Legume Generation

Boosting innovation in breeding for the next generation of legume crops for Europe

Progressing the breeding of phaseolus bean

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Legume Generation Report 13

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Contents

Summary

This report documents the progress that has been made up until August 2024 by Legume Generation to boost the breeding of phaseolus beans.

Our activities are guided by the expectations of the breeders of our innovation community. Our innovation community consists of 20 partners with currently 11 breeding and 13 pre-breeding programmes. By addressing their demands, we will gain improved phaseolus crops that compete better with other crops on farms. Phaseolus bean encompasses several crops which we consider in our work: Common bean (*Phaseolus vulgaris* L.) as snap (vegetable or French bean) and dry bean. There are two growing forms for each: bush and pole bean each. Common bean is also cultivated in intercropping for feed. The scarlet runner bean (*Phaseolus coccineus* L.) is a pole bean grown for both snap and dry beans.

The goal of our activities is twofold: to select promising lines for breeding programmes and to develop advanced breeding tools such as molecular markers and an integrated data management platform. To support breeding efforts, we are conducting field trials for two bean breeding sets, each addressing different phaseolus bean crops. In 2024, field trials were performed to propagate and preselect the material for the two bean panels to be tested in 2025 and 2026 at three European locations each. In parallel, indoor tests under controlled conditions were conducted to preselect drought tolerant candidates for further testing and analysis, including for 2025 in a Lemnatec automated phenotyping of 39 genotypes with 10 plants each. The indoor tests under controlled conditions focus on drought and heat stress tolerance, aiding the selection of climate-resilient genotypes.

Furthermore, we are developing key genetic resources to support the breeding of improved varieties: (1) Novel plants from an interspecific cross between *P. vulgaris* and *P. coccineus* as a new source for improved traits like disease resistances, (2) A common bean genotype that combines resistance genes against four major diseases, and (3) A multiparent advanced generation inter-Cross (MAGIC) population for use in both breeding and genetic analysis.

The commercial breeders in our bean innovation community are leading the innovation and driving the economic value creation. The knowledge and plant material shared with the breeders will ultimately contribute to the development of new bean genotypes and tools to breed for improved varieties, e.g., with multiple disease resistances. Improved bean varieties with a higher (economic) competitiveness to non-legume crops are in line with the overall European protein strategy as well as with the growing consumer demands for sustainable, locally produced plant protein-based food products.

Introduction

Phaseolus beans (often referred to a just ‘beans’) are the second most important cultivated legume species in the world (33 million ha, FAOStat 2020) and the most important legume for human consumption. Beans are a traditional food crop grown widely over Europe for grain (dry bean, 202,182 ha in 2017) and as a vegetable (green bean, 96,230 ha). In fact, 18 European traditional high quality dry bean production chains are protected by PGI and PDO. However, despite our substantial research base in Europe, the growing market for beans for sustainable healthy diets will be met especially from low-cost imports unless the European crop is revived. This revival is our goal. To reach it, the cultivation of phaseolus bean must be competitive in comparison to other crops. The partners involved in this goal, public institutions and private companies, form the innovation community to boost the breeding of phaseolus bean in Europe.

Phaseolus bean can be considered to encompasses several crops. For the species common bean (*Phaseolus vulgaris* L.), the usage forms snap and dry bean, both with the growing forms bush and pole bean can be considered as in total 4 crops, as the required traits often differ between the single crop. Traits such as resistances and tolerances to biotic and abiotic stress are of general importance, though. Common bean cultivated in intercropping for feed, i.e., pole bean with maize, can be considered as another crop. The species runner bean (*Phaseolus coccineus* L.) with the usage of the dry beans represents another phaseolus crop being part of our work.

Common bean yield is affected by many factors, including diseases, abiotic stress, growth habits, seed phenotype, specific climatic conditions, and adaptation to mechanisation. In addition, factors such as adaptation to organic farming systems, adaptation to processing, consumer preferences, quality traits, and chemical composition can impact bean crop expansion. Many of these characteristics can be improved by plant breeding methods. Specific traits to be improved by breeding include tolerance to drought and heat stress, resistance against anthracnosis, yield, pod quality traits for snap bean, and earliness to avoid the environmental conditions at the hot and dry peak of summer.

Basically, there are two ways to approach genetic improvement: taking advantage of existing diversity, and generating new diversity through crossing and recombination. We are using both strategies to boost bean breeding. To develop breeding programmes, breeders need these sources of characters (genotypes and variation), but also knowledge of character inheritance (genetic control), and tools for selecting the best genotypes. These tools can involve phenotyping methods, markers to assist selection, and available genomes.

The key activities are:

1. the collection of information about genetic resources from related projects;
2. the screening of panels of bean breeding lines and genetic resources, with one focus placed on a multilocation field trial of two bean breeding sets. The screening includes common bean and scarlet runner bean accessions to identify sources of resistance to biotic/abiotic stress, variation in plant/root architecture and in seed quality, and yield. Yield is important especially for dry beans, whereas for snap bean as vegetable the pod quality is of elevated significance;
3. the development and provision of new resources and new tools such as breeding populations (bi- and multi-parental), inter-specific lines, and validated user-friendly genetic markers for genomic assisted breeding;
4. the development of new bean genotypes and cultivars, suitable as either breeding and pre-breeding material, through recombination and pyramiding using cutting-edge methods, such as precision breeding, high-throughput phenotyping and high throughput genotyping complementing classical phenotypic selection.

Progress

The Bean Innovation Community (BIC)

Our innovation community serves as the basis in our bottom-up innovator-led approach to boost breeding. The BIC includes public research institutes and universities as well as private companies, whereby the latter as the breeding partners are driving the innovation and the economic value creation. We will strengthen existing collaborations and develop connections with the European legume community of researchers and breeders. The BIC has grown after project start with new members, and consists currently of 20 partners. They run in total 24 breeding and pre-breeding programmes.

We built upon existing programmes, results and material from previous works and research projects of our partners. This enables us to address the objective to boost the breeding of phaseolus bean on an advanced level, and directly support the breeders with material and tools in their breeding pipeline to develop improved novel varieties.

Phenotypic evaluation of selected genetic resources

Multi-location field trials to evaluate and to select superior genotypes

As phaseolus bean can be considered to encompasses several crops, we designed two bean breeding sets to better address the requirements of the several crops. By this, we extended and deepened the field trial experiments beyond that set out in our proposal:

Supported by results from previous projects (BRESOV, BEAN_ADAPT, INCREASE, and TOOLBEAN), SERIDA led the establishment of our **bean breeding set 1** (**BBS1**, 204 lines) from:

1. well-known breeding lines with characterised genes or QTL controlling important traits;
2. local traditional varieties (incl. cultivars from European Protected Geographical Indication); and
3. selected snap bean lines and dry beans from previous projects. Led by IPK and van Waveren, we also established a **bean breeding set 2** (**BBS2**, 197 lines) specifically focused on bush snap beans with high relevance to our central European breeders (Van Waveren, KWS, LIDEA) comprising breeders' elite material and preselected drought tolerant genotypes.

In total, 30 entries overlap between BBS1 (20 entries) and BBS2 (10 entries). These are grown and phenotyped in both panel and all locations. Additionally, at the location Paterno (Potenza), both BBSs will be grown and phenotyped in both years.

We are performing multilocation field trials (MLFT) for the two bean breeding sets (BBS1 and BBS2), each at three locations (Figure 1). The BBS1 is being phenotyped at SERIDA, Villaviciosa, Northern Spain, UNIBAS, Paterno (Potenza) South Italy, and at SZG, Gleisdorf, Austria. The BBS2 is being phenotyped at UNIBAS, Paterno (Potenza) South Italy, van Waveren, Rosdorf, Germany, and at IPK, Gatersleben, Germany.

The locations have different environmental conditions (temperature, precipitation and soils), and at one location (IPK Gatersleben) one of three replicates is grown with irrigation.

The field trial located in SERIDA is grown within an organic farming system. Taking these differences in environmental parameters between the locations into account, conclusions can be drawn for the suitability of the single genotypes for different environmental conditions, e.g., for the ability to cope with drought or heat stress.

For BBS1 a completely randomized design with three plots per line is being used in the MLFT. The plots comprise 10-12 plants distributed along a one-meter single row. For BBS2, an alpha-lattice design with 197 lines and 14 plants per plot in double rows is used.

We determined a unified descriptor (Table 1), which is used for the phenotyping of all the field trials. Phenotyping will be primarily focused on morpho- agronomic traits of high interest in breeding. This set included morphological, phenological and agronomic characters. These descriptors are based upon those used in previous projects (e.g., BRESOV and INCREASE), enabling a strong comparability. Additionally, the incidence of pests and diseases is recorded. With all this, a database will be available that will allow the identification of genotypes adapted to each region and resilient genotypes showing high yield stability.

Genotyping data for the two BBSs are either already available (BBS1: whole genome sequencing [WGS] mainly available with a few entries still to be sequenced), or will be generated (BBS2: genotyping-by-sequencing [GBS]). Combining phenotyping and genotyping in an association study will enable us to identify marker trait associations (GWAS, Genome-Wide Association Studies) with the aim to generate breeder friendly molecular markers.

Figure 1. Locations for the multi-location field trials (MLFT) for the 2 bean breeding sets (BBS1 and BBS2)

Table 1. Descriptors for the bean field trials, based upon the descriptors previously used in the projects INCREASE and BRESOV.

Object	Morphological trait	Type of trait	BRESOV	INCREASE
Plant	Plant determinacy	Qualitative	Yes	Yes
Plant	Plant growth habit (=type)	Qualitative	Yes	Yes
Pod	Flower: colour of standard	Qualitative	Yes	Yes
Pod	Flower: colour of wings	Qualitative	Yes	Yes
Pod	Pod cross-section	Qualitative	Yes	
Pod	Pod curvature	Qualitative	Yes	Yes
Pod	Pod colour at R8 stage	Qualitative	Yes	Yes
Pod	Pod suture string	Qualitative	Yes	
Seed	Pod length	Quantitative	Yes	
Seed	Pod with	Quantitative	Yes	
Seed	Seed coat patterns	Qualitative	Yes	Yes
Seed	Seed coat darker colour	Qualitative	Yes	
Seed	Seed coat lighter colour	Qualitative		
Seed	Seed shape	Qualitative	Yes	Yes
Seed	Seed length	quantitative		
Seed	Seed width	quantitative		
	Phenological characters			
Plot	Sowing date	Quantitative	Yes	Yes
Plot	Days to emerging	Quantitative	Yes	Yes
Plot	Emerged plants	Quantitative	Yes	Yes
Plot	Plants being harvested	Quantitative	Yes	Yes
Plot	Days to beginning of flowering 1	Quantitative	Yes	Yes
Plot	Days to beginning of flowering 2	Quantitative	Yes	Yes
Plot	Days to ending of flowering	Quantitative	Yes	Yes
Plot	Days to snap bean stage	Quantitative	Yes	Yes
Plot	Days till harvest	Quantitative	Yes	Yes
	Agronomic traits			
Plot	Number of seeds per pod	Quantitative	Yes	Yes
Plot	Total dry seed mass per plot	Quantitative	Yes	Yes
Plot	100 Seed Weight	Quantitative	Yes	Yes
Plot	Plant height	Quantitative	Yes	Yes
	Stress-related traits			
Plant	Abiotic stress	Qualitative	Yes	Yes
Plant	Pests	Qualitative	Yes	Yes
Plant	Fungi	Qualitative	Yes	Yes
Plant	Bacteria and virus	Qualitative	Yes	Yes
Plant	Seed diseases	Qualitative	Yes	Yes

Establishment of the panels

Compiling of BBS1

The **BBS1** is designed to study adaptation to cultivation in different European regions and to identify sources of traits for European breeding programmes. The BBS1 encompasses 204 entries, of which are 123 classified as pole beans and 72 as bush beans. Among the 204 entries, 50 are classified to be used as dry bean, 72 as snap beans, and the others are not determined in their usage. The BBS1 and BBS2 have 30 lines in common. The entries for BBS1 were selected considering (see also Figure 2).

1. Well-known breeding lines with characterised genes or QTLs that control important traits.
2. Local traditional varieties, including cultivars from the European Protected Geographical Indication (PGI).
3. Snap bean lines and dry beans selected from the BRESOV and INCREASE projects, e.g., lines with resistance to powdery mildew, local anthracnose races, and high yield or yield stability.
4. Other genotypes of interest for the Bean Innovative Community, such as genotypes with available genomes. For BBS1, whole genome sequencing (WGS) data will be used to associate phenotype with genotype. WGS for most entries are already available, the lacking ones will be sequenced.

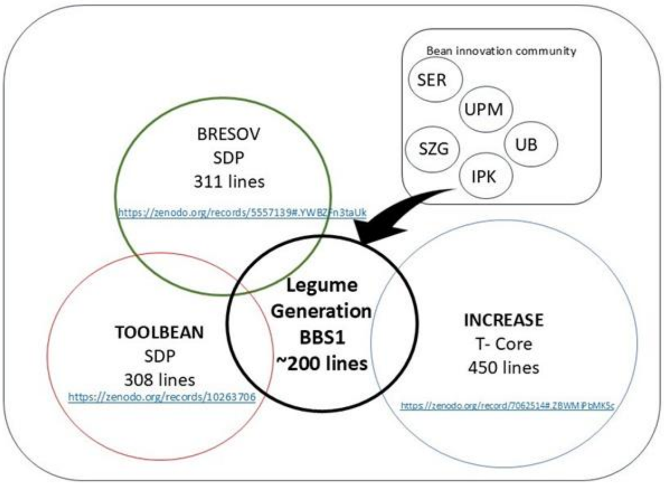

Figure 2. Establishing the BBS1 from available materials in the Bean Innovation Community and results obtained in previous projects (https://zenodo.org/records/10263706;https://zenodo.org/record/7062514#.ZBWMiPbMK5c;https://zenodo.org/record/5557139#.YWBZFn3taUk).

Compiling of BBS2

The **BBS2** is largely based on up-to-date breeders bush snap bean varieties and elite lines. The focus lies on bush snap beans as locally grown phaseolus crop for the central, eastern and northern European markets. The European breeders van Waveren and Pop Vriend contributed breeders' elite material and varieties, and the IPK from its genebank accessions. The BBS2 encompasses 197 entries: 60 genebank accessions, 117 modern breeders´ varieties and elite material, and 20 entries from the BBS1. Special attention was given to potential drought tolerant genotypes. Genotyping-by-sequencing (GBS) will be newly accomplished.

Field pre-trials in 2024 for seed propagation and selection

Multiplications were carried out in the field and greenhouses at SERIDA, UNIBAS and IPK, accompanied by phenotyping for selection to have seeds available for the MLFT, (Figure 3 -

Figure 5). In addition, some materials (accessions) were purified by self-pollination of individual plants to provide lines.

Two cycles of greenhouse crops were developed in 2024 in SERIDA facilities. Plant showing symptoms of diseases were removed. At the end, 66 lines with more than 250 seeds were available. In addition, a DOI was assigned to each line to record the exchanges using Easy-SMTA (standard material transfer agreement) and Global Information System established under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Finally, 365 lines of the BBS1 were distributed to partners (SZG, IPK van Waveren and UNIBAS) from SERIDA to develop the 2025 MLFT.

At UNIBAS, a subsample of 200 lines selected from the INCREASE project was multiplied. For each line, 15 seeds were sown in single row plots. 81 lines were further selected to be part of the BBS1.

At the IPK, in 2024 a field trial was conducted (

Figure 5), encompassing 160 sown seeds for 57 genebank accession and 16 breeders' lines of in total 73 bush beans for seed propagation, and phenotyping of 30 traits at the plots. All 73 lines from the 2024 field trial are part of BBS2. After the harvest, for 6 selected lines/accessions, identical with the ones for the drought stress pretrial in the Lemnatec automated phenotyping platform (see below), the complete plants including their root system were dug out, cleaned and the root morphology was phenotyped. Smaller differences in the length of the main root were scored, and differences in root nodule formation were observed.

Figure 3. Greenhouse at SERIDA with lines of BBS1 in multiplication

Figure 4. Field multiplication for BBS1 carried out in 2024 at UNIBAS.

Figure 5. Field trial of bush beans (Phaseolus vulgaris L.) at the IPK Gatersleben in 2024 for phenotyping and seed propagation

Multi-location field trial (MLFT) in 2025

Field phenotyping is in progress in all field location in 2025 (Figure 6 – Figure 9). The data will be compiled in a common database. This database will be a valuable tool for breeders to select the most suitable genotypes for their improvement programmes. Once the phenotyping and genotyping data are available, we will conduct association studies and mapping to identify marker-trait associations as basis for breeder friendly molecular markers. In parallel, pods, seeds and symptoms of diseases and pests will be documented with photos, and the images will be made available in a Zenodo repository. To help detect pests in bean crops, SERIDA has compilated a photo gallery (Figure 6).

Figure 6. a: Field trial at SERIDA within an organic farming system with plants at a young stage (09/06/2025); b: Field trial at SERIDA in organic farming system with plants at an advanced growing stage (26 June 2025)

Figure 7. Field trial at UNIBAS (26 July 2025)

Figure 8. Field trial at SZG (14 July 2025) revealing strong differences between the entries regarding susceptibility to root and leaf disease in BBS1

Figure 9. Phenotyping at the IPK´s common bean field trial showed elevated phenotypic diversity between the entries of BBS2 (left; 19/08/2025), as well as the phenotyping of the pods of BBS1 at Serida (right).

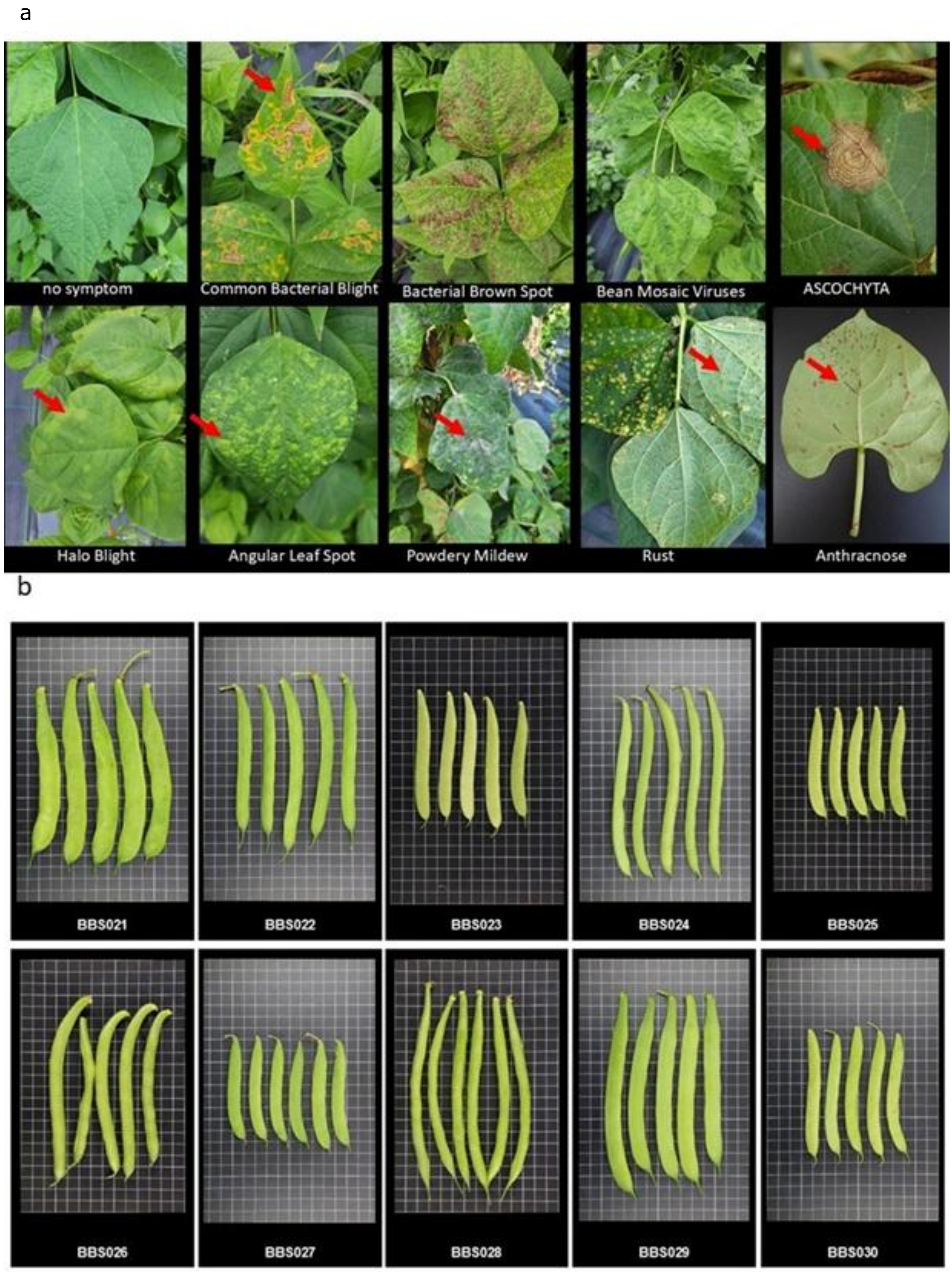

Figure 10. a: Image gallery to help in the identification of common bean diseases; b: Image gallery to record the phenotypic variation of the pods in BBS1. Both prepared by SERIDA.

Selection for drought stress tolerance at the IPK

Typically, drought tolerant plants are able to better survive drought periods, and to better and recover afterwards.

In a Lemnatec automated phenotyping platform at the IPK, in 2025 a drought stress trial is performed to select genotypes with drought tolerance and with favorable root architecture, e.g., for dry regions the ones with deep growing tap roots reaching deeper water resources (Figure 11 - Figure 12). The results will also serve as basis for further work to map genes/QTLs of root morphological traits to develop selection tools for breeding.

In 2024, a pre-experiment for drought tolerance and root architecture was conducted at the IPK (16 h light, with 24°C/16°C light/dark; "Potgrond P Substrate" (Klasmann, BayWa München, Germany). Six lines with 11 plants each (six drought stress + five control ones with normal watering) were included, in total 66 plants (Figure 12, Figure 13). Three lines were modern breeders' varieties provided by van Waveren and three lines were accessions from the IPKs genebank, selected based upon root morphology and drought response data previously obtained. The plants in the Lemnatec facility were phenotyped scoring root and shoot traits once per day to evaluate the vitality, growing speed, biomass and chlorophyll content as affected by drought stress. We selected a medium strong drought stress, with a strong impact on the plant growth and metabolism, but without endangering the survival of the plant. At the drought stressed plants, the soil water content was adjusted to approx. 40% rel. soil humidity (control: approx. 85% rel. humidity). The presence/absence of rhizobia was scored, and clear differences were found. The experiment was finished 82 days after sowing. Seeds were harvested, and from a subset of the panel (50%: six plants per line, with three plants each for drought stress and control condition) the roots were cleaned from soil, dried and phenotyped manually.

Significant differences between the genotypes were identified for shoot mass in both control and drought stress conditions, and for root mass under drought stress (Figure 13 - Figure 15). Under normally watered conditions, no significant differences were found between the genotypes (Figure 16).

The results helped to define the conditions and design for the main drought stress trial in 2025 with 390 plants (Figure 17; 39 entries with 10 plants each - 5 plants for drought stress and 5 plants as control, respectively).

Figure 11. Experimental set-up of the Lemnatec phenotyping platform for the pre-trial, involving a visiting researcher from Ghana, Dr. Isaac Amegbor (left), who is collaborating on common bean research with IPK and van Waveren. Two bush bean accessions showed climbing behavior in 2024 (right), leading to increased light intensity for the main trial in 2025 to prevent this.

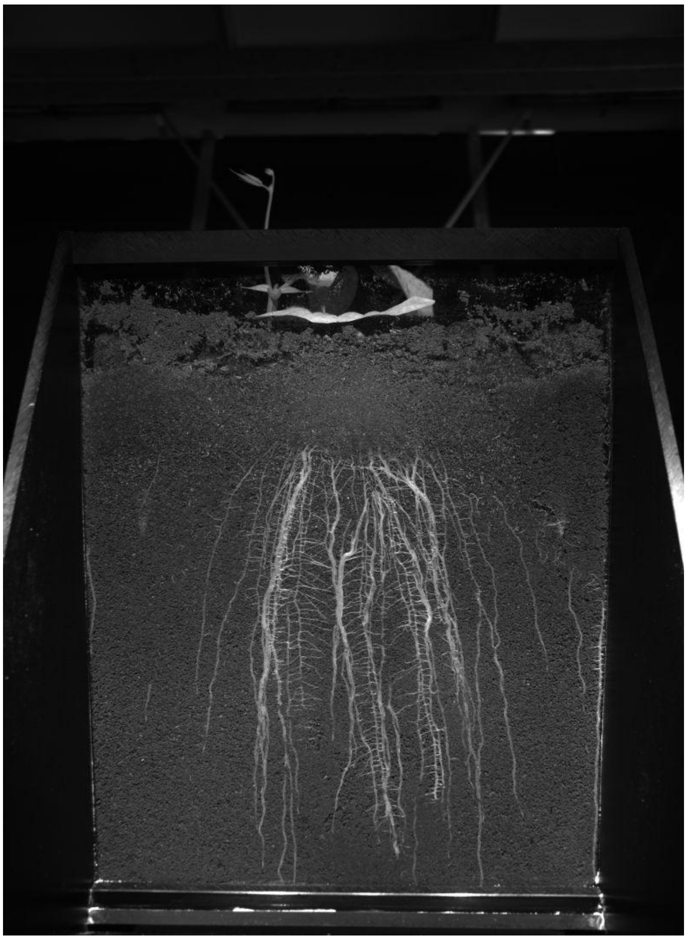

Figure 12. Root phenotyping in the IPK´s Lemnatec platform with automated imaging.

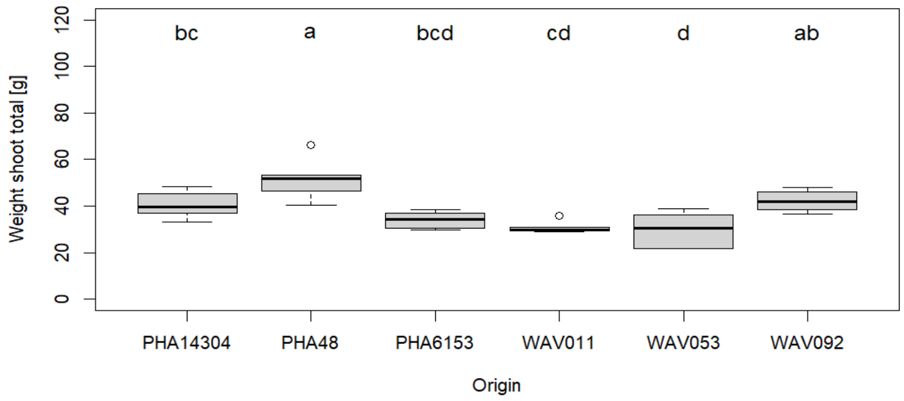

Figure 13. Total shoot weights (shoot and pods) for the individuals of the drought group per origin. Different letters at the top of each plot stand for significant differences between the origins in a Tukey test with α = 0.05. n = 6 individuals per origin.

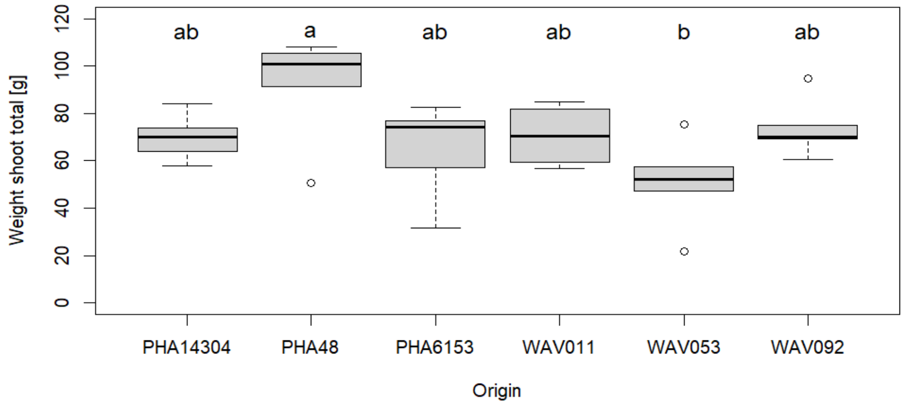

Figure 14. Total shoot weights (shoot and pods) for the individuals of the non-drought group per origin. Different letters at the top of each plot stand for significant differences between the origins in a Tukey test with α = 0.05. n = 5 individuals per origin (n = 4 for WAV011).

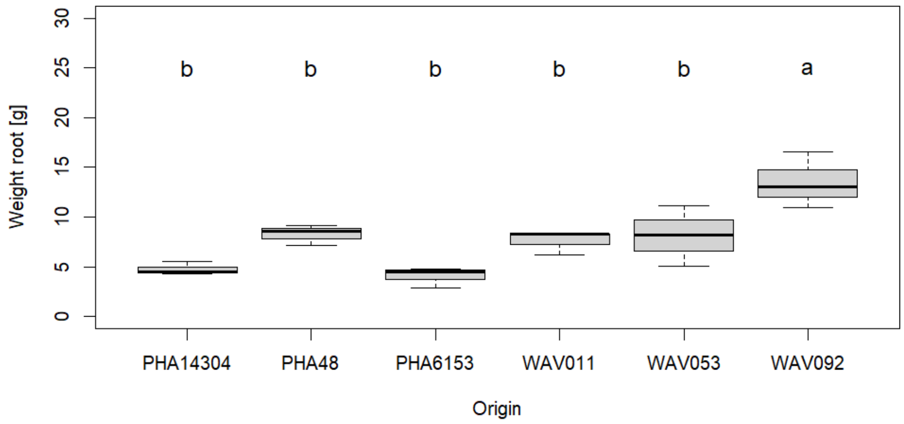

Figure 15. Root weights for the individuals of the drought group per origin. Different letters at the top of each plot stand for significant differences between the origins in a Tukey test with α = 0.05. n = 3 individuals per origin.

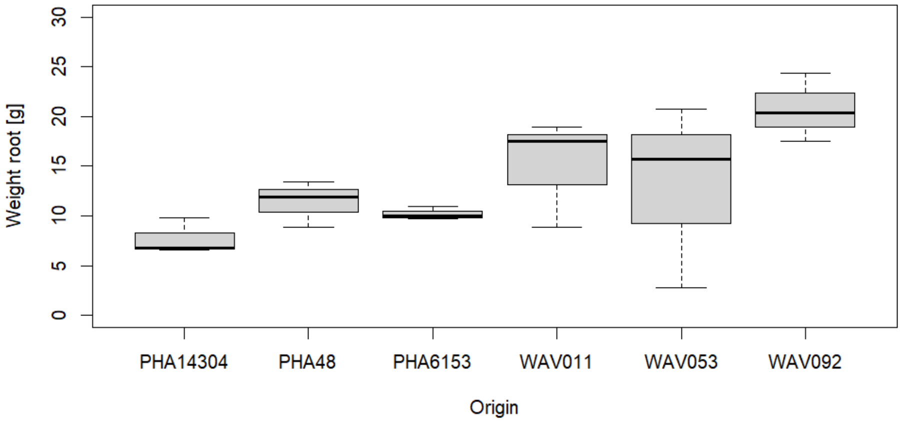

Figure 16. Root weights for the individuals of the non-drought group per origin. No significant differences between the origins were found in an ANOVA with α = 0.05. n = 3 individuals per origin.

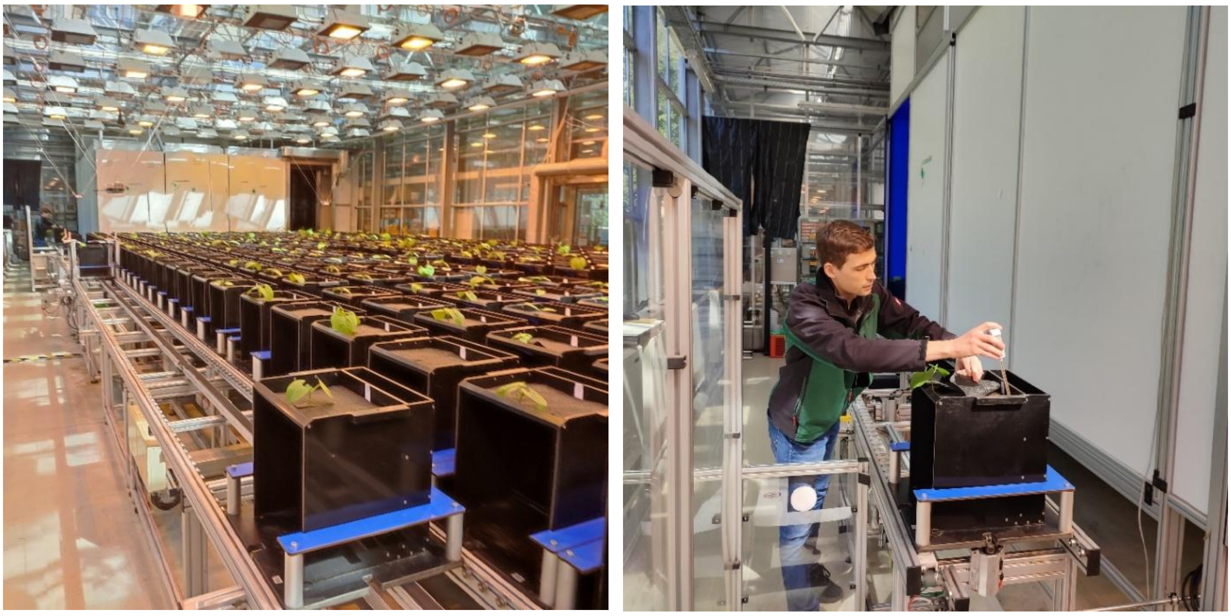

Figure 17. (left) Common bean plants from 39 entries are emerging in the 390 pots of the drought stress trial at the Lemnatec automated phenotyping platform at the IPK Gatersleben. Root and shoot development are recorded. (right) Setting up the conditions for the common bean drought stress trial at the Lemnatec automated phenotyping platform at the IPK Gatersleben. Immanuel Scheil, student at the University of Göttingen, is working on his master thesis using the drought stress trial.

To gain data for selection to breeding programmes, as well as to determine the most promising entries and the experimental conditions for a Lemnatec drought stress trial in 2025, a drought stress trial in the greenhouse was conducted in 2024 at the IPK (Figure 18). Drought stress was applied at an early developmental stage, as planned for the further experiments (6 d after germination). The panel consisted of 24 bush and 14 pole bean lines from three breeding partners: 11 lines from SERIDA, 11 lines from Pop Vriends and 13 lines from van Waveren, plus three accessions from the IPKs genebank. 256 plants from 38 lines/varieties were obtained from 304 seeds sown (eight seeds each line with four replicates for each treatment: drought stress vs. control). We separated the plants in the two groups drought stress vs. normal, and reduced watering for the drought stress samples to adjust a medium strong stress level. The soil water content was frequently measured, and adjusted to approx. 40% relative soil humidity. After 28 days of drought stress, all plants were watered fully and equally (approx. 85% rel. humidity). Traits were measured to evaluate the effect of drought stress on biomass, developmental speed, vitality/chlorophyll content, and yield (pods and seeds). A suitable level of drought stress was applied. These results serve as basis for selection of tolerant plant material for the breeders, but also for the further drought stress trials.

Figure 18. Drought stress pre-trial of 256 pole (left) and bush (right) beans during the winter 2024/2025 in the greenhouse.

A first analysis revealed strong yield differences (pods and seeds) between the lines after drought stress, i.e., in this trial several genotypes showed a tolerance to drought stress (Figure 19).

For most genotypes, the drought stress caused a delay in development, i.e., flowering time and time to maturity. Drought stress plants tended to reach the harvesting stage later, and yielded more than the control plants at these later time points. However, final harvestable yield was reduced – with few exceptions (Figure 20). The bush bean KWSNL-LG-009, and the pole beans KWSNL-LG-003, PHA_14304 and SDP308 performed non-significantly better under drought stress conditions, and are thus good candidates for further detailed investigation. As part of the further analysis, a gene expression analysis (RNA-Sequencing) is planned to identify differences between drought stressed and non-drought stressed plants to identify genetic factors for drought stress tolerance in common bean.

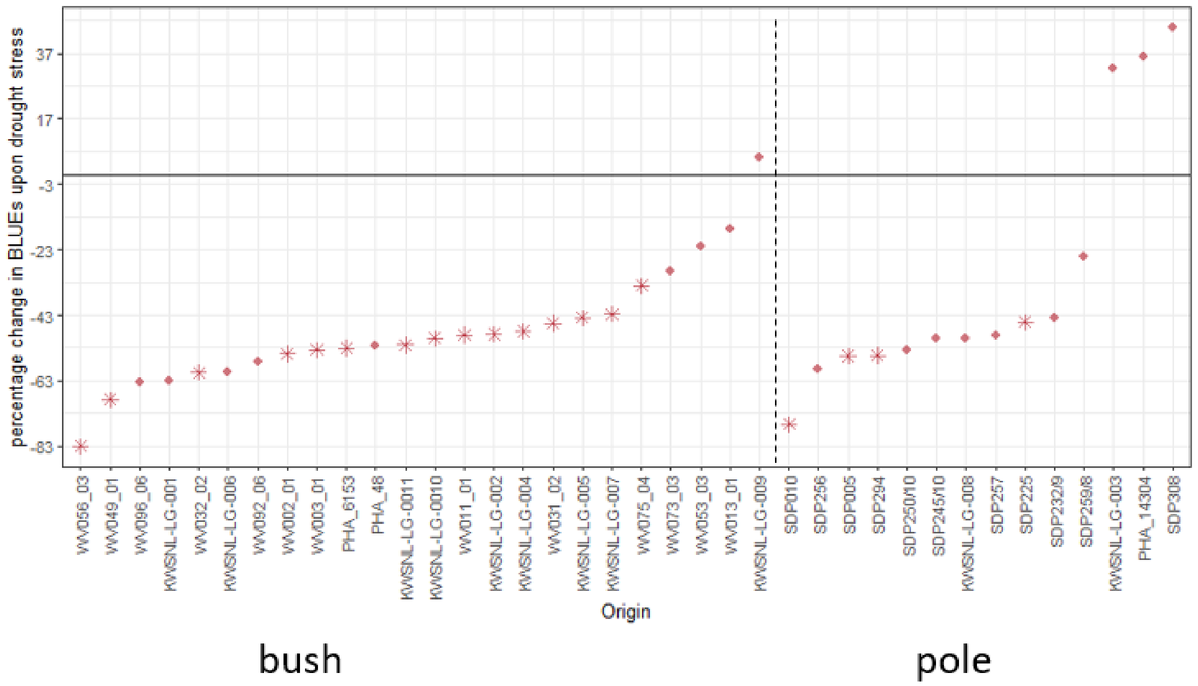

Figure 19. Difference in seed number (%) between drought stressed and control plants as representative seed yield trait for the 38 bean origins (lines, varieties, accessions) in the greenhouse trial. Asterices indicate significant differences in seed yield between drought stressed and control plants within an origin, whereas the origins without significant differences are visualized as diamonds.

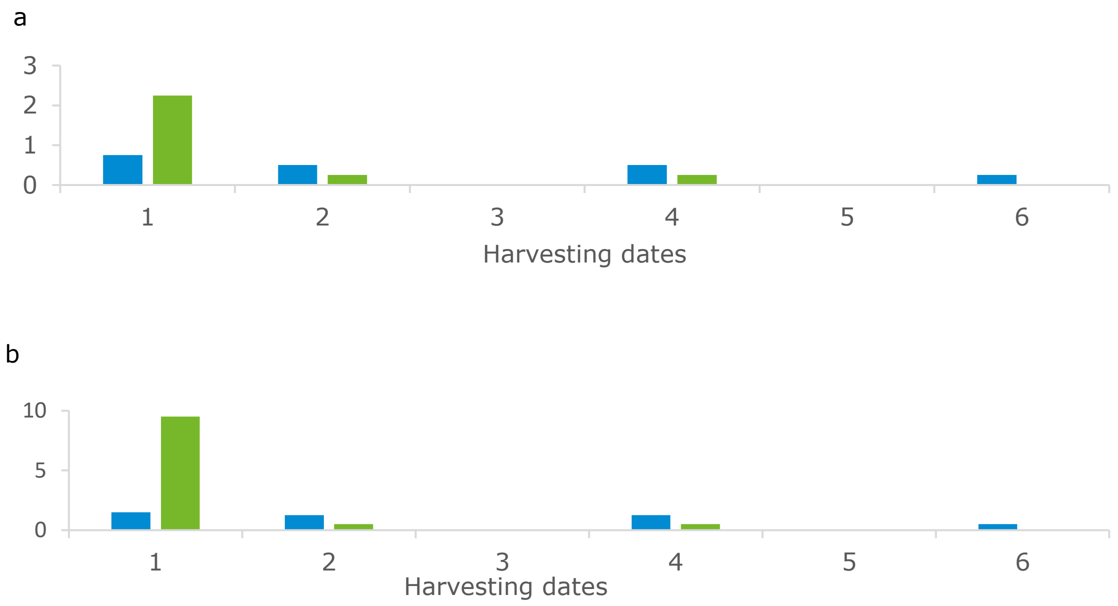

Figure 20. Taken the line WV092 as representative example, the mean values of the 4 replicates per line for the number of number of seeds (a) and pods (a) are calculated for each of the 6 harvesting dates ("1" is the first, "6" the last harvesting date). The values for the drought stressed plants are displayed in blue, for the control in orange. Drought stress led to a reduction as well as delay of seed production. A recovery after watering was observed, but with a mostly lower total seed yield.

Updating breeding tools and resources

Analysis to refine the selection of our plant material

To select lines from previous projects (INCREASE, BEAN_ADAPT) fitting best the requirements of European breeders, we selected at UNIVPM and SERIDA lines based on molecular diversity, more specifically on PAV (presence/absence variation, Figure 21) that were mapped using the pangenome developed within INCREASE as a reference. The selection process considered agronomic traits, and also diversity based on the PAV, in order to promote segregation for loci that can be hemizygous in F1 lines. Figure 21 shows in light blue the European Andean accessions; in red, European Mesoamerican accessions; in dark blue, the reference accession MIDAS which is one of the American Andean reference genomes used for the assembly of the pangenome. Colored rectangles refer to selected accessions for performing F1 crossing.

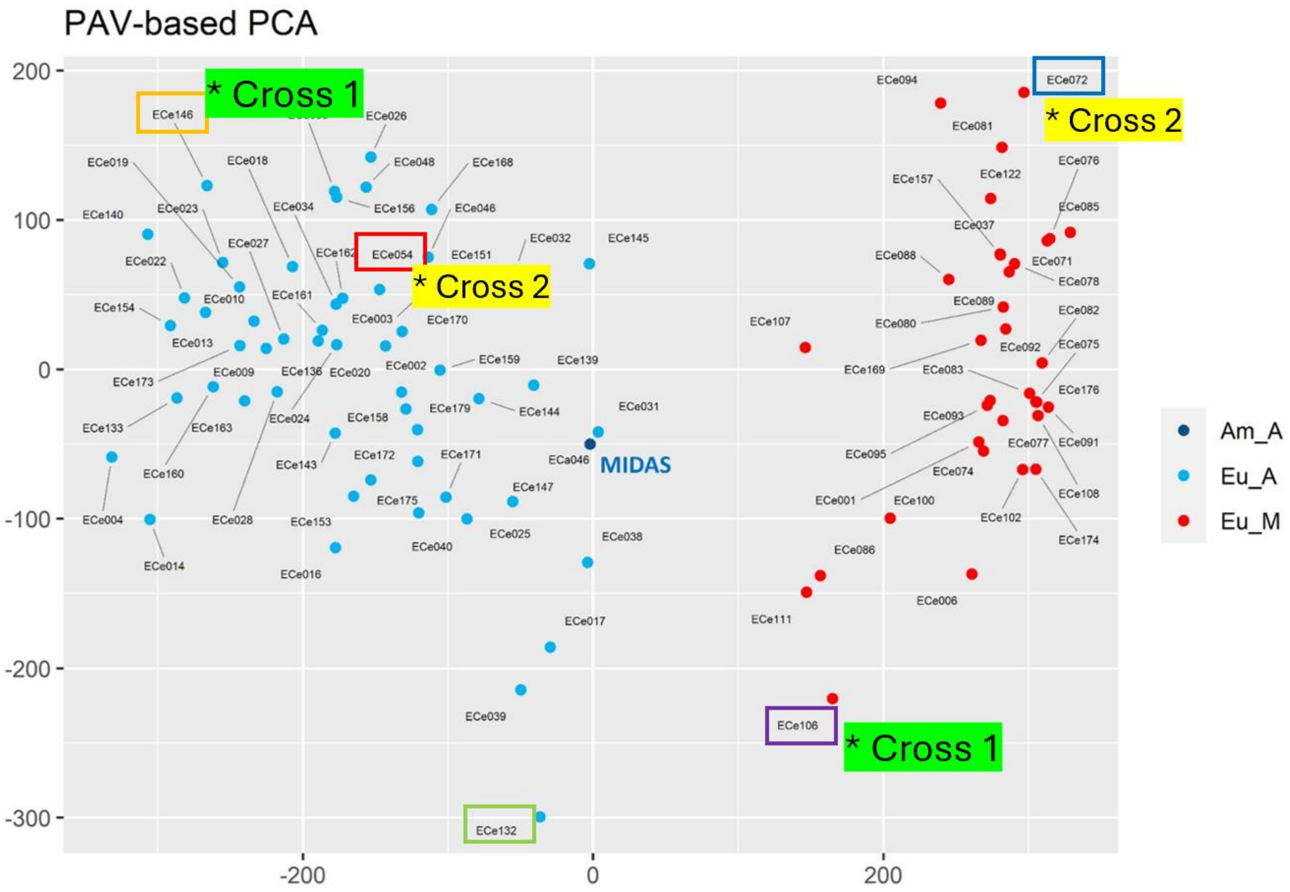

Figure 21. Molecular PAV (presence/absence variation) analysis of lines from previous projects (INCREASE, BEAN_ADAPT) to select lines fitting best the requirements of European breeders in Legume Generation.

Interspecific common bean and runner bean populations

Recombinant interspecific inbred population generated by SERIDA

Runner bean (*Phaseolus coccineus* L.) is part of the secondary gene pool of common bean (*Phaseolus vulgaris* L.) and presents interesting characteristics for the improvement of common bean as resistance/tolerance to biotic and abiotic stresses. Several segregating materials and homozygous lines (HYB lines) were derived from crossing (*P. coccineus* × *P. vulgaris*) × *P. vulgaris* at the SERIDA partner: F2Bc1, F3Bc1, F4Bc1, and F5Bc1 generations.

The accession V215 (SERIDA seed collection) was used as the *P. coccineus* parent. It is resistant to many diseases present (anthracnose, powdery mildew, rust, BCMV, BCMNV, white mold, common bacterial blight). V215 also has a highly developed root system and very large white seeds. The Michigan Dark Red Kidney (MDRK, maintained in the SERIDA seed collection) lines were used as *P. vulgaris* parents because these are susceptible to many races of anthracnose, powdery mildew, and rust,) and have a purple kidney seed. Our approach aims to obtain a new set of lines through self-pollination and selection, and the genetic characterization of this new resistance.

As we aim to generate a set of recombinant lines from the available segregating material obtained from (*P. coccineus x P. vulgaris*) x *P. vulgaris*, the following work was conducted (Figure 22):

1. The investigation of the available material in early generations. A total of 226 lines were rescued from previously generated material from the cross MDRK x (MDRK x V215).
2. The development of three generations of self-fertilization following the single seed descent method. In total, 130 interspecific inbred lines (generation > F6Bc1) with more than 5g of seeds were obtained. However, 96 of these lines did not produce pods or viable seeds, and thus could not be included in the further work.

The obtained set of lines is being multiplied in greenhouse and is subjected to primary characterization based on morphological and phenological descriptors. The same descriptors used in the MLFT evaluations will be used in this characterisation. Our aim is to select genotypes that show tolerance or resistance to biotic and abiotic stresses.

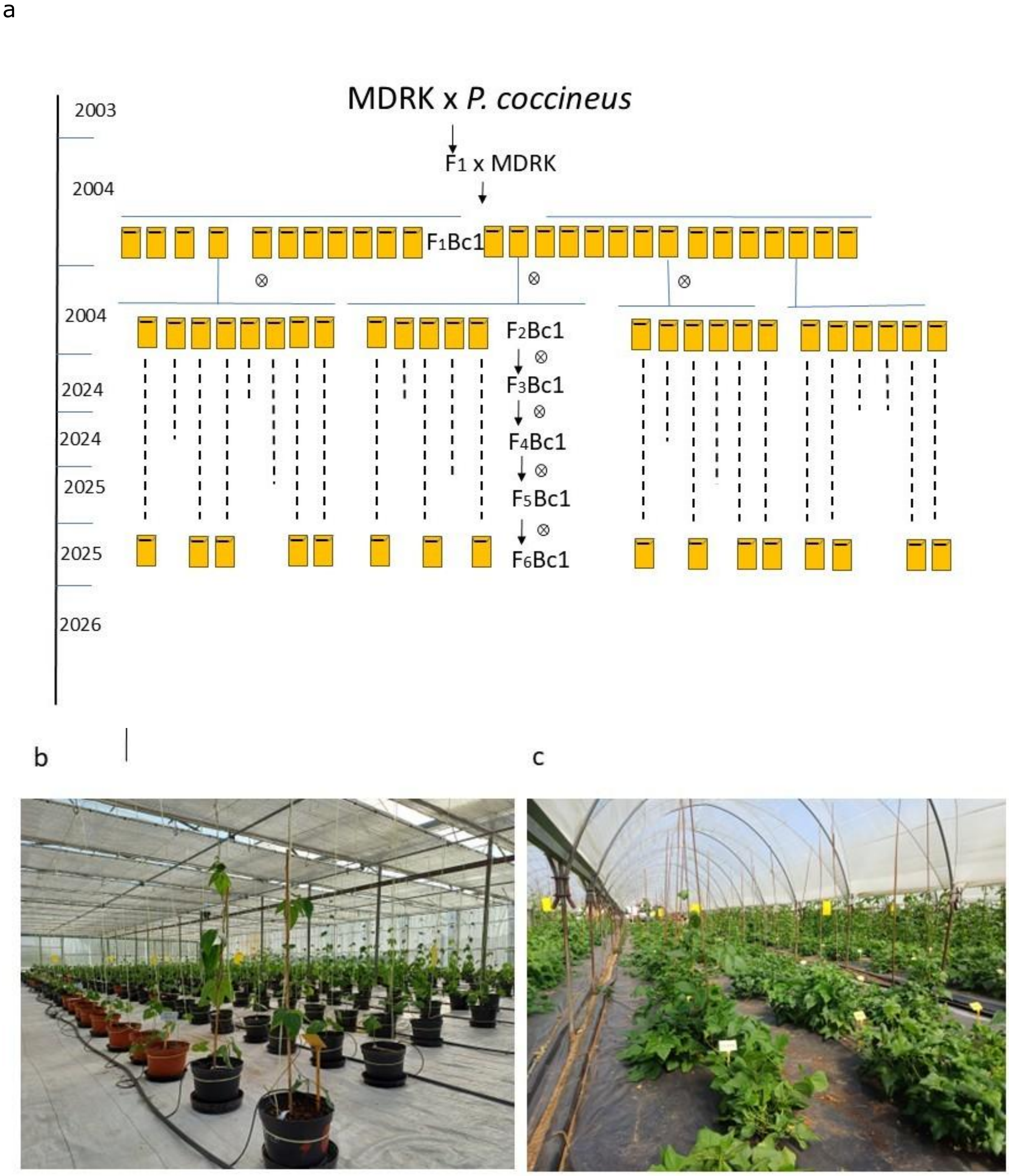

Figure 22. a: Scheme showing the plan to develop the interspecific inbred lines; b: Self-pollination of plants to obtain interspecific recombinant lines; c: Characterisation of 130 inter-specific lines and their two parents in the greenhouse

Ad-hoc F2 populations to characterize the resistance anthracnose and powdery mildew introgressed in P. vulgaris from P. coccineus

Two interspecific advanced lines with resistance to anthracnose (raza 38) and powdery mildew (under natural conditions) were identified at SERIDA, HYB020 and HYB028. To understand the resistance, a forward genetic analysis was carried out by SERIDA. The HYB028 line was crossed with Midas, a snap bean susceptible to race 38. The F3 progeny of each F2 plant was tested against the race 38. The observed segregation was

21R:40H:24S, which is expected for one gene underlying the resistant phenotype ($\chi^2_{1:2:1}$= 0.21, P =0.78). Plants with the heterozygous genotype (H) showed segregation that conformed to the 3R:1S ratio, indicating the dominant nature of this resistance.

Two bulks (resistant and susceptible) with 10 plants each were established and genotyped using whole-genome sequencing (WGS). Sequencing reads from different genotypes were aligned using the bean genome G19833 V2 (GCA_000499845.2). A total of 5,766,560 SNPs were detected and reduced to 5,432,040 SNPs after filtering (removing SNP with missing values and location in bean chromosomes). Bulked segregate analysis implemented with the QTLseqR package revealed a couple of regions significantly associated with resistance. These regions were located at the end of chromosome 7 and the beginning of chromosome 11. The involvement of these regions will be verified by linkage analysis between the resistance and markers located at this genomic position. For this purpose, new molecular markers (InDels) are being developed.

Multiparent advanced generation inter-cross (MAGIC) populations

Coordinated by UNIVPM, together with SERIDA the development of multiparental populations for breeding and genetic analysis was started with part of the crosses conducted by SERIDA and part by UNIVPM. MAGIC populations (Multi-parent Advanced Generation Inter-Cross) are used in plant genetic to create high genetic diversity and enable precise genetic mapping for improving complex traits in crops. In addition, the development of MAGIC can generate new genotypes of interest in plant breeding through recombination among the involved parents.

A MAGIC population with 8 parents was designed, and five potential parental genotypes were selected by SERIDA considering the phenotypic data from previous works and the genetic distance from the genotypic data (Table 2). Figure 25 shows the variation in seed phenotype for these five genotypes (Midas, BAT93, UI111, 5-593, and Xana), of which four are ultimately selected to generate half of the MAGIC population. Table 2 contains the main characteristics of the five selected genotypes. F1 seeds from three different crosses (XanaxUI111, MidasxBAT93, Xanax5-593) were obtained, and afterwards these F1 were used to obtain the second generation of crosses. In total, 126 and 184 seeds were obtained from two different crosses in this second step of crossing (XanaxUI111, MidasxBAT93), respectively.

Figure 23. Seed phenotypes in five bean genotypes selected by SERIDA to constitute the MAGIC population.

Table 2. Main characteristics in the five genotypes selected by SERIDA. ANT: anthracnose, CBB: common bacterial blight, BCMV: bean common mosaic virus, RUST: bean rust

Trait	UI111	BAT93	Xana	Midas	5-593
Gene pool	Mesoamerican	Mesoamerican	Andean	Mesoamerican	Mesoamerican
Use: snap / dry	Dry	Dry	Dry	Snap	Dry
Growth habits	Determinate	Indeterminate prostrate	Determinate prostrate	Determinate erect	Determinate
Seed traits	pinto middle	cream small	White Very large	white	black
Resistance to diseases	Under study	ANT, CBB, BCMV, RUST			Under study
genome available	Yes	Yes	No	No	Yes

Five potential bean genotypes were selected by UNIVPM based on molecular diversity, more specifically on PAV (presence/absence variation, Figure 21) and considering previous projects (INCREASE, BEAN_ADAPT) fitting best the requirements of European breeders. Two crosses have been mainly performed as depicted in Figure 21, where accessions are named after the code established in the BEAN_ADAPT project, coordinated by UNIVPM. However, other crosses, involving Andean x Mesoamerican have been performed based on flowering synchronisation. Four of these genotypes are ultimately selected to constitute the second half of the MAGIC population.

Those lines are also part of the INCREASE project. ECE132 has been also chosen, however, priority has been given to the crosses 1 and 2 (Figure 21) based on agronomic data. Crosses have been performed as Mesoamerican (in red) x Andean (Light blue) and among SSD lines with contrasting growth habit, under controlled greenhouse conditions in the summer 2024 at UNIVPM (see more details on passport data and molecular classification for the five parental lines at Bellucci et al. 2023: https://doi.org/10.1038/s41467-023-37332-z). In total, 89 F1 seeds were obtained by UNIVPM as shown in

The F1 seeds were quite homogeneous across the different F1 producing plants (Table 3) from the same cross, with slight differences in terms of colour intensity appearing. The 38 F1-plants from two crosses (Ece146xEce106, Ece54xEce072) were grown.

Table 3. Number of F1 seeds from crosses between Mesoamerican x Andean and among SSD lines with contrasting growth habit at the UNIVPM

Female x male	Gene pools	Number of seeds
Ece146 x Ece072	A x M	36
Ece146 x Ece106	A x M	19
Ece054 Ece072	A x M	19
Ece054 x Ece106	A x M	7
Ece132 x Ece106	A x M	8

The codes from BEAN_Adapt (Ece) are used which correspond to the INCREASE (INCBN) project codes as follows: Ece146 = INCBN_00183; Ece072 = INCBN_00144; Ece106 = INCBN_00168; Ece054 = INCBN_00141; Ece132 = INCB_00175

Breeding for resistance to biotic stress

Plant diseases usually weaken, damage, or even kill plants, resulting in a loss of total yield or market-appropriate products. Chemical plant protection treatments are expensive, have typically negative effects on the environment, and are not usable in organic farming. Besides our approaches to introgress resistances by interspecific crosses from *P. coccineus* to *P. vulgaris* (see above), SERIDA started to develop a programme to group resistance genes to anthracnosis (gene *Co-2*), powdery mildew (*Pm1*), bean common mosaic virus (gene *I*), and bean common mosaic necrotic virus (genes *I and bc-3*) in a bean genotype. The goal is to pyramid resistance genes in the dry bean market class Fabada with PGI 'Faba Asturiana' (https://faba-asturiana.org/; https://fabadelourenza.org/es) and demonstrate the efficiency of functional markers and precision breeding to accelerate breeding programmes.

Two homozygous lines with different resistance gene combinations served as the starting points: X4562 and A2648 (Figure 25). Both lines have similar seed phenotypes, classified as market class fabada and this market class has two PGI in the northern Spain. Genes involved in these resistance mechanisms have been well characterized. Reported functional markers are available to tag these genes in breeding programs.

Following the programme shown in Figure 25, we were able to obtain four F1Bc1 plants that possessed the four genes. 400 F2Bc1 were obtained from these four plants at SERIDA, which are currently (2025) self-pollinated. Two progenies showed determinate growth habit (fin fin) while the others two progenies showed a segregation indeterminate: determinate growth habit. In two progenies no symptoms of powdery mildew and BCMV infections were observed, suggesting that these are homozygous for the genes *Pm1* and *I*. The harvested plants will be phenotyped (yield and seed phenotype) and those selected will be evaluated for functional markers of the resistance genes managed. In the end, the goal is to have genotypes that combine resistance to anthracnose, BCMV, BCMNV, and powdery mildew in a seed phenotype within the fabada market class.

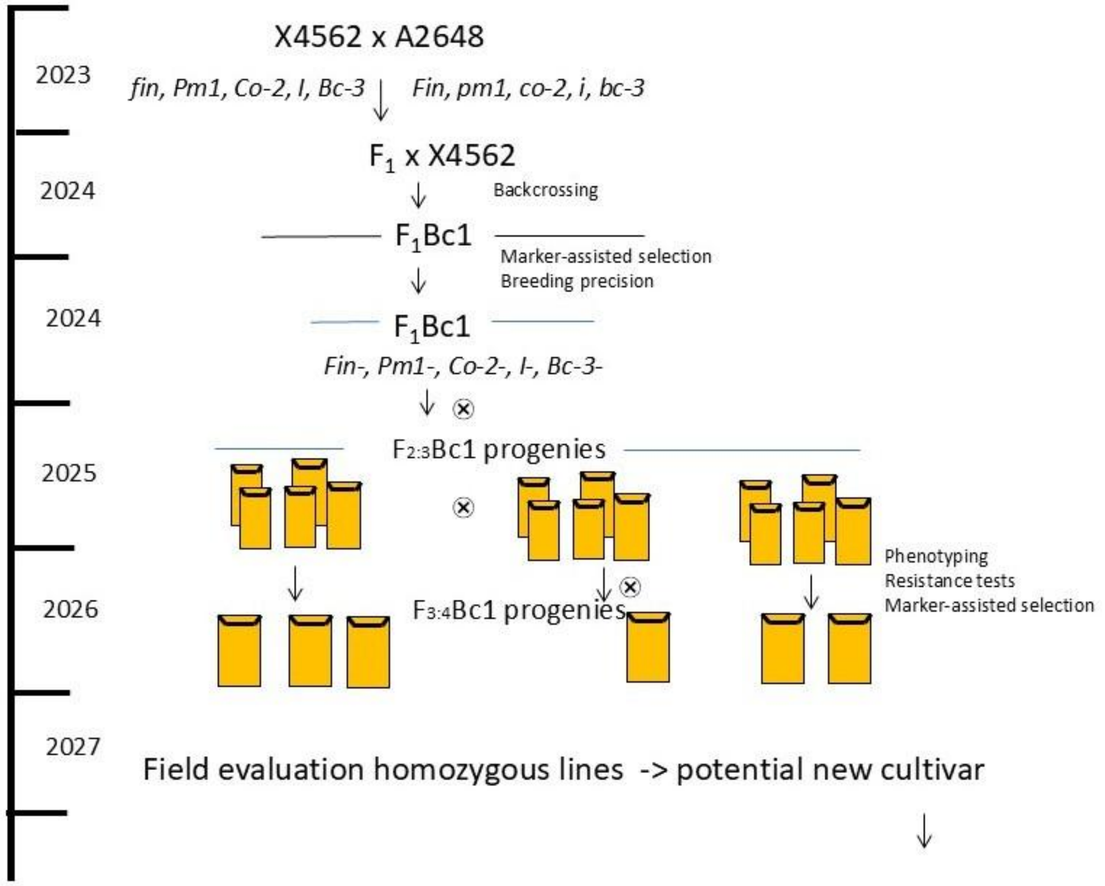

Figure 24. Overview of the breeding programme to pyramid resistance genes in the market class fabada;

Figure 25. Typical symptoms of anthracnose, bean common mosaic virus (BCMV) and powdery mildew in comparison to a non-infected plant.

Conclusions

We have started the selection and generation of improved plant material for phaseolus bean. This is being done in field trials, but also under controlled greenhouse and phytochamber conditions, and with directed crosses between selected parents. Additionally, the phenotypic data will together with genotypic data enable us to unravel the genetic background of important traits, and by this provide (molecular) tools for the breeding. Both, improved plant material and novel tools contribute to the objective to boost the breeding of phaseolus bean, and by this to boost the generation of more competitive legume crops in European agriculture. The breeders involved in our work can directly utilise the results for innovation, i.e., the breeding of novel, superior varieties. The generated material and the new tools (including our research data platform) can serve together with the created connections in our innovation community also as a basis to address future research needs not apparent yet. Examples might be new bean diseases, but also new demands by consumers and industry concerning the product (seeds, pods, biomass) harvested.