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# Nodulation performance and agronomic traits of European common bean (*Phaseolus vulgaris* L.) genetic resources

Eva Plestenjak [a], Vladimir Meglič [a], Lovro Sinkovič [a], Matevž Likar [b], Marjana Regvar [b] and Barbara Pipan [a]

[a]Crop Science Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia; [b]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

**ABSTRACT**
Field production of common beans benefit from root microbial associations, although they are generally considered to be weaker nitrogen fixers than other legumes. Therefore, the number of nodules on the roots of 64 accessions of the European common bean (*Phaseolus vulgaris* L.) and the relationship between the number of nodules per accession and other plant characteristics (growth type, earliness, seed yield, and total nitrogen content in seeds) were studied. The results indicated that growth type and earliness influenced the number of nodules per accession (the contributions of principal component analysis were 34.9% for Dim1 and 29.8% for Dim2). The average number of nodules per accession with indeterminate growth type was almost five times higher (20.1) than for accessions with determinate growth type (4.4). Common bean accessions with regular growth cycle length had the highest number of nodules (21.9). In contrast, nodulation efficiency, measured as seed yield per plant and total nitrogen content in seeds, were not correlated with the number of nodules per accession (correlation analysis, $r < 0.1$). Consequently, data on the nodulation efficiency of European common bean accessions are important for breeding programmes in conjunction with other agronomically important traits for commercial and/or organic cultivation systems.

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**KEYWORDS**
Agronomic traits; common bean; nitrogen fixation; nodules; symbiotic efficiency

## Introduction

Legumes are a large family of plants known for their edible immature pods, in which the seeds are stored. Some species are cultivated for their immature pods, which are prepared as vegetables. Nevertheless, legumes are primarily cultivated for their mature seeds. Due to their high-protein content, they are one of the most important sources of nutrients and are second in the human diet, after cereals (Graham and Vance 2003; Bellucci et al. 2014; Nadeem et al. 2021). In addition to their high-protein content, they are also rich in complex carbohydrates, fibre, vitamins and minerals and have low fat content (Pipan et al. 2017). The presence of legumes in the field is beneficial due to microbial associations in the roots, where symbiotic bacteria fix atmospheric nitrogen, which is then consumed by the host plant. The process of symbiotic nitrogen fixation provides sufficient amounts of nitrogen for plant growth, thereby negating the necessity for additional fertiliser inputs. Furthermore, the decomposition of leguminous plants enhances soil nitrogen availability. This is important from both an environmental and economic perspective, helping to increase yields and reduce cost (Barbosa et al. 2018; Beltayef et al. 2018; Jiang et al. 2020; Habinshuti et al. 2021).

According to botanical classification, the common bean (*Phaseolus vulgaris* L.) belongs to the legume family. It is an self-pollinated annual herbaceous plant with a small diploid genome ($2n = 2x = 22$). Since its discovery in the Americas, where the bean originated, it has spread all over the world. Two gene pools are known, depending on the place of origin, the main seed storage protein, e.g. phaseolin, seed size and weight. The specific phaseolin types S (*Sanilac*) and B (*Boyacá*) are of Mesoamerican origin, whereas the phaseolin types T (*Tendergreen*) and H (*Huevo de Huanchaco*) are of Andean origin. Phaseolin type C (*Contender*) represents a subgroup of mixed origin (Pipan and Meglič 2019). After domestication, beans spread around the world, and European bean germplasm is considered

**CONTACT** Eva Plestenjak eva.plestenjak@kis.si Crop Science Department, Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

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the secondary centre of bean diversification and is therefore of particular value for crop improvement (Angioi et al. 2010). Today, the common bean is the most popular bean consumed in Europe and the second most imported legume vegetable after soybean (Bellucci et al. 2014; De Ron et al. 2015; Bitocchi et al. 2017; CBI 2022). According to the Centre for the Promotion of Imports from Developing Countries (CBI) of the Ministry of Foreign Affairs (2022), Europe is a leading market for bean-based vegetable products. Common bean is mainly cultivated for their edible dry (mature) seeds, seeds at physiological maturity, and green pods (Lioi and Piergiovanni 2013; Nadeem et al. 2021). The characteristics of common beans that are preferred by consumers are contingent upon the specific plant, seed or pod characteristics that are desired. This depends mainly on the purpose of cultivation (for seeds, pods, or combined), the type of growth (determinate varieties that do not require support, interdeterminate and climbing – indeterminate varieties that require support for growth), the length of the vegetation period, resistance to individual diseases, and the amount of seeds and pods the crop can produce (Dawo et al. 2007; Nadeem et al. 2021).

Like all legumes, the common bean forms symbiotic associations with the *Rhizobia* genus of nodulating bacteria (Aguilar et al. 2022; Moura et al. 2022). The interaction between the plant and the bacteria results in the formation of a new structure on the roots of the plant called a nodule. It serves as a shelter for bacteria that differentiate into bacteroids, nitrogen-fixing cells that fix atmospheric $N_2$ into a plant-available organic form such as ammonia. Ammonia is then used by the host plant in exchange for nutrients and energy, so that the plants tend to develop a minimal number of nodules that nevertheless ensure sufficient ammonia for optimal growth. Leghemoglobin, a heme protein found in these root nodules, plays a crucial role in maintaining low oxygen levels, which is essential for the proper functioning of nitrogenase enzymes involved in nitrogen fixation (Mortier et al. 2012; Jiang et al. 2020). In addition to its association with promiscuous symbioses, the common bean is one of the model species (Aguilar et al. 2022). It may be associated with a number of *Rhizobium* species, but most of them appear to be biological nitrogen fixers with low efficiency (Nleya et al. 2009; Shamseldin and Velázquez 2020; Moura et al. 2022).

Nodulation efficiency and establishment are influenced by several factors, including environmental conditions, viz. soil properties, number of resident bacterial populations (Nleya et al. 2009; Mortier et al. 2012), and host plant characteristics, such as growth type (Dawo et al. 2007; Nleya et al. 2009; Barbosa et al. 2018). Under optimal environmental conditions, 73% of total plant nitrogen can be obtained through biological nitrogen fixation (Barbosa et al. 2018). Beans with indeterminate growth types have better biological nitrogen fixation ability than beans with determinate growth types (Barbosa et al. 2018). In addition, growth type is also related to productivity. Beans with indeterminate growth types are associated with a higher number of seeds per pod and more pods per plant than bean plants with determinate growth types (Dawo et al. 2007; Zoffoli et al. 2020). In addition, common bean has a short growth cycle and high nitrogen requirements compared to other legumes. Therefore, it would be highly desirable for a host plant to extend the growing season, but only as long as is optimal for the plant`s growth cycle to avoid drought and heat and to form nodules. In addition, the length of the growth cycle should allow bacteria to transform into bacteroids and actively produce organic nitrogen for the host plant (Farid and Navabi 2015; Andraus et al. 2016).

To gain new insights into the nodulation potential of a representative common bean collection, the ability of plants to nodulate and the relationships and differences between the number of nodules per accession and corresponding plant traits such as seed yield, growth type, growth cycle, and total nitrogen content in seeds were investigated.

## Materials and methods

### *Initial screening and collection assessment*

Following the study by Pipan and Meglič (2019), a subset of 63 accessions was selected from the 782 common bean accessions that differed in biological status, geographic origin, ancestral information, seed phenotypic characteristics, or genetic background. These accessions were obtained from ten gene banks, and the representative collection formed in this study included a total of 64 common bean (*Phaseolus vulgaris* L.) genetic resources, 63 of which originated from 13 European countries (Supplementary Table S1). In addition, one commercial variety of common bean, i.e. Parker, was included as the standard for determinate growth type, while accession KP-400 represented the standard for the indeterminate growth type. The list of accessions, their country of origin, and phaseolin type information are shown in Supplementary Table S1. Seeds were exchanged under the national project L4-7520 in accordance with the Seed Material Transfer Agreement (SMTA).

Field experiment

A field experiment was established in the second week of May 2017 at the Agricultural Institute of Slovenia experimental station in Jablje, Slovenia (302 m a.s.l.; 46.08°N 14.33°E). Mean monthly precipitation and mean temperature during the growing season were obtained from the National Meteorological Service of Slovenia and are shown in Figure 1. The soil type at the experimental site is classified as Umbric Planosols (Plu) with a predominant silt loam texture. In the uppermost 25 cm of the soil profile, the mean bulk density content of the soil was 1.60 g $cm^{-3}$ and the mean soil pH was 6.3. The mean concentrations of $P_2O_5$, $K_2O$, and MgO were 256 mg $kg^{-1}$, 200 mg $kg^{-1}$, and 145 mg $kg^{-1}$, respectively. The organic carbon content was 5.3%, while the available nitrate-nitrogen ($NO_3$–N) was 0.68%.

Each of the 64 accessions from the representative collection was grown in three replicates (at three microsites) in which 24 seeds (two rows of twelve seeds each) were sown on black polyethylene foil. The sowing distance was 10 cm in a row and 40–45 cm between rows, where a drip system was laid for irrigation. Since no information was available on the growth habit of individual common bean accessions at the time of sowing, poles were subsequently placed for all accessions with indeterminate and interderterminate growth types. No artificial rhizobia inoculum was used during the field experiment to promote nodulation.

During the growing season, the growth of each of the 64 accessions was monitored, and data on six traits were recorded. Days to emergence were determined as the number of days from sowing to the emergence of 50% of the seeds. Days to maturity were determined as the number of days from emergence to physiological maturity of 90% of the pods. Based on the timing of physiological maturity, accessions were classified into four groups according to the length of the growth cycle/earliness, as established by Andraus et al. (2016). The length of the growth cycle was determined as the number of days from the sowing date (May 15) to the day of excavation, which coincides with the day of physiological maturity of the pods.

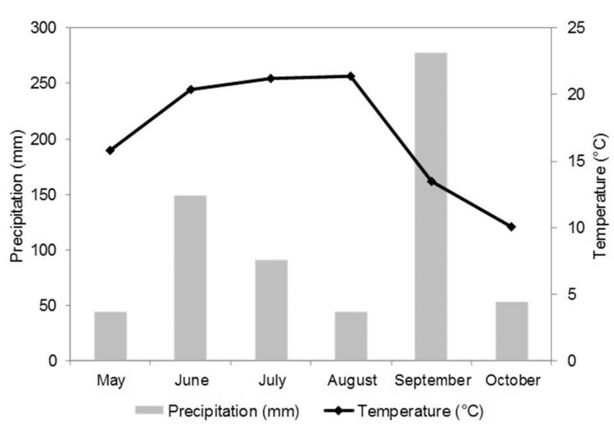



**Figure 1.** Climate data at experimental site Jablje during the 2017 growing season.

Common bean accessions were also grouped according to growth type through phenotypic observation at the stage of maximum flowering. Bean accessions with lower growth type and the ability to climb were classified as interdeterminate growth type (growing taller on poles) and those requiring poles for growth were classified as indeterminate growth type (climbing). Bean accessions that do not climb and grow as bushes were classified as determinate growth types.

At the time of physiological maturity, bean seeds were collected from individual plants per accession. Seed yield per plant was calculated from the total mass of all seeds (g) divided by the number of plants for each accession. To determine total nitrogen in the seeds, common bean seeds were homogenised using a laboratory ball mill (Retsch MM 400; GmbH, Germany) at a frequency of 30 Hz for 3 minutes. Total nitrogen content (%) was determined using the Kjeldahl method (Muñoz-Huerta et al. 2013).

The root system of the plants was excavated when the bean pods on the plants reached physiological maturity. The entire root ball was excavated, leaving the roots undamaged. The plants were then scored according to the number of nodules. Since this was a destructive method, several closely spaced plants were dug up at once at each microsite, and the least damaged representative plants were selected for analysis. The nodules were counted on the root system of each plant, and the average of the nodules on four plant roots represented the number of nodules per accession.

Statistical analysis

To determine the most informative variables between the number of nodules per accession, growth type, earliness, seed yield, and total nitrogen content in seeds, principal component analysis (PCA) was performed using the R program version 4.1.2 under the 'FactoMineR' and 'factoextra' packages. Correlation analyses between yield, total nitrogen content in seeds, and number of nodules per accession were performed using the R program under the 'ggpubr' package. Other statistical analyses and visual plots were performed with the R program under the 'Rcmdr' and 'ggplot2' packages.

## Results

The presence of nodules in the common bean collection was assessed by counting all nodules on the root systems per accession, as shown in Figure 2. Of all bean accessions sampled, 30% (19 accessions) did not

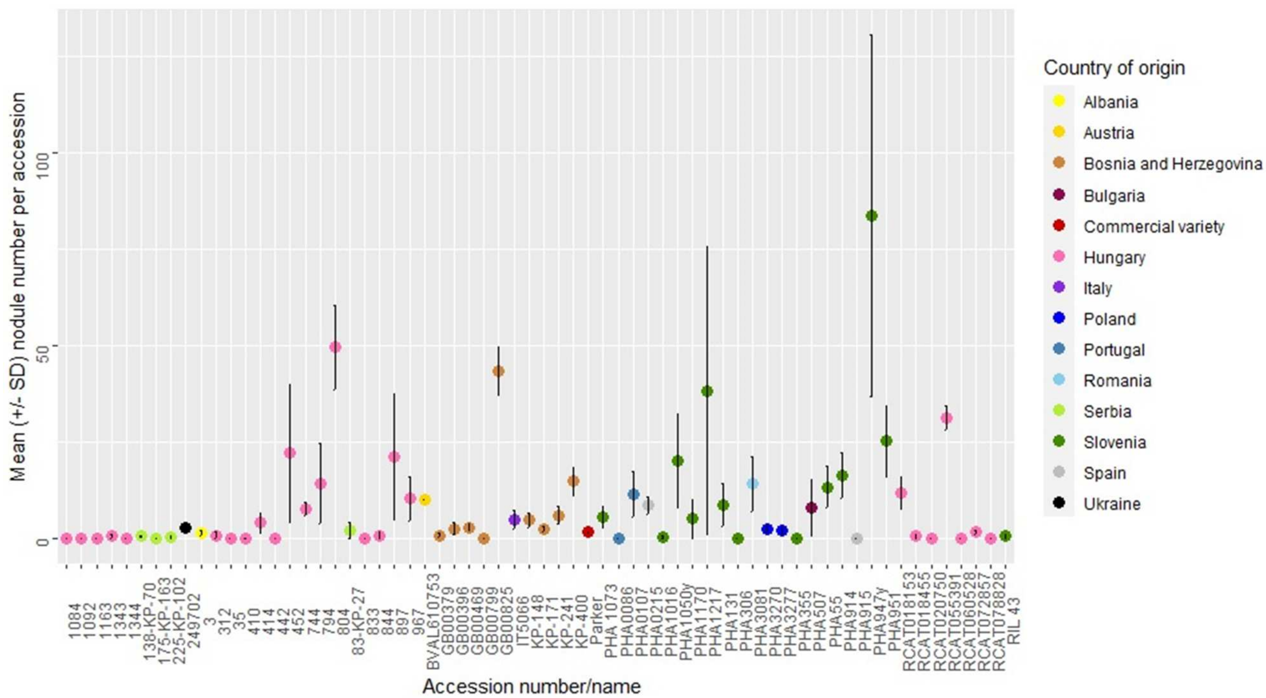



**Figure 2.** Number of nodules per accession (mean ± SD) in a representative common bean collection.

develop nodules on their roots (PHA306, PHA355, PHA0086, PHA915, PHA1016, 225-KP-102,833, 1084, 1344, 1092, 35, 410, 442, 1163, 175-KP-163, GB00799, RCAT060528, RCAT078828, and RCAT020750). The average number of nodules per accession in the collection was 8.4, while the highest number of nodules was 83.8 (accession PHA947y).

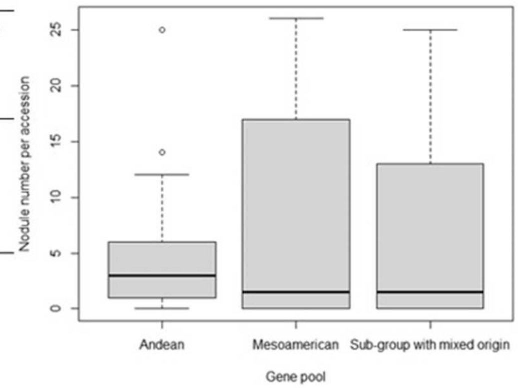
Common bean plants were arranged by country of origin and evaluated for differences in the number of nodules per accession. The country of origin of the studied accessions is listed in Supplementary Table S1. Common bean accessions originating from Slovenia had the highest number of nodules (16.6), followed by accessions from Romania (14.0), Austria (10.0), Bosnia and Herzegovina (8.5), and Bulgaria (8.0). Accessions from other countries had fewer than 8.0 nodules. The lowest number of nodules per accession was observed in those originating from Serbia (0.7). The number of nodules per accession in relation to phaseolin type (gene pool), growth type, and earlines is shown in Figure 3. Although data on phaseolin type were only available for 12 accessions, the possible relationship with the number of nodules per accession was also investigated. Phaseolin type is associated with the gene pool origin, as shown in Figure 3A. There was a tendency for the common bean accessions from the Mesoamerican gene pool (phaseolin types B – *Boyaca* and S – *Sanilac*) to have a higher number of nodules (8.1) than the accessions from the Andean gene pool (phaseolin types T – *Tendergreen* and H – *Huevo de Huanchaco*) (5.0) and those from the sub-group with mixed origin (phaseolin type C – *Contender*) (7.0).

Depending on growth habit, the common bean accessions were divided into three groups (Figure 3B). Most of the accessions showed a determinate growth type (36 accessions), 18 accessions showed an indeterminate growth type, and 10 accessions showed an interdeterminate growth type. The common bean accessions with interdeterminate growth type had the lowest number of nodules (2.6), while the median number of nodules was 0. The accessions with interdeterminate growth type had the least variability in the data for the number of nodules (interquartile range was 2.0), and only four of these accessions had not developed any nodules. The accessions with indeterminate growth type had the highest number of nodules (20.1), and the median was 10.0. Here, only one accession did not form nodules on the root system. However, the number of nodules per accession varied the most with an interquartile range of 25.0. The number of nodules per accession with determinate growth type was 4.4, with a median of 0. Of the 36 common bean accessions with determinate growth type, 14 accessions had not developed nodules.

Depending on the length of the growth cycle/earliness, the common bean accessions studied were classified into four groups (Figure 3C). The first group contained the earliest common bean accessions, *A*

| Phaseolin type | No. of analyzed ACC | No. of ACC without nodules | Mean nodule no. per ACC | Median nodule no. per ACC | Interquartile range of nodule no. per ACC |
|---|---|---|---|---|---|
| B | 1 | 0 | 11.2 | 10.0 | 16.7 |
| C | 3 | 1 | 7.0 | 1.5 | 12.0 |
| H | 1 | 0 | 8.5 | 7.5 | 6.0 |
| S | 2 | 1 | 6.6 | 0 | 11.7 |
| T | 5 | 1 | 4.3 | 3.0 | 5.0 |

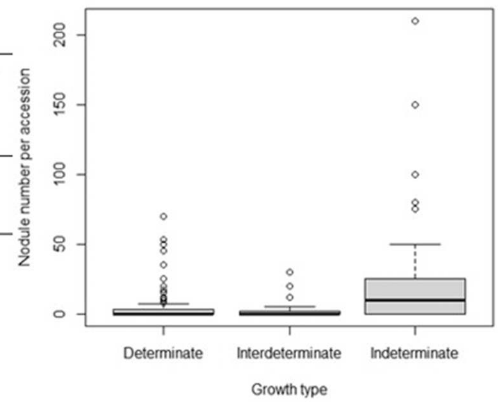
ACC - accession; phaseolin type B and S indicate germplasm origin from Mesoamerican gene pool; phaseolin type H and T indicate germplasm origin from Andean gene pool; phaseolin type C indicate sub-group with mixed origin.




*B*

| Growth type | No. of analyzed ACC | No. of ACC without nodules | Mean nodule no. per ACC | Median nodule no. per ACC | Interquartile range of nodule no. per ACC |
|---|---|---|---|---|---|
| Determinate | 36 | 14 | 4.4 | 0 | 3.0 |
| Interdeterminate | 10 | 4 | 2.6 | 0 | 2.0 |
| Indeterminate | 18 | 1 | 20.1 | 10.0 | 25.0 |

ACC - accession.




*C*

| Earliness | No. of analyze d ACC | No. of ACC without nodules | Mean nodule no. per ACC | Median nodule no. per ACC | Interquartile range of nodule no. per ACC |
|---|---|---|---|---|---|
| Early | 18 | 12 | 1.3 | 0 | 0 |
| Semi-early | 20 | 3 | 9.2 | 3.0 | 14.2 |
| Regular | 8 | 1 | 21.9 | 3.0 | 22.5 |
| Late | 18 | 3 | 8.6 | 2.0 | 7.0 |

ACC - accession.

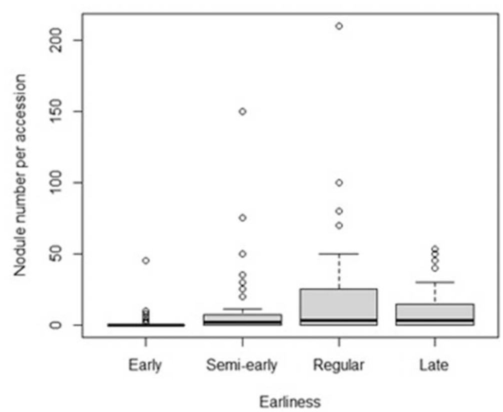



**Figure 3.** Number of nodules per accession in relation to: (*A*) phaseolin type (gene pool), (*B*) growth type, and (*C*) earlines.

whose growth cycle was completed after 101 growing days. The second group contained common bean accessions whose growth cycle was completed after 108 days, the third group after 115 days, and the last, fourth group contained accessions with the longest growth cycle of 147 days. Accessions with a regular growth cycle had the highest number of nodules (21.9), and the median was 3.0. However, the number of nodules varied the most among common bean accessions with a regular growth cycle, with an interquartile range of 22.5. Only one accession (833) with a regular growth cycle did not form any nodules. Common bean accessions with an early growth cycle had the lowest number of nodules (1.3), followed by those with a late growth cycle (mean of 8.6 and median of 2.0) and semi-early growth cycle (mean of 9.2 and median of 3.0). Among the common bean accessions with an early growth cycle, there was the highest number of accessions without nodules (12 accessions).

Accession PHA55 formed only a few empty pods (without seeds), so the seed yield was 0 g and the total nitrogen content in the seed was not measured. Accession KP-171 had a maximum yield of 36.09 g of seed per plant with an average of 2.3 nodules. A very low seed yield was observed in accession 414, which had an average of four nodules. Accession PHA947y had the highest number of nodules (average 83.8), while seed yield was relatively low (1.70 g of seed per plant). Yields of common bean accessions without nodules ranged from 0.09–28.21 g of seeds per plant. As can be seen in Figure 4A, there is no correlation between the number of nodules per accession and seed yield, as the r-value is 0.092 and the $p$-value is 0.47. A moderately strong correlation ($r = 0.48$, $p = 0.16$) between the number of nodules per accession and seed yield was observed in the interdeterminate growth type

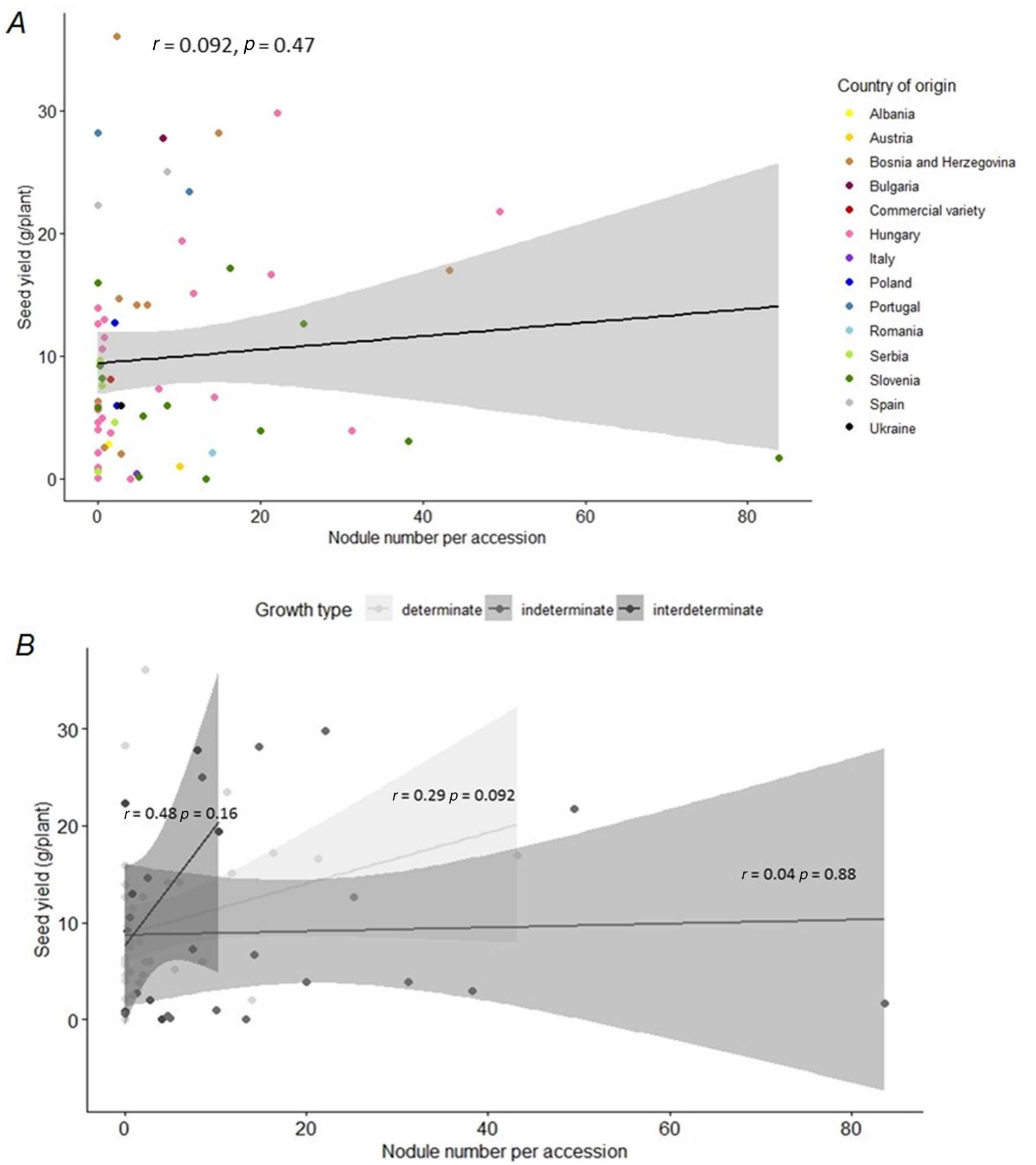



**Figure 4.** . Correlation between (*A*) number of nodules per accession and seed yield, and (*B*) number of nodules per accession and seed yield according to growth type.

accessions, while a weak correlation ($r = 0.29$, $p = 0.092$) was observed in the determinate growth type accessions (Figure 4B).

Nitrogen content in seeds of three common bean accessions (PHA55, BVAL610753, and IT5066) was not determined because the total seed yield was too low for nitrogen content analysis. Total nitrogen content in seeds of the accessions in the common bean collection ranged from 3.22% to 4.64%, regardless of the number of nodules per accession. For example, accession PHA947y with the highest number of nodules (83.8) had a total nitrogen content in the seeds of 3.36%. In contrast, accession RCAT072857 had the highest nitrogen content in seeds (4.64%) and formed an average of 1.5 nodules. Figure 5 shows that there is no correlation between the number of nodules per accession and the total nitrogen content in the seeds ($r = -0.064$, $p = 0.63$).

To obtain a different number of linear combinations for the variables number of nodules per accession, growth type, earliness, seed yield, and total nitrogen content in seeds, a principal component analysis (PCA) plot was performed. For the representative common bean collection studied, the contribution of principal component 1 (PC 1), PC 2, PC 3, PC 4, and PC 5 to the total variance was 34.9%, 29.8%, 15.4%, 12.4%, and 7.4%, respectively. The first three PCs together accounted for 80.1% of the total observed variability, with earliness accounting for the largest proportion (Figure 6). The variables that contributed most to the distribution of PC 1 were earliness, followed by growth type, and number of nodules per accession, while the variables that contributed most to the distribution of PC 2 were total nitrogen content in seeds, followed by seed yield. The distribution of PC 3 was mainly covered by the variables seed yield and total nitrogen content in seeds, while the distribution of PC 4 was mainly covered by the number of nodules per accession, followed by earliness, and growth type. The variables contributing most to the distribution of PC 5 were earliness, growth type, and total nitrogen content in seeds (Supplementary Table S2, Supplementary Figure S1). Seed yield and total nitrogen content in seeds seemed to be inversely connected, while the number of nodules per accession was independent (orthogonal) of both parameters.

## Discussion

A representative common bean collection was constituted of 64 accessions from different countries, representing sources that have been adapted to the European climate. These common bean phenotypes represent the most diverse source of agronomically

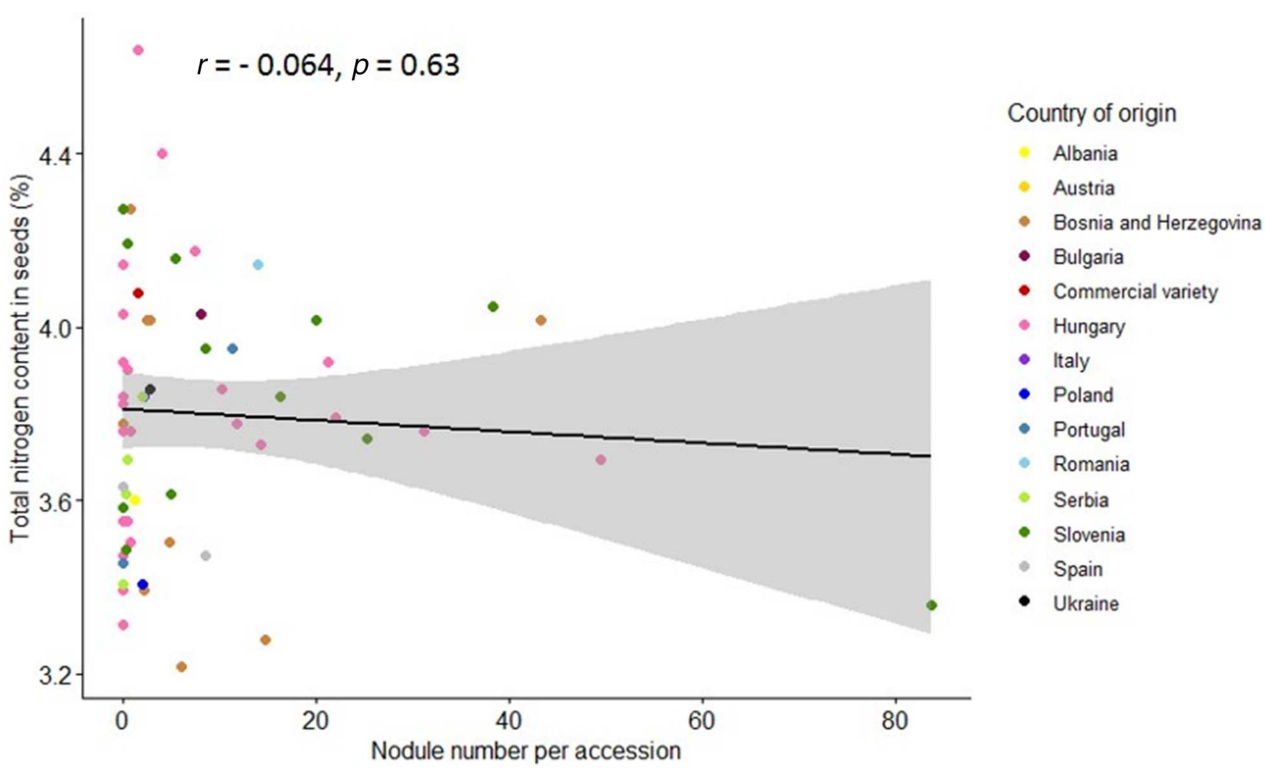



**Figure 5.** Correlation between number of nodules per accession and total nitrogen content in seeds for representative common bean collection.

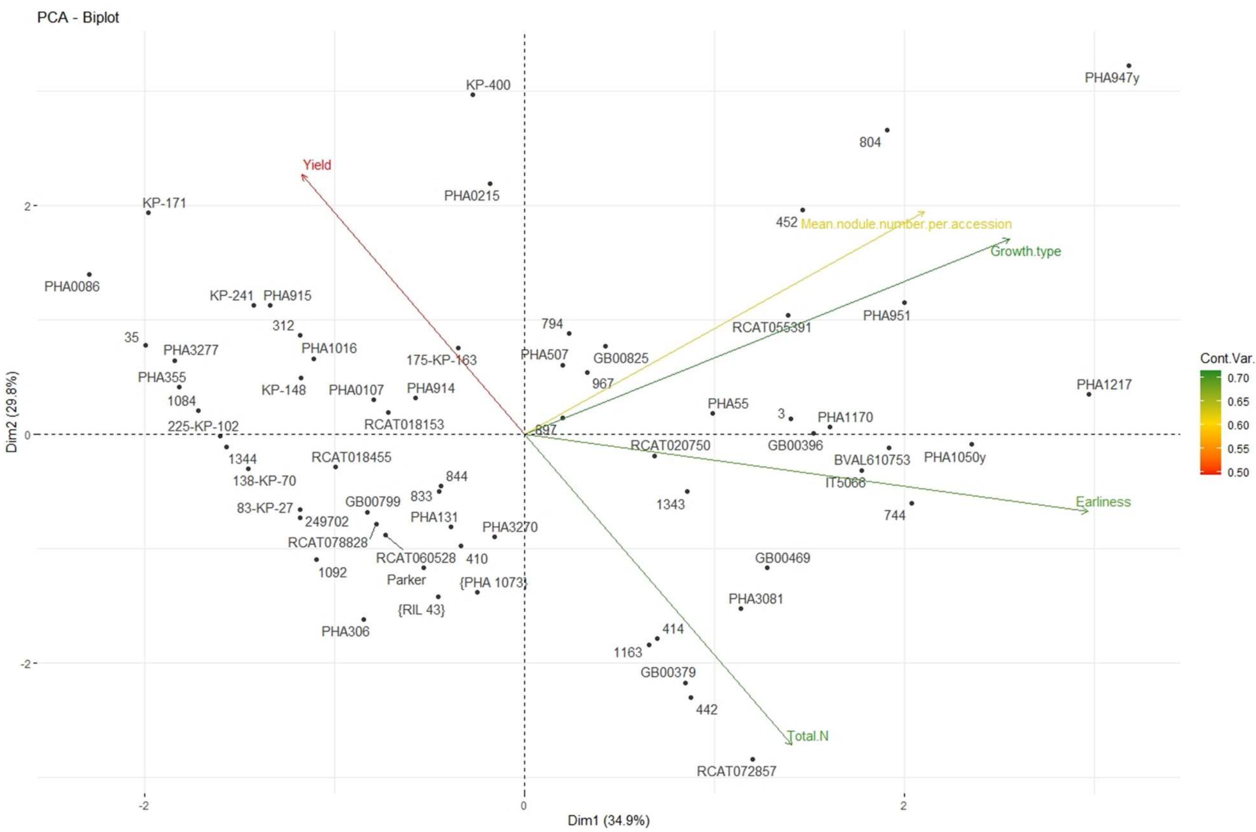



**Figure 6.** PCA distribution for earliness, growth type, number of nodules per accession, seed yield, and total nitrogen (N) content in seeds of a representative common bean collection.

important traits and alleles related to nutritional value and resistance potential. Therefore, this collection was used to determine the number of nodules per accession and to determine relationships between the number of nodules per accession and other plant traits. While studying nodulation in a single environment has inherent limitations, this is the initial investigation to examine a representative bean collection for nodulation ability in order to provide general information on the agronomic behaviour of accessions in the same location and at the same time.

The common bean accessions were initially analysed to determine if there was a relationship between the number of nodules and the country of origin and phaseolin type. With respect to phaseolin type (B, C, H, S, and T), for which data were available for only 12 accessions, these were divided into three gene pools. In Europe, the Andean gene pool is the predominant one, as was the case for six accessions from the collection with known phaseolin type (Belucci et al. 2014, Pipan and Meglič 2019). The findings of the study conducted by Knupp and Ferreira (2017) indicated that Mesoamerican accessions exhibited a greater capacity for nodulation than those of Andean origin. In contrast, Farid and Navabi (2015) reported a higher number of colourless nodules in Andean accessions, while Mesoamerican accessions had more red-coloured nodules, indicating their activity. Our results demonstrated no differences in nodulation ability according to gene pool. However, some differences between accessions were observed. The efficiency and establishment of symbiotic relationships are dependent on the genotype of the bacterial strain and the genotype of the common bean (Aguilar et al. 2022). It is possible that native bacterial strains that have adapted to specific soil properties may ultimately prevail over inoculated bacteria. The association of common bean genotypes that are adapted to these microorganisms results in a higher number of nodules (Milcheski et al. 2022). In the present study, the number of nodules per accession was not found to correlate with a country of origin in all countries represented. The highest number of nodules was observed in common bean accessions originating from Slovenia (16.6), while the lowest number was observed in those originating from Serbia (0.7).

Additionally, a relationship was identified between the number of nodules per accession and the growth type. In the present study, the roots of common bean accessions with determinate and interdeterminate growth types exhibited the lowest number of nodules (< 5). Accessions with indeterminate growth type had the highest mean (20.1) and median (10.0) number of nodules. Of the European common bean collection under study, 19 accessions failed to develop nodules on the root system. Of these, 14 common bean accessions exhibited determinate growth types, four exhibited interdeterminate growth types, and one exhibited an indeterminate growth type. Furthermore, the number of nodules differed among common bean accessions with the same growth type. These discrepancies can be attributed to the varying modulation capabilities of the common bean, environmental conditions, the quantity of bacteria present in the surrounding environment, and the specific type of nodulating bacteria (Nleya et al. 2009; Aguilar et al. 2022). The results obtained in this study are consistent with existing data indicating that accessions with indeterminate growth type form a higher number of nodules than those with determinate growth type (Nleya et al. 2009; Farid and Navabi 2015; Barbosa et al. 2018; Keller et al. 2022).

The second relationship that was verified was that between the number of nodules per accession and the growth cycle. Common bean accessions were classified into four groups according to the length of the growth cycle/earliness, i.e. early, semi-early, regular, and late. The results demonstrated that the roots of the accessions with a regular growth cycle exhibited the highest mean and median number of nodules. The mean number of nodules varied from 2.3–83.8 in the accessions with a regular growth cycle. Furthermore, there was one accession (PHA947y) with a regular growth cycle that had the highest number of nodules (83.8), while only one accession did not form any nodules (accession 833). In contrast, accessions with an early growth cycle had the lowest average number of nodules (1.3), with twelve of the 18 accessions failing to form any nodules. Jiang et al. (2020) identified a correlation between early growth cycle common bean accessions having early flowering and a shorter growing season, which is associated with a lower capacity for symbiotic N fixation. In general, carbon assimilated in the common bean and cannot be distributed to the roots after flowering. Consequently, the lack of carbon supply to the nodules results in a reduction in their activity (Mortier et al. 2012 Toso et al. 2017; Jiang et al. 2020). Furthermore, it is crucial to highlight that nodule formation is a highly energy-consuming process. Following the initial formation of nodules, the production of additional nodules is significantly inhibited (Mortier et al. 2012). Consequently, common beans with a regular growth cycle may produce more nodules on the roots (Keller et al. 2022). The findings of several studies on common beans, which indicate a correlation between plant growth type, growth cycle, and nodulation are in alignment with our results (Nleya et al. 2009; Andraus et al. 2016; Barbosa et al. 2018; Keller et al. 2022).

Based on seed yield per plant and total nitrogen content in seeds, the number of nodules per accession was compared with seed yield and total nitrogen content in seeds. In this study, the roots were evaluated based on the number of nodules present when the bean pods on the plants reached physiological maturity and nodule efficiency decreased. At this stage, plant metabolism and carbon production to supply nodules are minimal (Mortier et al. 2012; Toso et al. 2017; Jiang et al. 2020). However, the number of nodules is typically positively correlated with the fixed nitrogen content (Jiang et al. 2020). The present study did not reveal any significant correlations between the number of nodules per accession and seed yield ($r = 0.092$, $p = 0.47$) or the number of nodules per accession and total nitrogen content in seeds ($r = -0.064$, $p = 0.63$). Nevertheless, there was a moderately strong correlation was observed between the number of nodules per accession and seed yield in accessions with the interdeterminate growth type, while a weaker correlation was evident in accessions with the determinate growth type. This indicates that not all nodules are equally active, and it is also possible that different species of bacteria nodulate different genotypes of common bean (Beltayef et al. 2018). Additionally, common bean accessions exhibit variation in their capacity to utilise biologically fixed nitrogen for seed production (Barbosa et al. 2018).

The contribution of different traits or variables, including earliness, growth type, number of nodules per accession, seed yield, and total nitrogen content in seeds, was evaluated to identify differences among common bean accessions within the collection. The variables that contributed most to the observed variability among accessions were earliness, growth type, and number of nodules. Common bean plants that are capable of nodulation and efficient nitrogen fixation by symbiotic bacteria are particularly valuable and desirable for organic farming. It is therefore evident that further studies are required to gain a deeper understanding of the factors influencing crop yield and growth, particularly in relation to the number of nodules, their activity and the different growth types of common beans. This will enable the improvement of bean production in the future.

## Conclusion

The representative common bean collection exhibited several agronomically important traits and alleles related to nutritional value and resistance potential, thus representing valuable genetic variability in Europe. In addition, almost two-thirds of the accessions examined in this study formed nodules, but the number of nodules per accession was highly variable. Nevertheless, our results show that the number of nodules varied among accessions with different growth types and growth cycles, i.e. earliness. Common bean accessions with a regular length of plant growth cycle had the highest average number of nodules compared to accessions with other growth cycles, e.g. early, semi-early, and late growth cycles. Accessions with indeterminate growth types also differed in the number of nodules from the accessions with determinate growth types. The average number of nodules per accession with indeterminate growth type was nearly five times that of accessions with determinate growth type. In our study, nodulation efficiency, measured as seed yield per plant, was independent of the number of nodules per accession. Common bean accessions also differed in their ability to utilise biologically fixed nitrogen for seed production. Results of the present study provide novel insights into the relationship between growth type, growth cycle, nitrogen content in seeds, and nodulation ability, which is of particular importance for breeding programmes and organic farming systems.

## Author contributions

**Conceptualisation**: Barbara Pipan, Vladimir Meglič, Marjana Regvar and Matevž Likar. **Methodology**: Barbara Pipan and Matevž Likar; **Formal analysis and investigation**: Matevž Likar, Lovro Sinkovič and Eva Plestenjak; **Writing – original draft preparation**: Eva Plestenjak and Lovro Sinkovič; **Writing – review and editing**: Vladimir Meglič, Barbara Pipan, Matevž Likar and Marjana Regvar; **Funding acquisition**: Vladimir Meglič and Barbara Pipan. All authors read and approved the final manuscript.

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## Notes on contributors

***Eva Plestenjak*** is a PhD student at the Biotechnical Faculty, University of Ljubljana (Slovenia). Her research is conducted at the Crop Science Department in the Agricultural Institute of Slovenia, where she studies composite populations of common bean.

***Dr. Vladimir Meglič*** is a research associate at the Department of Crop Science, at the Agricultural Institute of Slovenia. His research interests lie in the fields of plant breeding and molecular plant biology.

***Dr. Lovro Sinkovič*** is a research associate at the Department of Crop Science, at the Agricultural Institute of Slovenia. His research is focused on the nutritional characteristics of different crops.

***Dr. Matevž Likar*** is an assistant professor at the Biotechnical faculty, University of Ljubljana (Slovenia). His research is focused on fungal endophytes, with emphasis on mycorrhiza.

***Dr. Marjana Regvar*** is a university professor at the Biotechnical faculty, University of Ljubljana (Slovenia). Her research deals with physiology and morphology of plants.

***Dr. Barbara Pipan*** is a research associate at the Department of Crop Science, at the Agricultural Institute of Slovenia. Her research interests lie in the field of plant genetics and breeding.

## ORCID

*Eva Plestenjak* http://orcid.org/0000-0002-9707-828X
*Vladimir Meglič* http://orcid.org/0000-0002-7739-0145
*Lovro Sinkovič* http://orcid.org/0000-0002-6123-8085
*Matevž Likar* http://orcid.org/0000-0002-3086-6433
*Marjana Regvar* http://orcid.org/0000-0002-2623-1099
*Barbara Pipan* http://orcid.org/0000-0002-3860-119X

## References

Aguilar OM, Collavino MM, Mancini U. 2022. Nodulation competitiveness and diversification of symbiosis genes in common beans from the American centers of domestication. Sci Rep. 12:1–9. doi:10.1038/s41598-022-08720-0.

Andraus MP, Cardoso AA, Ferreira EPB. 2016. Differences in nodulation and grain yield on common bean cultivars with different growth cycles. Commun Soil Sci Plant Anal. 47:1148–1161. doi:10.1080/00103624.2016.1166376.

Angioi SA, Rau D, Attene G, Nanni L, Bellucci E, Logozzo G, Negri V, Spagnoletti Zeuli PL, Papa R, 2010. Beans in Europe: origin and structure of the European landraces of *Phaseolus vulgaris* L. Theor Appl Genet. 121:829–843. doi:10.1007/s00122-010-1353-2.

Barbosa N, Portilla E, Buendia HF, Raatz B, Beebe S, Rao I. 2018. Genotypic differences in symbiotic nitrogen fixation ability and seed yield of climbing bean. Plant Soil. 428:223–239. doi:10.1007/s11104-018-3665-y.

Bellucci E, Bitocchi E, Rau D, Rodriguez M, Biagetti E, Giardini A, Attene G, Nanni L, Papa R. 2014. Genomics of origin, domestication and evolution of *phaseolus vulgaris*. In: Tuberosa R., Graner A., Frison E., editors. Genomics of plant genetic resources. Dordrecht: Springer Netherlands; p. 483–507.

Beltayef H, Melki M, Saidi W, Samaali S, Muscolo A, Cruz C, Garoui T 2018. Betterment of biological nitrogen fixation in snap bean under Mediterranean semi-arid conditions. Bulg J Agric Sci. 24:244–251.

Bitocchi E, Rau D, Bellucci E, Rodriguez M, Murgia ML, Gioia T, Santo D, Nanni L, Attene G, Papa R. 2017. Beans (*Phaseolus* ssp.) as a model for understanding crop evolution. Front Plant Sci. 8:722. doi:10.3389/fpls.2017.00722.

CBI. 2022. The European market potential for common dry beans.In: CBI, Ministry of Foreign Affairs. https://www.cbi.eu/market-information/grains-pulses-oilseeds/dried-kidney-beans/market-potential.

Dawo MI, Sanders FE, Pilbeam DJ. 2007. Yield, yield components and plant architecture in the F3 generation of common bean (*Phaseolus vulgaris* L.) derived from a cross between the determinate cultivar 'prelude' and an indeterminate landrace. Euphytica. 156:77–87. doi:10.1007/s10681-007-9354-1.

De Ron AM, Papa R, Bitocchi E, González AM, Debouck DG, Brick MA, Fourie D, Marsolais F, Beaver J, Geffroy V, et al. 2015. Common bean. In: De Ron AM, editor. Grain legumes. New York: Springer New York; p. 1–36.

Farid M, Navabi A. 2015. $N_2$ fixation ability of different dry bean genotypes. Can J Plant Sci. 95:1243–1257. doi:10.4141/cjps-2015-084.

Graham PH., Vance CP. 2003. Legumes: importance and constraints to greater Use. Plant Physiol. 131:872–877. doi:10.1104/pp.017004.

Habinshuti SJ, Maseko ST., Dakora FD 2021. Inhibition of N2 fixation by N fertilization of common bean (phaseolus vulgaris L.) plants grown on fields of farmers in the eastern cape of South Africa, measured using 15N natural abundance and tissue ureide analysis. Front Agron. 3:692933. doi:10.3389/fagro.2021.692933.

Jiang Y, MacLean DE, Perry GE, Marsolais F, Hill B, Pauls KP. 2020. Evaluation of beneficial and inhibitory effects of nitrate on nodulation and nitrogen fixation in common bean (*Phaseolus vulgaris*). Legume Sci. 2:e45. doi:10.1002/leg3.45.

Keller Beat, Ariza-Suarez Daniel, Portilla-Benavides Ana Elisabeth, Buendia Hector Fabio, Aparicio Johan Steven, Amongi Winnyfred, Mbiu Julius, Msolla Susan Nchimbi, Miklas Phillip, Porch Timothy G, et al. 2022. Improving Association Studies and Genomic Predictions for Climbing Beans With Data From Bush Bean Populations. Frontiers in Plant Science. 13. http://dx.doi.org/10.3389/fpls.2022.830896.

Knupp AM, Ferreira EPB., Araújo AP. 2017. Variability of nodulation traits in Andean and Mesoamerican common bean gene pools. Pesquisa Agropecuária Brasileira. 52:252–260. doi:10.1590/s0100-204x2017000400005.

Lioi L, Piergiovanni AR. 2013. European common bean. In: Singh M., Upadhyaya H.D., Singh Bisht I., editor. Genetic and genomic resources of grain legume improvement. London: Elsevier Inc; p. 11–40.

Milcheski VF, Senff SE, Orsi N, Botelho GR, Fioreze ACC. 2022. Influence of common bean genotypes and rhizobia interaction for nodulation and nitrogen fixation. Rev Ciênc Agrovet. 21:8–15. doi:10.5965/223811712112022008.

Mortier V, Holsters M, Goormachtig S. 2012. Never too many? How legumes control nodule numbers. Plant Cell Environ. 35:245–258. doi:10.1111/j.1365-3040.2011.02406.x.

Moura FT, Ribeiro RA, Helene LCF, Nogueira MA, Hungria M. 2022. So many rhizobial partners, so little nitrogen fixed: The intriguing symbiotic promiscuity of common bean (*Phaseolus vulgaris* L.). Symbiosis. 86:169–185. doi:10.1007/s13199-022-00831-6.

Muñoz-Huerta RF, Guevara-Gonzalez RG, Contreras-Medina LM, Torres-Pacheco I, Prado-Olivarez J, Ocampo-Velazquez RV. 2013. A review of methods for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. Sensors. 13:10823–10843. doi:10.3390/s130810823.

Nadeem MA, Yeken MZ, Shahid MQ, Habyarimana E, Yılmaz H, Alsaleh A, Hatipoğlu R, Çilesiz Y, Khawar KM, Ludidi N, etal. 2021. Common bean as a potential crop for future food security: an overview of past, current and future contributions in genomics, transcriptomics, transgenics and proteomics. Biotechnol Biotechnol Equip. 35:759–787. doi:10.1080/13102818.2021.1920462.

Nleya T, Walley FL, Vandenberg A. 2009. Response of determinate and indeterminate common bean genotypes to *rhizobium* inoculant in a short season rainfed production system in the Canadian prairie. J Plant Nutr. 32:44–57. doi:10.1080/01904160802531001.

Pipan B, Meglič V. 2019. Diversification and genetic structure of the western-to-eastern progression of European phaseolus vulgaris L. germplasm. BMC Plant Biol. 19:442. doi:10.1186/s12870-019-2051-0.

Pipan B, Šuštar-Vozlič J, Meglič V, Dolničar P. 2017. Breeding prospective of common bean (*phaseolus vulgaris*) for central and southeastern European production area. Acta Hortic. 1326:189–194. doi:10.17660/ActaHortic.2021.1326.25.

Shamseldin A., Velázquez E. 2020. The promiscuity of *phaseolus vulgaris* L. (common bean) for nodulation with rhizobia: a review. World J Microbiol Biotechnol. 36:63. doi:10.1007/s11274-020-02839-w.

Toso V, Andriolo JL, Lerner MA, Schmitt OJ, Cardoso FL. 2017. Nitrogen in plant growth and yield of common bean. J Plant Nutr. 40:2006–2013. doi:10.1080/01904167.2017.1346116.

Zoffoli BC, Brito LF, Straliotto R, Araújo AP. 2020. Early nitrogen supplementation stimulates the nodulation and growth of common bean plants inoculated with rhizobium. Acta Sci Agron. 43:1–13. doi:10.4025/actasciagron.v43i1.55105.