai, T. (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (Triticum aestivum L.). Scientific Reports, 8(1), 1–15. https://doi.org/10.1038/s41598-018-21441-7

Asfaw, A., Blair, M. W., & Struik, P. C. (2012). Multienvironment quantitative trait Loci analysis for photosynthate acquisition, accumulation, and remobilization traits in common bean under drought stress. *G3 (Bethesda, Md.)*, *2*(5), 579–595. https://doi.org/10.1534/g3.112.002303

Asfaw, A., Blair, M. W., & Struik, P. C. (2012). Multienvironment Quantitative Trait Loci Analysis for Photosynthate Acquisition, Accumulation, and Remobilization Traits in Common Bean under Drought Stress. G3: Genes, Genomes, Genetics, 2(5), 579–595. https://doi.org/10.1534/g3.112.002303

Azhar, M. T., & Rehman, A. (2018). Overview on effects of water stress on cotton plants and productivity. In Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress in Plants. Elsevier Inc. https://doi.org/10.1016/B978-0-12-813066-7.00016-4

Bacelar, E. L. V. A., Moutinho-Pereira, J. M., Gonçalves, B. M. C., Brito, C. V. Q., Gomes-Laranjo, J., Ferreira, H. M. F., & Correia, C. M. (2012). Water use strategies of plants under drought conditions. In Plant Responses to Drought Stress: From Morphological to Molecular Features (Vol. 9783642326). https://doi.org/10.1007/978-3-642-32653-0_6

Basu, S., Ramegowda, V., Kumar, A., & Pereira, A. (2016). Plant adaptation to drought stress [version 1; referees: 3 approved]. F1000Research, 5(0), 1–10. https://doi.org/10.12688/F1000RESEARCH.7678.1

Berny Mier Y Teran, J. C., Konzen, E. R., Palkovic, A., Tsai, S. M., Rao, I. M., Beebe, S., & Gepts, P. (2019). Effect of drought stress on the genetic architecture of photosynthate allocation and remobilization in pods of common bean (*Phaseolus vulgaris* L.), a key species for food security. BMC Plant Biology, 19(1), 1–15. https://doi.org/10.1186/s12870-019-1774-2

Bitocchi, E., Nanni, L., Bellucci, E., Rossi, M., Giardini, A., Zeuli, P. S., Logozzo, G., Stougaard, J., McClean, P., Attene, G., & Papa, R. (2012). Mesoamerican origin of the common bean (*Phaseolus vulgaris* L.) is revealed by sequence data. Proceedings of the National Academy of Sciences of the United States of America, 109(14). https://doi.org/10.1073/pnas.1108973109

Bitocchi, E., Rau, D., Bellucci, E., Rodriguez, M., Murgia, M. L., Gioia, T., Santo, D., Nanni, L., Attene, G., & Papa, R. (2017). Beans (*Phaseolus*sp.) as a model for understanding crop evolution. Frontiers in Plant Science, 8(May), 1–21. https://doi.org/10.3389/fpls.2017.00722

Broughton, W. J., Hern, aacute, Ndez, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus*spp.) - Model food legumes. Plant and Soil, 252(1), 55–128. http://www.ingentaconnect.com/content/klu/plso/2003/00000252/00000001/05102542

Camargo, A. V., Mackay, I., Mott, R., Han, J., Doonan, J. H., Askew, K., Corke, F., Williams, K., & Bentley, A. R. (2018). Functional mapping of quantitative trait loci (QTLS) associated with plant performance in a wheat MAGIC mapping population. Frontiers in Plant Science, 9(July). https://doi.org/10.3389/fpls.2018.00887

Chequer, F. M. D., Gisele Augusto Rodrigues de Oliveira, Ferraz, E. R. A., Cardoso, J. C., Zanoni, M. V. B., & Oliveira, D. P. de. (2013). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. Intech, i(tourism), 13. https://doi.org/10.1016/j.colsurfa.2011.12.014

Couchoud, M., Salon, C., Girodet, S., Jeudy, C., Vernoud, V., & Prudent, M. (2020). Pea Efficiency of Post-drought Recovery Relies on the Strategy to Fine-Tune Nitrogen Nutrition. Frontiers in Plant Science, 11(February), 1–15. https://doi.org/10.3389/fpls.2020.00204

Darkwa, K., Ambachew, D., Mohammed, H., Asfaw, A., & Blair, M. W. (2016). Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. Crop Journal, 4(5), 367–376. https://doi.org/10.1016/j.cj.2016.06.007

Deduced, F., Variability, P. E., & Europe, I. I. (1988). Dissemination Pathways of Common Bean. New York, 42(June 1986), 86–104.

DeWald, D. B., Mason, H. S., & Mullet, J. E. (1992). The soybean vegetative storage proteins VSPα and VSPβ are acid phosphatases active on polyphosphates. Journal of Biological Chemistry, 267(22), 15958-15964.

Duran, M. M. (2016). Identificación de QTLs de frijol común (*Phaseolus vulgaris*) asociados a tolerancia a sequía.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., & Huang, J. (2017). Crop production under drought and heat stress: Plant responses and management options. Frontiers in Plant Science, 8(June), 1–16. https://doi.org/10.3389/fpls.2017.01147

Hershko A, Ciechanover A. The ubiquitin system for protein degradation. *Annu Rev Biochem*. 1992;61:761-807. doi:10.1146/annurev.bi.61.070192.003553.

Galván, M. Z., Menéndez-Sevillano, M. C., De Ron, A. M., Santalla, M., & Balatti, P. A. (2006). Genetic diversity among wild common beans from northwestern Argentina based on morpho-agronomic and RAPD data. Genetic Resources and Crop Evolution, 53(5), 891–900. https://doi.org/10.1007/s10722-004-0981-2

Guo, Z., Chen, D., & Schnurbusch, T. (2015). Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. Journal of Experimental Botany, 66(11), 3099–3111. https://doi.org/10.1093/jxb/erv117

Holsinger, K. E., & Weir, B. S. (2009). Genetics in geographically structured populations: defining, estimating and interpreting F(ST). *Nature reviews. Genetics*, *10*(9), 639–650. https://doi.org/10.1038/nrg2611

Hoyos-Villegas, V., Song, Q., & Kelly, J. D. (2017). Genome-wide Association Analysis for Drought Tolerance and Associated Traits in Common Bean. The Plant Genome, 10(1), plantgenome2015.12.0122. https://doi.org/10.3835/plantgenome2015.12.0122

Huang, D., Wu, W., Abrams, S. R., & Cutler, A. J. (2008). The relationship of drought-related gene expression in *Arabisopsis thaliana* thaliana to hormonal and environmental factors. Journal of Experimental Botany, 59(11), 2991–3007. https://doi.org/10.1093/jxb/ern155

Jagadish, K. S. V., Kavi Kishor, P. B., Bahuguna, R. N., von Wirén, N., & Sreenivasulu, N. (2015). Staying alive or going to die during terminal senescence — An enigma surrounding yield stability. Frontiers in Plant Science, 6(NOVEMBER), 1–14. https://doi.org/10.3389/fpls.2015.01070

Jha, U. C., Bohra, A., & Nayyar, H. (2020). Advances in "omics" approaches to tackle drought stress in grain legumes. Plant Breeding, 139(1), 1–27. https://doi.org/10.1111/pbr.12761

Jha, U. C., Bohra, A., & Nayyar, H. (2020). Advances in "omics" approaches to tackle drought stress in grain legumes. Plant Breeding, 139(1), 1–27. https://doi.org/10.1111/pbr.12761

Johnson, S. M., Cummins, I., Lim, F. L., Slabas, A. R., & Knight, M. R. (2015). Transcriptomic analysis comparing stay-green and senescent Sorghum bicolor lines identifies a role for proline biosynthesis in the stay-green trait. Journal of Experimental Botany, 66(22), 7061–7073. https://doi.org/10.1093/jxb/erv405

Kamal, N. M., Gorafi, Y. S. A., Abdelrahman, M., Abdellatef, E., & Tsujimoto, H. (2019). Stay-green trait: A prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. International Journal of Molecular Sciences, 20(23). https://doi.org/10.3390/ijms20235837

Kusaba, M., Tanaka, A. & Tanaka, R. (2013). Stay-green plants: what do they tell us about the molecular mechanism of leaf senescence. Photosynth Res 117, 221–234 https://doi.org/10.1007/s11120-013-9862-x

Lafitte, Honor & Blum, Abraham & Atlin, Gary. (2003). Using secondary traits to help identify drought-tolerant genotypes.

Lan, W., & Miao, Y. (2019). New aspects of HECT-E3 ligases in cell senescence and cell death of plants. Plants, 8(11). https://doi.org/10.3390/plants8110483

Lee, J. H., & Kim, W. T. (2011). Regulation of abiotic stress signal transduction by E3 ubiquitin ligases in *Arabisopsis thaliana*. Molecules and cells, 31(3), 201–208. https://doi.org/10.1007/s10059-011-0031-9

Leelapon, O., Sarath, G. & Staswick, P.E. A single amino acid substitution in soybean VSPα increases its acid phosphatase activity nearly 20-fold. Planta 219, 1071–1079 (2004). https://doi.org/10.1007/s00425-004-1294-6

Li, L. F., & Olsen, K. M. (2016). To Have and to Hold: Selection for Seed and Fruit Retention During Crop Domestication. In Current Topics in Developmental Biology. https://doi.org/10.1016/bs.ctdb.2016.02.002

Lim, C.W., Park, C., Kim, J. *et al*. (2017). Pepper CaREL1, a ubiquitin E3 ligase, regulates drought tolerance via the ABA-signalling pathway. Sci Rep 7, 477 https://doi.org/10.1038/s41598-017-00490-4

Lin, Q., Yang, J., Wang, Q., Zhu, H., Chen, Z., Dao, Y., & Wang, K. (2019). Overexpression of the trehalose-6-phosphate phosphatase family gene AtTPPF improves the drought tolerance of *Arabisopsis thaliana* thaliana. BMC Plant Biology, 19(1), 1–15. https://doi.org/10.1186/s12870-019-1986-5

Luche, H. de S., Silva, J. A. G. da, Maia, L. C. da, Oliveira, A. C. de, Luche, H. de S., Silva, J. A. G. da, Maia, L. C. da, & Oliveira, A. C. de. (2015). Stay-green: a potentiality in plant breeding. Ciência Rural, 45(10), 1755–1760. https://doi.org/10.1590/0103-8478cr20140662

Luoni, S. B., Astigueta, F. H., Nicosia, S., Moschen, S., Fernandez, P., & Heinz, R. (2019). Transcription factors associated with leaf senescence in crops. Plants, 8(10), 1–28. https://doi.org/10.3390/plants8100411

Mason, H. S., & Mullet, J. E. (1990). Expression of two soybean vegetative storage protein genes during development and in response to water deficit, wounding, and jasmonic acid. The Plant cell, 2(6), 569–579. https://doi.org/10.1105/tpc.2.6.569

Moghaddam, S. M., Mamidi, S., Osorno, J. M., Lee, R., Brick, M., Kelly, J., Miklas, P., Urrea, C., Song, Q., Cregan, P., Grimwood, J., Schmutz, J., & Mcclean, P. E. (2016). Genome-Wide Association Study Identifies Candidate Loci Underlying Agronomic Traits in a Middle American Diversity Panel of Common Bean. november, 1–21. https://doi.org/10.3835/plantgenome2016.02.0012

Mukeshimana, G., Butare, L., Cregan, P. B., Blair, M. W., & Kelly, J. D. (2014). Quantitative trait loci associated with drought tolerance in common bean. Crop Science, 54(3), 923–938. https://doi.org/10.2135/cropsci2013.06.0427

Myers, J. R., Aljadi, M., & Brewer, L. (2018). The Importance of Cosmetic Stay-Green in Specialty Crops. Plant Breeding Reviews, 42, 219–256. https://doi.org/10.1002/9781119521358.ch6

National Human Genome Research Institute. (2020). Genome-Wide Association Studies Fact Sheet. Extracted from: https://www.genome.gov/about-genomics/fact-sheets/Genome-Wide-Association-Studies-FactSheet#:~:text=A%20genome%2Dwide%20association%20study%20is%20an%20approach%20that%20involves,associated%20with%20a%20particular%20disease.

Nuñez Barrios, A., Hoogenboom, G., & Nesmith, D. S. (2005). Drought sress and the distribution of vegetative and reproductive traits of a bean cultivar. Scientia Agricola, 62(1), 18–22. https://doi.org/10.1590/s0103-90162005000100004

Oladosu, Y., Rafii, M. Y., Samuel, C., Fatai, A., Magaji, U., Kareem, I., Kamarudin, Z. S., Muhammad, I., & Kolapo, K. (2019). Drought Resistance in Rice from Conventional to Molecular Breeding: A Review. International Journal of Molecular Sciences, 20(14). https://doi.org/10.3390/ijms20143519

Pereira, Wendell Jacinto, Melo, Arthur Tavares de Oliveira, Coelho, Alexandre Siqueira Guedes, Rodrigues, Fabiana Aparecida, Mamidi, Sujan, Alencar, Sérgio Amorim de, Lanna, Anna Cristina, Valdisser, Paula Arielle Mendes Ribeiro, Brondani, Claudio, Nascimento-Júnior, Ivanildo Ramalho do, Borba, Tereza Cristina de Oliveira, & Vianello, Rosana Pereira. (2020). Genome-wide analysis of the transcriptional response to drought stress in root and leaf of common bean. Genetics and Molecular Biology, 43(1), e20180259. Epub March 16, 2020.https://doi.org/10.1590/1678-4685-gmb-2018-0259

Pinto, R.S., Lopes, M.S., Collins, N.C. *et al*. (2016). Modelling and genetic dissection of staygreen under heat stress. Theor Appl Genet 129, 2055–2074. https://doi.org/10.1007/s00122-016-2757-4

Ponnu, J., Wahl, V., & Schmid, M. (2011). Trehalose-6-phosphate: connecting plant metabolism and development. Frontiers in plant science, 2, 70. https://doi.org/10.3389/fpls.2011.00070

Raggi, L., Caproni, L., Carboni, A., & Negri, V. (2019). Genome-Wide Association Study Reveals Candidate Genes for Flowering Time Variation in Common Bean (*Phaseolus vulgaris* L.). Frontiers in Plant Science, 10(July), 1–14. https://doi.org/10.3389/fpls.2019.00962

Rama Reddy, N. R., Ragimasalawada, M., Sabbavarapu, M. M., Nadoor, S., & Patil, J. V. (2014). Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. BMC Genomics, 15(1). https://doi.org/10.1186/1471-2164-15-909

Rendón-Anaya, M., Montero-Vargas, J. M., Saburido-Álvarez, S., Vlasova, A., Capella-Gutierrez, S., Ordaz-Ortiz, J. J., Aguilar, O. M., Vianello-Brondani, R. P., Santalla, M., Delaye, L., Gabaldón, T., Gepts, P., Winkler, R., Guigó, R., Delgado-Salinas, A., & Herrera-Estrella, A. (2017). Genomic history of the origin and domestication of common bean unveils its closest sister species. Genome Biology, 18(1), 1–17. https://doi.org/10.1186/s13059-017-1190-6

Rosales-Serna, R., Kohashi-Shibata, J., Acosta-Gallegos, J. A., Trejo-López, C., Ortiz-Cereceres, J., & Kelly, J. D. (2004). Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. Field Crops Research. https://doi.org/10.1016/S0378-4290(03)00161-8

Schmit, R., de MELO, R. C., Trevisani, N., Guidolin, A. F., & Coimbra, J. L. M. (2019). Screening and agronomic benefits of the stay-green trait in common bean genotypes. Bioscience Journal, 35(3), 869–877. https://doi.org/10.14393/BJ-v35n3a2019-42022

Schmutz, J., McClean, P. E., Mamidi, S., Wu, G. A., Cannon, S. B., Grimwood, J., Jenkins, J., Shu, S., Song, Q., Chavarro, C., Torres-Torres, M., Geffroy, V., Moghaddam, S. M., Gao, D., Abernathy, B., Barry, K., Blair, M., Brick, M. A., Chovatia, M., … Jackson, S. A. (2014). A reference genome for common bean and genome-wide analysis of dual domestications. Nature Genetics, 46(7), 707–713. https://doi.org/10.1038/ng.3008

Sedlar, Marjetka Kidrič, Jelka Šuštar-Vozlič, Barbara Pipan, Tanja Zadražnik and Vladimir Meglič (June 4th 2019). Drought Stress Response in Agricultural Plants: A Case Study of Common Bean (*Phaseolus vulgaris* L.), Drought - Detection and Solutions, Gabrijel Ondrasek, IntechOpen, DOI: 10.5772/intechopen.86526. Available from: https://www.intechopen.com/books/drought-detection-and-solutions/drought-stress-response-in-agricultural-plants-a-case-study-of-common-bean-em-phaseolus-vulgaris-em-

Sekhon, R. S., Saski, C., Kumar, R., Flinn, B. S., Luo, F., Beissinger, T. M., Ackerman, A. J., Breitzman, M. W., Bridges, W. C., de Leon, N., & Kaeppler, S. M. (2019). Integrated genome-scale analysis identifies novel genes and networks underlying senescence in maize. Plant Cell, 31(9), 1968–1989. https://doi.org/10.1105/TPC.18.00930

Singh, S. P. (2007). Drought Resistance in the Race Durango Dry Bean Landraces and Cultivars. 1219–1225. https://doi.org/10.2134/agronj2006.0301

Sivsakthi, K., Marques, E., Kalungwana, N., Carrasquilla-Garcia, N., Chang, P. L., Bergmann, E. M., Bueno, E., Cordeiro, M., Sani, S. G. A. S., Udupa, S. M., Rather, I. A., Mir, R. R., Vadez, V., Vandemark, G. J., Gaur, P. M., Cook, D. R., Boesch, C., von Wettberg, E. J. B., Kholova, J., & Penmetsa, R. V. (2019). Functional dissection of the chickpea (Cicer arietinum l.) stay-green phenotype associated with molecular variation at an ortholog of mendel's i gene for cotyledon color: Implications for crop production and carotenoid biofortification. International Journal of Molecular Sciences, 20(22). https://doi.org/10.3390/ijms20225562

Suárez, R., Wong, A., Ramírez, M., Barraza, A., Orozco, M. D. C., Cevallos, M. A., Lara, M., Hernández, G., & Iturriaga, G. (2008). Improvement of drought tolerance and grain yield in

common bean by overexpressing trehalose-6-phosphate synthase in rhizobia. Molecular Plant-Microbe Interactions, 21(7), 958–966. https://doi.org/10.1094/MPMI-21-7-0958

Thomas, H., & Ougham, H. (2014). The stay-green trait. Journal of Experimental Botany, 65(14), 3889–3900. https://doi.org/10.1093/jxb/eru037

Trapp, J. J. (2015). Genetics of drought tolerance in common bean (*Phaseolus vulgaris* L.). May, 135.

Trott, A. R., Welch, T. C., Hundy, G. F., Trott, A. R., Welch, T. C., & Qadri, B. (2016). Chapter 4 如何撰写开题报告. Refrigeration and Air Conditioning, 6(December 2015), 59–87. https://doi.org/10.1016/b978-075064219-4/50002-9

Villordo-Pineda, E., González-Chavira, M. M., Giraldo-Carbajo, P., Acosta-Gallegos, J. A., & Caballero-Pérez, J. (2015). Identification of novel drought-tolerant-associated SNPs in common bean (*Phaseolus vulgaris*). Frontiers in Plant Science, 6(JULY), 1–9. https://doi.org/10.3389/fpls.2015.00546

Wang, H., & Schippers, J. H. M. (2019). The role and regulation of autophagy and the proteasome during aging and senescence in plants. Genes, 10(4). https://doi.org/10.3390/genes10040267

Williams, B., Njaci, I., Moghaddam, L., Long, H., Dickman, M. B., Zhang, X., & Mundree, S. (2015). Trehalose Accumulation Triggers Autophagy during Plant Desiccation. PLoS Genetics, 11(12), 1–17. https://doi.org/10.1371/journal.pgen.1005705

Wingler, A., Delatte, T. L., O'Hara, L. E., Primavesi, L. F., Jhurreea, D., Paul, M. J., *et al*. (2012). Trehalose 6-phosphate is required for the onset of leaf senescence associated with high carbon availability. *Plant Physiol.* 158, 1241–1251. doi: 10.1104/pp.111.191908

Woo, H. R., Kim, H. J., Nam, H. G., & Lim, P. O. (2013). Plant leaf senescence and death - regulation by multiple layers of control and implications for aging in general. Journal of Cell Science, 126(21), 4823–4833. https://doi.org/10.1242/jcs.109116

Wright S. 1951. The genetical structure of populations. Annals of eugenics 15: 323–354.

Wu, J., Wang, L., Fu, J., Chen, J., Wei, S., Zhang, S., Zhang, J., Tang, Y., Chen, M., Zhu, J., Lei, L., Geng, Q., Liu, C., Wu, L., Li, X., Wang, X., Wang, Q., Wang, Z., Xing, S., … Wang, S. (2020). Resequencing of 683 common bean genotypes identifies yield component trait associations across a north–south cline. Nature Genetics, 52(1), 118–125. https://doi.org/10.1038/s41588-019-0546-0

Yıldırım, K., & Kaya, Z. (2017). Gene regulation network behind drought escape, avoidance and tolerance strategies in black poplar (Populus nigra L.). Plant Physiology and Biochemistry, 115, 183–199. https://doi.org/10.1016/j.plaphy.2017.03.020

Zhang, N., Yin, Y., Liu, X., Tong, S., Xing, J., Zhang, Y., Pudake, R. N., Izquierdo, E. M., Peng, H., Xin, M., Hu, Z., Ni, Z., Sun, Q., & Yao, Y. (2017). The E3 ligase TaSAP5 alters drought stress responses by promoting the degradation of DRIP proteins. Plant Physiology, 175(4), 1878–1892. https://doi.org/10.1104/pp.17.01319

Zhang, X., Wang, M., Wu, T., Wu, C., Jiang, B., Guo, C., & Han, T. (2016). Physiological and molecular studies of staygreen caused by pod removal and seed injury in soybean. Crop Journal, 4(6), 435–443. https://doi.org/10.1016/j.cj.2016.04.002

6. Appendices

6.1 Appendix 1-Supplementary tables

Table 3. Candidate genes found in high differentiate regions according to F_{st} analysis

Chromosome	Window start	Window end	Fst value	Gene start	Gene end	ID	*P. vulgaris* annotation	*A.thaliana* Id	Uniprot annotation
Pv01	**50450001**	**50500000**	0.2077	50457754	50459936	Phvul.001G254650	ENTH/ANTH/VHS superfamily protein	AT4G40080	Putative clathrin assembly protein At4g40080
			0.2077	50459299	50459952	Phvul.001G254700	ENTH/ANTH/VHS superfamily protein	AT4G40080	Putative clathrin assembly protein At4g40080
			0.2077	50461858	50464100	Phvul.001G254800	beta-1,4-N acetylglucosaminyltransferase family protein	AT1G12990	Beta-1,4-N-acetylglucosaminyltransferase family protein
			0.2077	50466401	50467110	Phvul.001G254900			
			0.2077	50467496	50468510	Phvul.001G255000			
			0.2077	50469815	50470364	Phvul.001G255100			
			0.2077	50473987	50475046	Phvul.001G255200	RING/U-box superfamily protein	AT1G67856	RING-type domain-containing protein
			0.2077	50477799	50482760	Phvul.001G255300	Protein of unknown function (DUF707)	AT1G67850	Lysine ketoglutarate reductase trans-splicing protein (DUF707)
			0.2077	50489729	50490292	Phvul.001G255400	sulfur E2	AT1G67810	SufE-like protein 2, chloroplastic (Protein SULFUR E 2)
			0.2077	50493914	50499798	Phvul.001G255500	Melibiase family protein	AT3G26380	Alpha-galactosidase (EC 3.2.1.22) (Melibiase)
	50500001	**50550000**	0.2273	50501181	50506664	Phvul.001G255600	O-fucosyltransferase family protein	AT3G26370	O-fucosyltransferase family protein

		0.2273	50509723	50517280	Phvul.001G255700	sphere organelles protein-related	AT1G13030	Coilin (Atcoilin)
		0.2273	50516516	50519922	Phvul.001G255800	mitogen-activated protein kinase phosphatase 1	AT3G55270	Protein-tyrosine-phosphatase MKP1 (EC 3.1.3.48) (Mitogen-activated protein kinase phosphatase 1) (AtMKP1)
		0.2273	50524833	50530075	Phvul.001G255900	Copine (Calcium-dependent phospholipid-binding protein) family	AT1G67800	E3 ubiquitin-protein ligase RGLG5
		0.2273	50530440	50537553	Phvul.001G256000	embryo defective 1745	AT1G13120	Protein GLE1 (AtGLE1) (Protein EMBRYO DEFECTIVE 1745)
		0.2273	50539605	50543384	Phvul.001G256100		AT3G01680	Protein SIEVE ELEMENT OCCLUSION B (AtSEOb) (Protein SIEVE ELEMENT OCCLUSION-RELATED 1) (AtSEOR1)
		0.2273	50542843	50548919	Phvul.001G256200	P-glycoprotein 18	AT3G28390	ABC transporter B family member 18 (ABC transporter ABCB.18) (AtABCB18) (P-glycoprotein 18) (Putative multidrug resistance protein 20)
50550001	**50600000**	0.2162	50554368	50557400	Phvul.001G256300	terminal EAR1-like 1	AT3G26120	Terminal EAR1-like 1
		0.2162	50563222	50567084	Phvul.001G256400	Pyridoxal-5\'-phosphate-dependent enzyme family protein	AT3G26115	D-cysteine desulfhydrase 2, mitochondrial
		0.2162	50567651	50572423	Phvul.001G256500	ARM repeat superfamily protein	AT1G13160	RING-type domain-containing protein
		0.2162	50573390	50580574	Phvul.001G256600	OSBP(oxysterol binding protein)-related protein 1D	AT1G13170	OSBP(Oxysterol binding protein)-related protein 1D
		0.2162	50584333	50588802	Phvul.001G256700	TCP-1/cpn60 chaperonin family protein	AT1G24510	TCP-1/cpn60 chaperonin family protein
		0.2162	50589495	50592622	Phvul.001G256800	Pectate lyase family protein	AT1G67750	Probable pectate lyase 5 (EC 4.2.2.2)
		0.2162	50597366	50599541	Phvul.001G256900	Pectate lyase family protein	AT1G67750	Probable pectate lyase 5 (EC 4.2.2.2)

	50850001	**50900000**	0.2342	50850386	50853825	Phvul.001G260500		AT1G24310	Nuclear pore complex protein NUP54 (Nucleoporin 54)
			0.2342	50856102	50858271	Phvul.001G260600	Galactose oxidase/kelch repeat superfamily protein	AT1G67480	F-box/kelch-repeat protein
			0.2342	50858356	50858695	Phvul.001G260700			
			0.2342	50864321	50867591	Phvul.001G260800	Minichromosome maintenance (MCM2/3/5) family protein	AT1G67440	Minichromosome maintenance (MCM2/3/5) family protein
			0.2342	50868792	50870742	Phvul.001G260900	Microsomal signal peptidase 12 kDa subunit (SPC12)	AT4G40042	Uncharacterized protein At4g40042 (Fragment)
			0.2342	50872503	50874426	Phvul.001G261000	Ribosomal protein L22p/L17e family protein	AT1G27400	60S ribosomal protein L17-1
			0.2342	50876320	50880498	Phvul.001G261100	ELMO/CED-12 family protein	AT1G67400	ELMO/CED-12 family protein
			0.2342	50885737	50890681	Phvul.001G261200	microtubule-associated protein 65-8	AT1G27920	65-kDa microtubule-associated protein 8
			0.2342	50890931	50891702	Phvul.001G261300	nuclear factor Y, subunit C13	AT5G43250	CBFD_NFYB_HMF domain-containing protein
			0.2342	50892606	50896565	Phvul.001G261400	translocase of outer membrane 20 kDa subunit 3	AT3G27080	Mitochondrial import receptor subunit TOM20-3 (Translocase of outer membrane 20 kDa subunit 3)
Pv05	**550001**	**600000**	0.2205	555865	568960	Phvul.005G006700	Zincin-like metalloproteases family protein	AT5G51540	HTH myb-type domain-containing protein
			0.2205	569854	570576	Phvul.005G006800		AT2G30695	Trigger_N domain-containing protein
			0.2205	571839	575049	Phvul.005G006900		AT2G30695	Trigger_N domain-containing protein
			0.2205	576958	579658	Phvul.005G007000	Eukaryotic protein of unknown function (DUF842)	AT2G31725	Uncharacterized protein
			0.2205	581044	582865	Phvul.005G007100	Leucine-rich repeat (LRR) family protein	AT1G33590	Leucine-rich repeat (LRR) family protein

			0.2205	588339	590099	Phvul.005G007200	disease resistance family protein / LRR family protein	AT2G34930	LRRNT_2 domain-containing protein
			0.2205	596253	596720	Phvul.005G007300			
			0.3356	608469	608936	Phvul.005G007400			
			0.3356	614712	615181	Phvul.005G007500			
			0.3356	622975	623987	Phvul.005G007600	Plant invertase/pectin methylesterase inhibitor superfamily protein	AT5G62350	Plant invertase/pectin methylesterase inhibitor superfamily protein (Ripening-related protein-like) (Ripening-related protein-like; contains similarity to pectinesterase)
	650001	**700000**	0.3544	652182	652808	Phvul.005G007700	Plant invertase/pectin methylesterase inhibitor superfamily protein	AT5G51520	Invertase (Plant invertase/pectin methylesterase inhibitor superfamily protein) (Ripening-related protein-like)
			0.3544	663738	666995	Phvul.005G007800	Plant invertase/pectin methylesterase inhibitor superfamily	AT3G47400	Probable pectinesterase/pectinesterase inhibitor 33 [Includes: Pectinesterase inhibitor 33 (Pectin methylesterase inhibitor 33); Pectinesterase 33 (PE 33) (EC 3.1.1.11) (Pectin methylesterase 33) (AtPME33)]
			0.3544	670006	675996	Phvul.005G007900	NAC 007	AT1G12260	NAC 007
	700001	**750000**	0.3291	707892	710826	Phvul.005G008100	Tetratricopeptide repeat (TPR)-like superfamily protein	AT2G29760	Pentatricopeptide repeat-containing protein At2g29760, chloroplastic (Protein ORGANELLE TRANSCRIPT PROCESSING 81)
			0.3291	711852	717390	Phvul.005G008200	Glycosyl hydrolases family 32 protein	AT1G12240	Beta-fructofuranosidase (EC 3.2.1.26)
			0.3291	719827	725719	Phvul.005G008300	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein	AT5G51460	Trehalose 6-phosphate phosphatase (EC 3.1.3.12)
			0.3291	739318	742827	Phvul.005G008400	Major facilitator superfamily protein	AT1G04570	Major facilitator superfamily protein

		0.3291	746355	749557	Phvul.005G008500	RNI-like superfamily protein	AT5G07670	AT5G07670 protein
1000001	**1050000**	0.2011	1003662	1006971	Phvul.005G011600	ABA-responsive element binding protein 3	AT3G56850	ABSCISIC ACID-INSENSITIVE 5-like protein 2
		0.2011	1009546	1017596	Phvul.005G011700	far-red elongated hypocotyls 3	AT3G22170	Protein FAR-RED ELONGATED HYPOCOTYL 3
		0.2011	1016106	1016456	Phvul.005G011800			
		0.2011	1028117	1032426	Phvul.005G011900	Plant invertase/pectin methylesterase inhibitor superfamily	AT5G04970	Probable pectinesterase/pectinesterase inhibitor 47
		0.2011	1032805	1035289	Phvul.005G012000	root hair specific 12	AT3G10710	Putative pectinesterase/pectinesterase inhibitor 24
		0.2011	1036786	1039441	Phvul.005G012100	ribosomal protein L3 plastid	AT3G17465	50S ribosomal protein L3-2, mitochondrial
		0.2011	1045458	1048898	Phvul.005G012200	Ethylene insensitive 3 family protein	AT3G20770	Protein ETHYLENE INSENSITIVE 3
1400001	**1450000**	0.2187	1400762	1403225	Phvul.005G016000	RING/U-box superfamily protein	AT3G03550	RING-H2 finger protein ATL51
		0.2187	1406275	1423210	Phvul.005G016100	DNA replication helicase, putative	AT1G08840	DNA replication ATP-dependent helicase/nuclease JHS1
		0.2187	1427172	1429815	Phvul.005G016200	zinc ion binding	AT2G44580	Zinc ion binding protein
		0.2187	1434007	1435335	Phvul.005G016300	Late embryogenesis abundant protein, group 1 protein	AT1G32560	Late embryogenesis abundant protein 6
		0.2187	1440819	1442176	Phvul.005G016400	alpha/beta-Hydrolases superfamily protein	AT1G68620	Probable carboxylesterase 6
		0.2187	1443971	1449481	Phvul.005G016500	disease resistance protein (TIR-NBS-LRR class), putative	AT5G17680	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase
1450001	**1500000**	0.282	1451753	1456371	Phvul.005G016600	DCD (Development and Cell Death) domain protein	AT2G32910	DCD (Development and Cell Death) domain protein

		0.282	1457827	1461531	Phvul.005G016700	Ribosomal L18p/L5e family protein	AT1G08845	Ribosomal L18p/L5e family protein
		0.282	1463127	1467372	Phvul.005G016800	Ribosomal protein L18e/L15 superfamily protein	AT5G64670	Ribosomal protein L18e/L15 superfamily protein
		0.282	1472062	1472724	Phvul.005G016850	RING/U-box superfamily protein	AT5G07040	Putative RING-H2 finger protein ATL69
		0.282	1473409	1473633	Phvul.005G016900	Chloroplast Ycf2;ATPase, AAA type, core	ATCG00860	Protein Ycf2
		0.282	1475070	1480689	Phvul.005G017000	Protein kinase superfamily protein	AT1G74320	Probable choline kinase 2
		0.282	1493992	1494966	Phvul.005G017100		AT4G28230	Uncharacterized protein
		0.282	1496685	1497622	Phvul.005G017200	AGAMOUS-like 82	AT5G58890	Agamous-like MADS-box protein AGL82
1750001	**1800000**	0.2655	1755532	1759079	Phvul.005G020301			
		0.2655	1764818	1767029	Phvul.005G020400	WIP domain protein 5	AT1G51220	Zinc finger protein WIP5
		0.2655	1781391	1787271	Phvul.005G020500		AT2G26840	Holliday junction resolvase MOC1, chloroplastic
		0.2655	1789494	1793992	Phvul.005G020600	Putative methyltransferase family protein	AT2G26810	Putative methyltransferase family protein
1800001	**1850000**	0.2302	1806435	1809832	Phvul.005G020800		AT3G52110	Remorin_C domain-containing protein
		0.2302	1813240	1813440	Phvul.005G020900		AT3G52105	DIS3-exonuclease-like protein
		0.2302	1825952	1830397	Phvul.005G021000	delta subunit of Mt ATP synthase	AT5G13450	ATP synthase subunit O, mitochondrial
		0.2302	1832630	1833672	Phvul.005G021100	DNA ligase 1	AT1G08130	DNA ligase 1
		0.2302	1837701	1840507	Phvul.005G021200	DNA ligase 1	AT1G08130	DNA ligase 1
		0.2302	1844506	1846174	Phvul.005G021250			

	1850001	**1900000**	0.2157	1861333	1862324	Phvul.005G021300	microtubule-associated proteins 70-3	AT2G01750	Microtubule-associated protein 70-3
			0.2157	1883871	1884323	Phvul.005G021400			
Pv10	**40250001**	**40300000**	0.2336	40254618	40255522	Phvul.010G121301	Ribosomal RNA processing Brix domain protein	AT4G01560	Glycosyltransferase
			0.2336	40260565	40262990	Phvul.010G121400	photosystem I light harvesting complex gene 2	AT3G61470	Photosystem I chlorophyll a/b-binding protein 2, chloroplastic
			0.2336	40263909	40267363	Phvul.010G121500	purple acid phosphatase 29	AT5G63140	Probable inactive purple acid phosphatase 29
			0.2336	40272872	40274653	Phvul.010G121600	Protein of unknown function (DUF1295)	AT2G46890	3-oxo-5-alpha-steroid 4-dehydrogenase (DUF1295)
			0.2336	40276891	40281329	Phvul.010G121700		AT2G46900	Uncharacterized protein
			0.2336	40284713	40291417	Phvul.010G121800	Calcineurin-like metallo-phosphoesterase superfamily protein	AT1G53710	Protein kinase domain-containing protein
			0.2336	40295008	40295669	Phvul.010G121900	ROTUNDIFOLIA like 9	AT1G53708	ROTUNDIFOLIA like 9

Table 4. Candidate genes identified thought Genome-Wide Association Analysis.

Chromosome	Window start	Window end	Gene start	Gene end	ID	*P. vulgaris* annotation	*A.thaliana* Id	Uniprot annotation
Chr01	10460000	10470000	10469775	10470233	Phvul.001G075900	No annotation		
	50830000	50840000	50832574	50835622	Phvul.001G260200	Cysteine proteinases superfamily protein	AT3G57810	OVARIAN TUMOR DOMAIN-containing deubiquitinating enzyme 4 (OTU domain-containing protein 4) (EC 3.4.19.12) (Deubiquitinating enzyme OTU4)
			50838135	50843093	Phvul.001G260300	ARM repeat superfamily protein	AT1G24330	U-box domain-containing protein 6 (EC 2.3.2.27) (Plant U-box protein 6) (RING-type E3 ubiquitin transferase PUB6)
	50840000	50850000	50847436	50850120	Phvul.001G260400	no annotation	AT5G20165	Protein kish
			50850386	50853825	Phvul.001G260500		AT1G24310	Nuclear pore complex protein NUP54 (Nucleoporin 54)
Chr04	3150000	3160000	3157686	3159839	Phvul.004G027700	basic helix-loop-helix (bHLH) DNA-binding family protein	AT3G28857	Transcription factor PRE5
Chr05	480000	490000	489922	493059	Phvul.005G006000	P-loop containing nucleoside triphosphate hydrolases superfamily protein	AT1G33970	Immune-associated nucleotide-binding protein 9
	490000	500000	495711	503125	Phvul.005G006100	COP9 signalosome subunit 6A	AT5G56280	COP9 signalosome complex subunit 6a
	520000	530000	515969	520294	Phvul.005G006200	P-loop containing nucleoside triphosphate hydrolases superfamily protein	AT1G33970	Immune-associated nucleotide-binding protein 9
			522660	524583	Phvul.005G006300	P-loop containing nucleoside triphosphate hydrolases superfamily protein	AT1G33970	Immune-associated nucleotide-binding protein 9

			526506	532539	Phvul.005G006400	ankyrin repeat-containing 2B	AT2G17390	Ankyrin repeat domain-containing protein 2B
	540000	550000	536759	548906	Phvul.005G006500	peroxin 5	AT5G56290	Peroxisome biogenesis protein 5
			548927	554228	Phvul.005G006600	Vacuolar sorting protein 9 (VPS9) domain	AT3G19770	Vacuolar protein sorting-associated protein 9A
	550000	560000	555865	568960	Phvul.005G006700	Zincin-like metalloproteases family protein	AT5G51540	Mitochondrial intermediate peptidase, mitochondrial
	560000	570000	569854	570576	Phvul.005G006800	No annotation	AT2G30695	Trigger_N domain-containing protein
			571839	575049	Phvul.005G006900	No annotation	AT2G30695	Trigger_N domain-containing protein
			576958	579658	Phvul.005G007000	Eukaryotic protein of unknown function (DUF842)	AT2G31725	Expressed protein
	600000	610000	608469	608936	Phvul.005G007400	No annotation		
			614712	615181	Phvul.005G007500	No annotation		
Chr10	1250000	1260000	1254077	1260168	Phvul.010G008800	homogentisate phytyltransferase 1	AT2G18950	Homogentisate phytyltransferase 1, chloroplastic
	40460000	40470000	40467177	40470443	Phvul.010G123600	Protein of unknown function (DUF567)	AT3G14260	Protein LURP-one-related 11
			40470380	40472726	Phvul.010G123700	Eukaryotic translation initiation factor 2B (eIF-2B) family protein	AT1G53900	eIF-2B GDP-GTP exchange factor subunit alpha
			40475957	40476547	Phvul.010G123800	No annotation		
	40480000	40490000	40480603	40488838	Phvul.010G123900	ARID/BRIGHT DNA-binding domain-containing protein	AT3G43240	AT-rich interactive domain-containing protein 4
	40490000	40500000	40495457	40496329	Phvul.010G124000	Protein of unknown function (DUF581)	AT1G53903	FCS-Like Zinc finger 18

			40509908	40510997	Phvul.010G124100	RING/U-box superfamily protein	AT3G14250	RING-type domain-containing protein
	42290000	42300000	42290472	42292211	Phvul.010G140900	Pyridoxal phosphate phosphatase-related protein	AT1G17710	Inorganic pyrophosphatase 2
			42295982	42305244	Phvul.010G141050	Pyridoxal phosphate phosphatase-related protein	AT1G17710	Inorganic pyrophosphatase 2
	42480000	42490000	42474970	42480327	Phvul.010G142600	peptidase M20/M25/M40 family protein	AT1G51760	IAA-amino acid hydrolase ILR1-like 4
			42483141	42486072	Phvul.010G142700	peptidase M20/M25/M40 family protein	AT1G51760	IAA-amino acid hydrolase ILR1-like 4
			42486730	42491985	Phvul.010G142800	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein	AT3G21310	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
	42490000	42500000	42494816	42501294	Phvul.010G142900		AT3G21320	EARLY FLOWERING protein
	42610000	42620000	42612137	42614661	Phvul.010G144600	HAD superfamily, subfamily IIIB acid phosphatase	AT4G25150	Acid phosphatase-like protein
	42620000	42630000	42621151	42624169	Phvul.010G144700	IQ-domain 26	AT3G16490	Signal peptidase I
			42633755	42638337	Phvul.010G144800	nuclear poly(a) polymerase	AT4G32850	Nuclear poly(A) polymerase 4

6.2 Appendix 2-Supplementary figures

Figure 18. Accession NGB9300. A: 1WOT, B: 2 WOT, C: 3WAR

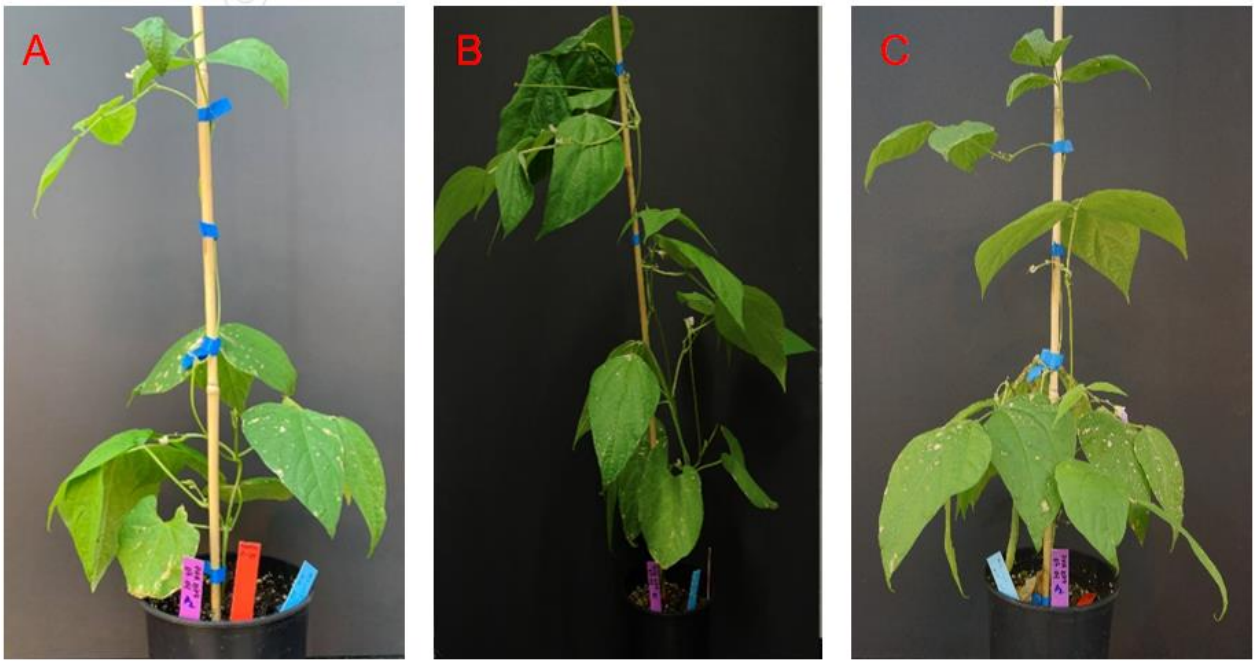

Figure 19. Accession PHA1077. A: 1WOT, B: 2 WOT, C: 3WAR

Figure 20. Accession PHA2682. A: 1WOT, B: 2 WOT, C: 3WAR