



Enhancing Legume Cultivars through Agronomy, Breeding, and Genetics

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Abstract: Legumes are a multipurpose crop species, with a great economic value, which, worldwide, are commonly cultivated for human food, livestock fodder, industrial raw materials, and soil health enhancement. Over the last few decades, numerous research projects have been conducted for the genetic improvements of legumes, in order to meet the Sustainable Development Goals of 2015–2030. Remarkable progress has been made in legume genetics, genomics, and breeding. The first references to the genome of legume plants were published in 2010, and these were the complete draft genome sequences of *Glycine max*. The chromosome scale high-quality genome assembly and annotations are available for many legume species today, including *Glycine max*; *Lotus japonicus*; *Medicago ruthenica* (L.); *Medicago truncatula*; *Phaseolus lunatus*; *Mucuna pruriens*; *Vicia sativa*; *Trifolium pratense*; *Lupinus angustifolius*; *Cajanus cajan*; *Vigna radiata* ssp.; and *Cicer arietinum*. Large-scale transcriptomic, genotyping, and phenotyping data have been generated from this diverse panel of legume cultivars for their varietal improvements. This Special Issue presents a collection of a variety of articles that cover the recent progress that has been made in legume genetics, genomics, and breeding. The authors have addressed the applications of phenotypic and genotypic diversity for the selection of the best cultivars; of morphological traits for the selection of the best local variety, the estimation of the agronomic performances of resistant and susceptible cultivars; the nutritional characteristics of the seed protein; genome-wide association studies on agronomic traits and isolation; and the characterization and function studies of many agronomically important genes.

Keywords: GWAS; genetic diversity; agronomic practices; gene identifications



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1. Introduction

The Leguminosae family is usually known as the fabaceae, legume, pea, or bean family. It is one of the most agriculturally important and is the third-largest family (based on the number of species within this family) of the flowering plants. Some economically important and well-known cultivated common legume species are: soybeans, beans, peanuts, chickpeas, lentils, tamarind, clover, alfalfa, lupins, carob, and mesquite, etc. [1] Legumes are commonly grown in tropic and sub-tropic regions and they contribute to roughly 30% of the world's primary crop production. Legumes are one of the key players in sustainable agriculture and food security. Their unique features, such as fixing nitrogen from the atmosphere through a symbiotic relationship with the soil bacteria of this plant family, are incredible for maintaining soil fertility and reducing the use of synthetic fertilizers in agriculture. Therefore, legume crop cultivation, in a crop rotation system, is common practice in many countries for their sustainable agriculture and for reducing the crop production cost.

The world population reached 8 billion at the end of 2022. To ensure the food security of the global population, sustainable agriculture, with an increase in food production, is urgently required. To meet these global challenges, the legume research community around

the globe has initiated a large number of research projects that have spanned many decades, in order to improve legume variety. Remarkable progress has been made and many of these projects are underway in the fields of agronomy, breeding, and genetics. In this section, the key methods that are used for legume crop improvements are briefly described.

For example, in legume agronomy research, the optimization of crop management practices (e.g., crop rotation, tillage, fertilization, and irrigation) is applied for improving their yields and quality; intercropping legumes with other crops improves the soil fertility and reduces the pest pathogen pressure that leads to higher yields. Likewise, reduced tillage practices lead to an increase in the soil's organic matter and improve the nutrient preservation in the soil, and, as a result, the crops grow better and the yield is boosted. Different breeding techniques are frequently applied for these legume crop improvements, and among them, hybrid breeding; participatory plant breeding; genomic selection; marker-assisted selection; trait development; multi-trait selection; and biotechnology are notable. The high-quality genome sequences of many important legume species are available, including those of *Glycine max* [2]; *Lotus japonicus*; *Medicago ruthenica* (L.) [3]; *Medicago truncatula* [4]; *Phaseolus lunatus* [5]; *Mucuna pruriens* [6]; *Vicia sativa* [7]; *Trifolium pratense* [8]; *Lupinus angustifolius* [9]; *Cajanus cajan* [10]; *Vigna radiata* ssp. [11]; and *Cicer arietinum* [12]. These genomic resources have allowed researchers to identify and characterize the agronomically important genes and genetic pathways that are underlying various traits. Furthermore, reference genome sequences, high-throughput genotyping technologies, and the phenotypic data of legume genotypes allow for the efficient identification and tracking of the genetic variations that are associated with important traits.

2. Special Issue Overview

2.1. Trial for Agronomic Performance

Trials for agronomic performance are conducted in order to evaluate the performance of new crop cultivars/varieties/landraces, or/and existing cultivars/variety/landraces, under different growing conditions. These trials are typically conducted under open-field conditions, and often the trials are performed in controlled environments, viz., greenhouses, glass houses, or even in vitro systems. Then, the performance of each variety is evaluated based on the research objectives. Typically, the performances are evaluated based on factors such as yield, biotic–abiotic stress, heat tolerance, and many other desirable traits. Field trials (agronomic trials) offer valuable information for farmers, seed companies, researchers, and other stakeholders, and support the identification of the most promising crop varieties, for their further development and commercialization.

The agronomic performances of legume cultivars under different conditions helps to improve our understanding of the genetic basis of legume performance. Therefore, legume breeders/scientists have evaluated these legume cultivars under different agronomic conditions, with this knowledge having potential for yield improvement, disease resistance, and the other desirable traits of legumes. In this Special Issue, several articles have demonstrated the agronomical performances of legume varieties under different environments.

For example, the parasitic weed broomrape (*Orobanche crenata*) is a major problem in the Mediterranean Basin for faba bean cultivations. This problem can be managed by adjusting their agronomic practices and adaptations. To address this, Rubiales et al. [13] evaluated 15 faba bean (*Vicia faba*) accessions (broomrape-resistant and susceptible accession) under multi-environment field trials. Additionally, the main objective of their research was to assess the revalorization possibility of faba bean cultivations in the Mediterranean region, by evaluating the agronomic performance and yield stability of broomrape resistance and susceptible genotypes under different environments. This study suggested that there is a high potential for faba bean cultivations in Mediterranean rain-fed farming systems, reinforcing the value of adopting broomrape-resistant cultivars. The varieties that showed the highest superiority indexes were Quijote, Navio6, Baraca, and FaraonSC, with grain

yields greater than the grand means, which can be deployed as parents in breeding for a higher yield and wider adaptability.

Biotic and abiotic stress seriously affects faba bean yield [14–17], and it is difficult to grow these beans under higher-than-normal temperatures, with a lack of moisture and changes in the soil fertility [18–20]. The faba bean genotype performs differentially under different environmental conditions, therefore, the genotype–environment interaction is very important for faba bean breeders [21–23] to select the varieties of faba bean that are the best-suited to the soil and climatic conditions. In view of this point, Janauskaite and Razbadauskiene [7] conducted their research to evaluate and compare the physiological traits, productivity, and seed quality of faba bean varieties that were grown under Boreal climate conditions. Their results revealed that the faba bean varieties Fuego and Isabell, which distinguished themselves from the other varieties that were tested by having the highest seed yields, were the most suitable for cultivation within the Boreal climate zone.

Biotic and abiotic constraints hamper lentil production worldwide [24,25]. Understanding the adaptation of lentil genotypes in differing agro-ecological zones assists breeders in the expansion of lentil cultivations. Rubiales et al. [13] reported that early sowings of lentils are prone to broomrape infection in the Mediterranean region. Thus, delaying the sowing of early maturing cultivars is recommended for Mediterranean regions, in order to avoid broomrape infection. However, this can expose lentils to high temperatures during the seed-filling stage, which leads to a yield loss. However, Rubiales et al. [13] identified some lentil accessions that were resistant to broomrape infection, had a good heat tolerance, and were suitable for commercial cultivation in Mediterranean regions.

The nutritional characteristics of the seed protein are some of the best agronomic traits that are used to evaluate the quality of legume cultivars and their added economical value. Elamine et al. [26] estimated the total amino acid composition of seeds from 23 different wild legume cultivars that were collected from southern Spain. Their results revealed that the presence of canavanine (a non-proteic amino acid) was heterogeneous among the tested legume cultivars. These legume cultivars could be useful in the domestication and breeding programs for new varieties with improved nutritional and functional properties, as well as a potential source of canavanine.

2.2. Phenotypic and Genotypic Diversity for Selections

Phenotypic and genotypic diversity are most important features for selecting the breeding materials in breeding programs. Typically, phenotypic diversity is the observable characteristics of a plant, such as its yield, disease resistance, heat tolerance, and many other agronomic traits. Genotypic diversity refers to the genetic variation underlying the DNA level within a population of plants. This phenotypic and genotypic diversity can be evaluated by using various techniques, such as a morphological characterization, molecular markers, and genome sequencing. This information assists breeders in identifying individual plants with unique characteristics and helps them to make decisions in the selection of the parents for further breeding programs.

Legume breeders/researchers frequently use phenotypic and genotypic diversity data when they are selecting the breeding materials for breeding programs. By identifying and utilizing diverse materials, legume breeders can increase the efficiency and effectiveness of their breeding efforts and develop new legume varieties with improved traits. In this Special Issue, Peláez et al. [5] evaluated a large number of lablab accessions, based on their morpho-agronomic traits. Their results demonstrated that the lablab accessions were highly diverse in terms of their traits, and the authors suggested 14 agronomic traits that could successfully discriminate all the lablab accessions that were used in their study. Therefore, these traits were suggested to be used in further lablab breeding programs. Letting et al. [27] characterized locally adapted (Northwest Andes of Colombia) lablab (*Lablab purpureus* (L.) Sweet) accessions using phenotypic diversity, and recommend some of the best locally adapted accessions for commercial use to the local farmers. The study that was conducted by Letting and his co-workers involved screening a diverse panel of

Liborino accessions under three different agro-ecological regions, and carried out a trait uniformity test and the multiplication and selection of the best genotypes for further trials and commercial use.

2.3. Marker-Assisted Selections

Plant breeding comprises the selection and crossing of genetically diverse parents with desirable traits, in order to generate progenies that show a mixture of these desirable traits. Traditional breeding methods are often time-consuming, labor-intensive, and costly. In contrast, marker-assisted selection (MAS) is a faster, cost-effective, and more efficient method for the selection of desirable traits. MAS is a powerful tool that accelerates the legume breeding process to develop new cultivars with improved traits. This section aims to summarize the recent advances in MAS applications for legume cultivar improvements.

A genome-wide association study [28] has been carried out to identify the QTLs in the soybean genome, which are associated with HSW (hundred-seed weight) and identify the candidate genes in LD blocks, where the most significant SNPs are located. Jo and his co-authors suggested GmCYP78A57 as a potential candidate gene that regulates the seed size of soybeans, and identified the QTL on chromosome 16, which had a small allelic impact on the seed size. Additionally, the authors assumed that there may be another gene on chromosome 16, with an additive genetic effect that reduced the seed size in combination with GmCYP78A57. These findings offer valuable insights for the development of molecular markers that can be utilized in soybean breeding programs, in order to produce novel cultivars with an enhanced or decreased seed weight and an improved yield.

The genome-wide GBS approach was applied to identify the significant SNPs and candidate genes that are associated with the P (phosphorus) uptake and P utilization efficiency traits in mungbean [29]. In this study, a total of 116 SNPs in 61 protein-coding genes were identified, of which, 16 were found to enhance the P uptake and utilization efficiency. In total, six genes were found to have a high expression in the root, shoot apical meristem, and leaf, indicating their roles in the P regulation. Chromosome six was identified as a hotspot region that harbored several major QTLs/genes which regulated the P uptake and utilization efficiency traits in mungbean, making it a target for genomics-assisted breeding to improve the response to a phosphorus deficiency in mugbean.

2.4. Gene Identifications and Characterizations

Gene identification, characterization, and assessments of their functions are key steps for crop improvement. Recent advances in molecular biology techniques, such as next-generation sequencing and functional genomics, have accelerated the gene identification and characterization of crop plants. These advances have led to the development of new breeding strategies, genetic engineering techniques, and functional genomic approaches which can be used to enhance crop productivity and sustainability.

This section provides a review of the gene functions and characterizations of legume species. The *Arabidopsis thaliana* gene, *ore1*, is well known for its ability to enhance the tolerance to various types of oxidative stress, and to delay leaf senescence. However, the function of this gene within the soybean system is currently unknown. To investigate the function of the *At-ore1* gene in soybeans, La et al. [30] utilized an agrobacterium-mediated transformation approach to transfer this gene into soybeans. The results indicated that *At-ore1* has an opposing role in leaf senescence, depending on the ABA/IAA balance, potentially through an increase in the GmNAC081- or GmNAC065-mediated H₂O₂ regulation.

The Ferredoxin-NADP reductase (FNR) gene is involved in the NADPH production, carbon assimilation, antioxidation, and cross-talk between the chloroplasts and mitochondria in plants. Shen et al. [31] examined the functional response of the soybean FNR gene (GmFNR) during a soybean mosaic virus (SMV) infection. A bean pod mottle virus (BPMV)-based gene construct (BPMV-GmFNR) was applied to silence the GmFNR gene, in

both the resistant and susceptible soybean lines. The results showed that the expression of GmFNR decreased to 50% in the susceptible line, and 40% in the resistant line. This experiment confirmed that the silencing of the GmFNR gene reduces the photosynthetic capacity and catalase (CAT) activity in both susceptible and resistant lines.

Plasma membrane intrinsic proteins (PIPs) are involved in regulating the water and solute movement across the plasma membrane. Liu et al. [32] explored soybean PIPs (GmPIPs) gene expressions in salt stress. This study showed that the GmPIP1- and GmPIP2-type genes might associate with each other to function combinedly in the plant cell, and a salt-stress environment could boost a part of their interactions. This finding provided new insights into the soybean PIP-isoform interactions that lead to the potentially functional homo- and hetero-tetramers for salt tolerance.

3. Conclusions

In conclusion, legumes are important crops in terms of food security, soil fertility, and sustainability. However, there are many challenges that limit the productivity and adaptability of legume cultivations around the globe. Agronomy, breeding, and genetic approaches are capable of enhancing legume cultivars for a better yield. By improving cultivation management practices, and developing new or improved varieties, these limitations can be overcome and achieve sustainable goals. Agronomic practices, for example, such as soil fertility, water, pest, disease, and crop management, can enhance the growth and yield of legumes. Breeding practices, such as cross-breeding and marker-assisted breeding, can boost the development of new or improved cultivars with desired traits, such as yield, quality, disease resistance, and stress tolerance. Genetic methods, such as genomic selection and gene editing, opens the door for enhancing legume cultivars. Therefore, agronomy, breeding, and genetic methods can contribute to the sustainable gains of legume production and the achievement of global food and nutrition security goals.

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