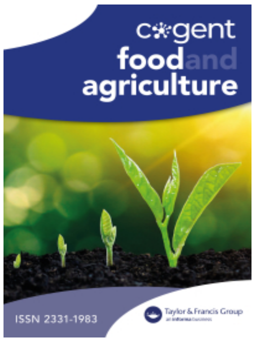

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Combining multi-criteria decision analysis with agro-morphological-biochemical-molecular traits of interest for use in breeding in promising common bean breeding lines (*Phaseolus vulgaris* L.)

Lovro Sinkovič, Hourieh Tavakoli Hasanaklou, Mohamed Neji, Eva Plestenjak, Peter Dolničar, Vladimir Meglič and Barbara Pipan

Department of Crop Science, Agricultural Institute of Slovenia, Ljubljana, Slovenia

ABSTRACT

Common bean is one of the most important legumes in the world. This study analyzed 26 promising breeding materials of *Phaseolus vulgaris* L. for 12 quantitative and 15 qualitative traits to identify elite lines for possible registration of new varieties. High variability was observed for most quantitative traits, with coefficients of variation (CV) from 8.87 to 67.04% and Shannon-Weaver diversity index (H') values from 1.21 to 1.54. Qualitative traits had H' values from 0.54 to 1.47. Seed yield per plant and total soluble solids showed considerable CVs (>42%), indicating significant variability among breeding lines. The H' value also illustrated diversity among quantitative traits, with 100 seed weight showing the highest diversity (H'=1.54). Factor analysis of mixed data (FAMD) and hierarchical clustering on principal components (HCPC) grouped the breeding materials into two clusters and subgroups. The number of seed colors and the main seed color were key differentiators. Using a Composite Performance Index (CPI) from multi-criteria decision analysis (MCDA), the breeding materials were ranked, and five elite breeding lines were selected: KIS6B, KIS23B, KIS22B, KIS8B and KIS7B (CPI = 44.5–53.1). These finings offer valuable insights into the diversity of promising common bean breeding lines and provide a solid basis for the final selection and registration of new varieties.

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a valuable vegetable plant whose seeds and pods are rich in proteins, fiber, vitamins, minerals, and antioxidants that are essential for human nutrition. It is cultivated worldwide and can be adapted to different cultivation methods and environments (Savić et al., 2021). This crop (snap bean or dry bean) occupies an important place in the traditional diet of many European countries. Domestication of the common bean led to the development of two distinct gene pools, namely Mesoamerican and Andean. These gene pools show remarkable differences in terms of phenotype, biochemistry, genotype, and geographical and reproductive barriers (Long et al., 2020). Complex interactions between natural selection, human intervention, and environmental factors have shaped this genetic diversity (Pipan & Meglič, 2019; Savić et al., 2021), resulting in a rich reservoir of genetic variation underlying the agronomic performance and resilience of common bean genotypes (Chávez-Servia et al., 2016; Pipan & Meglič, 2019). Agromorphological markers are highly valued for their directness, simplicity, and cost-effectiveness in the characterization of bean germplasms (Ibrahim Bio Yerima et al., 2020). As they encompass both quantitative and qualitative traits, they are the first and most crucial step in identifying available genetic resources and maximizing their effectiveness in breeding programs (Kouam et al., 2023; Pipan et al., 2023).

The agromorphological diversity of common bean genotypes encompasses a wide range of traits related to plant morphology, growth habits, phenology, seed and pod yield components, and responses

CONTACT Barbara Pipan barbara.pipan@kis.si Department of Crop Science, Agricultural Institute of Slovenia, Ljubljana, Slovenia

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to biotic and abiotic stresses (Kouam et al., 2023). Promoting this diversity through innovative breeding measures is crucial for ensuring the adaptability and sustainability of new bean varieties. In common bean cultivation, numerous biotic and abiotic stress factors contribute to yield reductions (Assefa et al., 2019). Climate change, soil quality and water availability are the most important global stress factors alongside pests, diseases and weeds. The bean pod weevil (*Apion godmani* Wagner) is a major economic threat, as it infests fields and damages pods and growing seeds (Blair et al., 2006; Vieira et al., 2009). In the field, larvae penetrate pod walls, feed on cotyledons, and reduce the nutritional quality of seeds (Vieira et al., 2009). Previous studies have shown that their behavior and development are influenced by environmental factors and host characteristics (Singh & Schwartz, 2011). Several studies have shown a correlation between trypsin inhibitor activity and infestation of different bruchids in legumes, emphasizing the importance of trypsin inhibitors in conferring resistance to different bruchids. In addition, the considerable genetic variability observed in the genotypes for these traits provides an opportunity to improve both yield and biotic tolerance through hybridization and subsequent selection. By utilizing this genetic diversity, plant breeders can identify and breed promising genotypes with improved biotic tolerance, which represents an economically and efficiently feasible solution to agricultural challenges (Bornowski et al., 2023).

The aim of this study was to characterize the diversity of several agromorphological-biochemical-molecular traits in common bean breeding materials and to select elite materials for the registration of new varieties. Different groups of qualitative and quantitative traits were analyzed, ranging from plant morphology and growth habit to seed yield, flowers, pod, and seed size, and determination of trypsin inhibitors and total soluble solids. The correlation between trypsin inhibitor activity and molecular SCAR marker was also investigated, particularly for bruchid disease susceptibility. The results of this study will provide valuable information for the final selection and registration of new varieties in the national breeding program for common beans.

2. Materials and methods

2.1. Plant material and data collection

A set of 26 breeding materials (24 breeding lines of generation F7 or higher and two new varieties) of the common bean (*Phaseolus vulgaris* L.) from the ongoing public breeding program at the Agricultural Institute of Slovenia was studied (Table 1). This breeding material is managed by the leading common bean breeder Barbara Pipan (corresponding author) in accordance with the established guidelines of the Agricultural Institute of Slovenia and the Public Service in Vegetables and Herbs. One hundred seeds from each breeding line/variety were sown for comprehensive agromorphological-biochemical-molecular analysis. Adjacent plants were planted 10 cm apart within the row and 80 cm between rows on a black polyethylene cover placed on the soil before planting. For the climbing types, the wooden poles were placed along with the plants. Field screening was conducted during the 2023 growing season in the experimental fields of the Jablje Infrastructure Center (46°30′17.4″ N, 15°37′34.6″ E; 320 m a.s.l.), where a sub-alpine climate prevails and standard bean cultivation practices are applied. Ten representative plants from each breeding line were selected for evaluation.

Data were collected for a total of 28 traits, including 12 quantitative traits (growing period (days) [GP]; time of flowering (50% of plants with at least one flower) (days) [TF]; seed size: length (mm) [SSLA]; seed size: width (mm) [SSWA]; seed size: height (mm)

Table 1. List of the common bean (*Phaseolus vulgaris* L.) breeding material examined with the corresponding codes.

Breeding material	Code	Status	Breeding material	Code	Status
228_4aa_ca	KIS1B	breeding line	189_4bc_bb	KIS14B	breeding line
302_2ba_bc	KIS2B	breeding line	91_4aa_ac	KIS15B	breeding line
227_2ac_cb	KIS3B	breeding line	91_4aa_bb	KIS16B	breeding line
227_2ac_ab	KIS4B	breeding line	32_aa_cb	KIS17B	breeding line
227_2ac_aa	KIS5B	breeding line	385x425	KIS18B	breeding line
239_5ca_bc	KIS6B	breeding line	KIS Marcelijan	KIS19S	variety
239_5ca_bb	KIS7B	breeding line	KIS Amand	KIS20S	variety
239_5ca_ba	KIS8B	breeding line	316x498	KIS21B	breeding line
239_5cb_bb	KIS9B	breeding line	425x417	KIS22B	breeding line
220_5cc_aa	KIS10B	breeding line	428xčeš	KIS23B	breeding line
196_1bc_cb	KIS11B	breeding line	491x498	KIS24B	breeding line
72_6ab_ca	KIS12B	breeding line	306x452	KIS25B	breeding line
174_1aa_ac	KIS13B	breeding line	425x301	KIS26B	breeding line

[SSHA]; 100 seeds weight (g) [SW100]; technologically mature pod length (cm) [ATMPL]; technologically mature pod width (mm) [ATMPW]; seed yield per plant (g) [TY]; percentage of trypsin inhibition (%TIn) [INH]; trypsin units inhibited (TUI/mg sample) [TUI]; and total soluble solids (°Brix) [TSS]) and 15 qualitative traits (plant growth type [PGT]; flower: color of standard [FCSS]; flower: color of wing [FCW]; pod length (excluding beak) [PL]; pod shape of cross-section (through seed) [PSCS]; pod ground color [PGC]; pod stringiness on ventral suture [PSV]; pod fiber hardness [PFH]; pod color (fresh pods) [PFC]; pod color (mature pods) [PMC]; seed: number of colors [SNC]; seed main color (largest area) [SMC]; seed predominant secondary color [SPSC]; seed shape [SH]; and a functional DNA marker (SCAR-SW6-800R) [Gen]). Data were accurately recorded during the respective phenophases according to the descriptor lists for Phaseolus vulgaris L. established by the Community Plant Variety Office (CPVO Phaseolus vulgaris L. Protocol for Tests on Distinctness, Uniformity & Stability: French Bean, 2013) and the PHASELIEU Project (De La Caudra et al., 2001). All qualitative descriptors were assessed visually, while quantitative descriptors were measured using a digital caliper (to +/− 0.1 mm), tape measure (to +/− 1 mm), or electronic laboratory balance (to +/− 0.01 g). The determination of biochemical traits (INH, TUI, and TSS) and a molecular trait (Gen) is described below.

2.2. Biochemical analysis: total soluble solids and trypsin inhibitor activity in common bean pods

Total soluble solids were determined in freshly squeezed juice from technologically mature pods using a digital refractometer (ORF 2WM, Kern Optics, Kern&SWohn GmbH, Germany) in three replicates and expressed as °Brix. To measure the trypsin inhibitor activity (TIA), representative pods were homogenized using a laboratory ball mill (Retsch MM400, GmbH, Germany), and 400 mg of the homogenized pod paste was used for extraction with 10 mL of 0.006 N NaOH solution and mixed with a vortex. For optimal extraction, ultrasonic treatment (PRO, 40 Hz, ASonic Ultrasonic cleaning bath, Slovenia) was performed for 15 minutes at room temperature. The sample extracts were centrifuged at 17,500 *g* for 20 min at 4 °C and filtered with syringe filters (0.45 µm, Whatman™). The pH of the extracts was in the range of 8.4 and 10.0. The TIA assay described by Kakade et al. (1974) was used for determination, and the results were expressed as the percentage of trypsin inhibition (%TIn) and trypsin units inhibited (TUI/mg sample).

2.3. Molecular analysis: SCAR marker genotyping for association with trypsin inhibitors

Samples of fresh and healthy plant tissues (60–100 mg) were used for DNA extraction, according to the protocol described by Pipan et al. (2013). DNA concentrations of each isolate were determined using a fluorimeter (Qubit 3.0; Thermo Fisher Scientific, MA, USA) and diluted to a final uniform concentration of 5.6 ng/µL. The molecular marker SCAR-SW6-800R has been used to show the association between common bean genotypes and bruchid infestation (Blair et al., 2006). PCR reactions were performed in a final volume of 11.5 µL containing 8.4 ng of genomic DNA and the following reagents, with initial concentrations of 1 µL 10× PCR buffer (Biotools, Spain), 0.2 µL of a mixture of 10 mM dNTPs (Sigma-Aldrich, USA), 0.5 µL 50 mM $MgCl_2$ (Biotools, Spain), 0.25 µL forward primer (Sigma-Aldrich, USA), 0.25 µL reverse primer (Sigma-Aldrich, ZDA) and 0.5 µL DNA polymerase (Biotools, Spain). PCR analyses were performed using a thermal cycler (Veriti, Thermo Fisher Scientific) under the following touchdown conditions, depending on the primer pair: 94 °C for 2 min, 45 cycles at 94 °C for 1 min, 55 °C for 40 s, 72 °C for 40 s, followed by one cycle at 72 °C for 2 min. Fragment analysis was performed with a genetic analyser (3130XL, Applied Biosystems), and allele lengths were determined by comparison with an internal size standard (GeneScan-350ROX, Applied Biosystems) using GeneMapper 4.0 software (Applied Biosystems) (Pipan & Meglič, 2019).

2.4. Data analysis

To evaluate the variability within and between the common bean breeding materials, data on agromorphological-biochemical-molecular traits were analyzed using the R-programming environment (R Core Team, 2023). First, descriptive statistics were used to illustrate variations in both the quantitative and qualitative traits. For this purpose, the *Pastecs* package was used to calculate descriptive statistics (Grosjean et al., 2018), including the coefficient of variation (CV), standard error (SE), maximum (Max) and minimum (Min) values, and mean for quantitative traits. In addition, we used the *Diverse* package (Guevara et al., 2016) to determine the frequency distribution and estimate diversity levels using the Shannon-Weaver diversity index (H′) (Shannon & Weaver, 1949) for all traits. The *CorrPlot* package was used to calculate the Spearman coefficient (ρ) to assess pairwise patterns of associations between traits. The *Daisy* function in the *Cluster* package (Maechler, 2018) was used to calculate the Gower distance (Gower, 1971) to estimate the similarity between pairwise breeding materials based on all traits analyzed. The obtained matrix was then used for hierarchical clustering to visualize the differentiation patterns between breeding materials. Furthermore, we performed a factor analysis of mixed data (FAMD) using the FactoMineR and Factoextra packages (Kassambara & Mundt, 2020) to investigate the differentiation patterns between breeding materials and to identify the main factors responsible for this differentiation. We then employed hierarchical clustering based on principal components (HCPC), which is commonly used as a complement to factor analysis, to further categorize breeding materials into more specific groups. Finally, we used a comprehensive multi-criteria decision analysis (MCDA) to evaluate and rank breeding materials based on their suitability for the final selection and registration of new varieties. Our analysis included all qualitative and quantitative traits to ensure a thorough evaluation of the performance of each breeding material. First, we normalized the quantitative traits to a common scale of 0 to 1 using a min-max normalization technique to ensure consistent treatment of all quantitative criteria. In addition, qualitative weights reflecting the relative importance of each categorical trait and quantitative weights representing the importance of numerical measurements were provided. To integrate these weights into the decision-making process, they were normalized to allow fair comparisons between the different criteria. We then calculated the weighted sum of the normalized feature values for each alternative, considering the preference direction for each quantitative feature. This resulted in composite scores reflecting the overall performance of each breeding material (Composite Performance Index; CPI). CPI was calculated as follows:

$$\mathrm{CPI} = \sum_{i=1}^{n}\left(Wqi \times Qi\right) + \sum_{j=1}^{m}\left(Wqj \times Qj \times Dj\right),$$

where *n* is the number of qualitative characteristics, m is the number of quantitative characteristics, W*qi* and Qi are the normalized weight and normalized value of qualitative trait *i*, *Wqj* and *Qj* are the normalized weight and normalized value to quantitative trait *j* and *Dj* is the direction of preference for the quantitative characteristic j, where 1 means that higher values are preferred and 0 means that lower values are preferred. Finally, the alternatives are ranked based on their CPI, with a score indicating better suitability.

3. Results

3.1. Evaluation of breeding material based on quantitative traits

A set of 12 quantitative traits related to agromorphological (flower, pod, seed) and biochemical (pod) parameters was determined in 26 breeding lines of common beans. These breeding lines showed high diversity in all parameters of descriptive statistics (Table 2). The growth period varied from 74 to 106 days, with a mean of 97 days, whereas the flowering time ranged from 28 to 48 days, with a mean of 36 days. Pod length ranged from 10.4 to 28.2 cm

Table 2. Summary of descriptive statistics for 12 quantitative traits of the common bean breeding material studied.

Group of parameters	Part of the plant	Trait	Unit	Min	Max	Mean	SE	CV (%)	H′
Agromorphological parameters	Flower	GP	days	74.00	106.00	96.54	1.70	9.04	1.31
		TF	days	28.00	48.00	36.31	0.90	12.11	1.37
	Pod	ATMPL	cm	10.40	28.20	15.18	0.90	29.70	1.21
		ATMPW	mm	9.23	22.95	15.02	0.60	22.02	1.37
	Seed	SSLA	mm	11.13	15.99	13.89	0.30	10.51	1.53
		SSHA	mm	5.71	8.38	7.29	0.10	8.87	0.76
		SSWA	mm	3.98	6.86	5.39	0.10	13.45	1.30
		SW100	g	20.81	50.90	33.58	1.70	25.98	1.54
		TY	g	0.58	15.82	6.63	0.90	67.04	1.51
Biochemical parameters	Pod	INH	%TIn	30.89	68.29	44.79	2.10	23.95	1.48
		TUI	TUI/mg sample	0.04	0.12	0.07	0.02	28.24	1.43
		TSS	°Brix	1.97	11.33	5.52	0.50	42.29	1.37

SE: standard error; CV: coefficient of variation; H′: Shannon-Weaver diversity index; GP: growing period; TF: time of flowering; ATMPL: technologically mature pod length; ATMPW: technologically mature pod width; SSLA: seed size length; SSHA: seed size height; SSWA: seed size width; SW100: 100 seeds weight; TY: seed yield per plant; INH: percentage of trypsin inhibition; TUI: trypsin units inhibited; TSS: total soluble solids.

and pod width from 9.2 to 23.0 mm, with a mean of 15.2 cm and 15.0 mm, respectively. For seed size parameters, seed length, height and width ranged from 11.1 – 16.0 mm, 5.7 – 8.4 mm and 4.0 – 6.9 mm, respectively. The 100 seed weight varied between 20.8 and 50.9 g with a mean of 33.6 g. The seed yield per plant ranged significantly from 0.6 to 15.8 g/plant with a mean of 6.6 g/plant. Among the biochemical parameters determined on the pods, the total soluble solids content varied considerably between 2.0 and 11.3°Brix, with a mean of 5.5°Brix. The percentage of trypsin inhibition ranged from 30.9 – 68.3%TIn and the trypsin units inhibited from 0.04 – 0.12 TUI/mg sample, with a mean of 44.8%TIn and 0.07 TUI/mg sample, respectively. Among the quantitative traits, the highest coefficient of variation (CV) was observed for seed yield per plant (67.0%), followed by total soluble solids (42.3%), pod length (29.7%), trypsin units inhibited (28.2%), 100 seeds weight (26.0%), percentage of trypsin inhibition (24.0%), and pod width (22.0%). In contrast, the lowest CV values were observed for seed height, growing period, seed length, flowering time, and seed width (8.9, 9.0, 10.5, 12.1%, and 13.5%, respectively). The Shannon-Weaver index (H′) showed significant variability among these 12 quantitative traits, ranging from 0.76 for seed height (SSHA) to 1.54 for 100 seed weight (SW100) (Table 2).

The distribution frequency of these 26 bean breeding lines for the 12 quantitative traits examined (in relation to plants, seeds, and pods) is shown in Figure 1. According to the FAO. (2024), the length of the total growing period for most bean varieties varies depending on the use of the product, and is 60 to 90 days for green beans (snaps) and 90 to 120 days for dry beans. In this study, all breeding lines had a growth period of less than 107 days. Further distribution showed that the growth period of the six breeding lines was between 74 and 93 days, 12 between 96 and 100 days and eight between 103 and 106 days. According to the time of flowering, seven breeding lines were very early (28 – 34 days), 16 breeding lines were early (35 – 40 days) and three breeding lines were early to medium (42 – 48 days). Seed yield per plant was generally lower for climbing type breeding lines (0.6 – 13.0 g, mean 5.3 g) and slightly higher for dwarf lines (1.0 – 15.8 g, mean 7.6 g). Based on seed length, 19 breeding lines can be classified as those with medium seeds between 10 and 15 mm, and seven breeding lines as those with large seeds (> 15 mm). Similarly, the bean breeding lines can be divided into two groups according to the 100 seeds weight, that is, low- and medium-weight. The low-weight seeds group included breeding lines with a 100 seed weight of 20.0 – 35.0 g (14 breeding lines or 54%) and the medium-weight seeds group included breeding lines with a 100 seed weight of 35.0 to 51.0 g (12 breeding lines or 46%). The distribution of the length and width of mature pods was highly variable and reflected the main breeding objectives, namely, climbing bean types for pods and dwarf bean types for dry seeds. Thus, 19 breeding lines formed shorter pods with pod lengths of 10 – 18 cm, while six of them formed longer pods with pod lengths of 18 – 28 cm. Similarly, 14 breeding lines (mainly dwarf types) formed narrower pods with widths of 9 – 15 mm, while 12 breeding lines (mainly climbing types) formed wider pods with widths of 16 – 23 mm (Figure 1).

The biochemical parameters determined for the pods also showed high variability between the bean breeding lines. The percentage of trypsin inhibition was below 50%TIn (30.9 – 49.8%TIn) in 18 breeding lines and above 50%TIn (50.3 – 68.3%TIn) in eight lines. Based on the trypsin units inhibited, 17 breeding lines could be classified as low TUI/mg sample (0.041 – 0.077) and nine as high TUI/mg sample (0.081 – 0.119). Although the sugar content in bean pods is generally low compared to that in some other vegetables or fruits, large differences in total soluble solids (TSS) content were found between the breeding lines. These can be divided into three groups according to TSS content: low, medium, and high. The low-sugar pod group included breeding lines with a TSS of 2.0 – 4.8°Brix (14 breeding lines), the medium-sugar pod group lines with a TSS of 5.5 – 9.4°Brix (11 breeding lines) and the high-sugar pod group lines with a TSS of 10.9 – 11.3°Brix (two breeding lines) (Figure 1).

3.2. Evaluation of breeding material based on qualitative traits

A set of 15 qualitative traits related to agromorphological (plant, flower, pod, and seed) and molecular parameters were determined in 26 common bean breeding materials, as shown in Figure 2. In terms of plant growth type, 15 breeding lines belonged to the dwarf bean type with a bush growth habit, while 11 had a climbing growth habit (H′=0.68). The flower color of the standard was white in 14 breeding lines, purple in 10, and green in two (H′=0.69). Only two flower wing colors were observed: 16 breeding lines had white wings and 10 had purple wings (H′=0.64). Pod length varied considerably between the breeding lines studied. In particular, the pod length was very

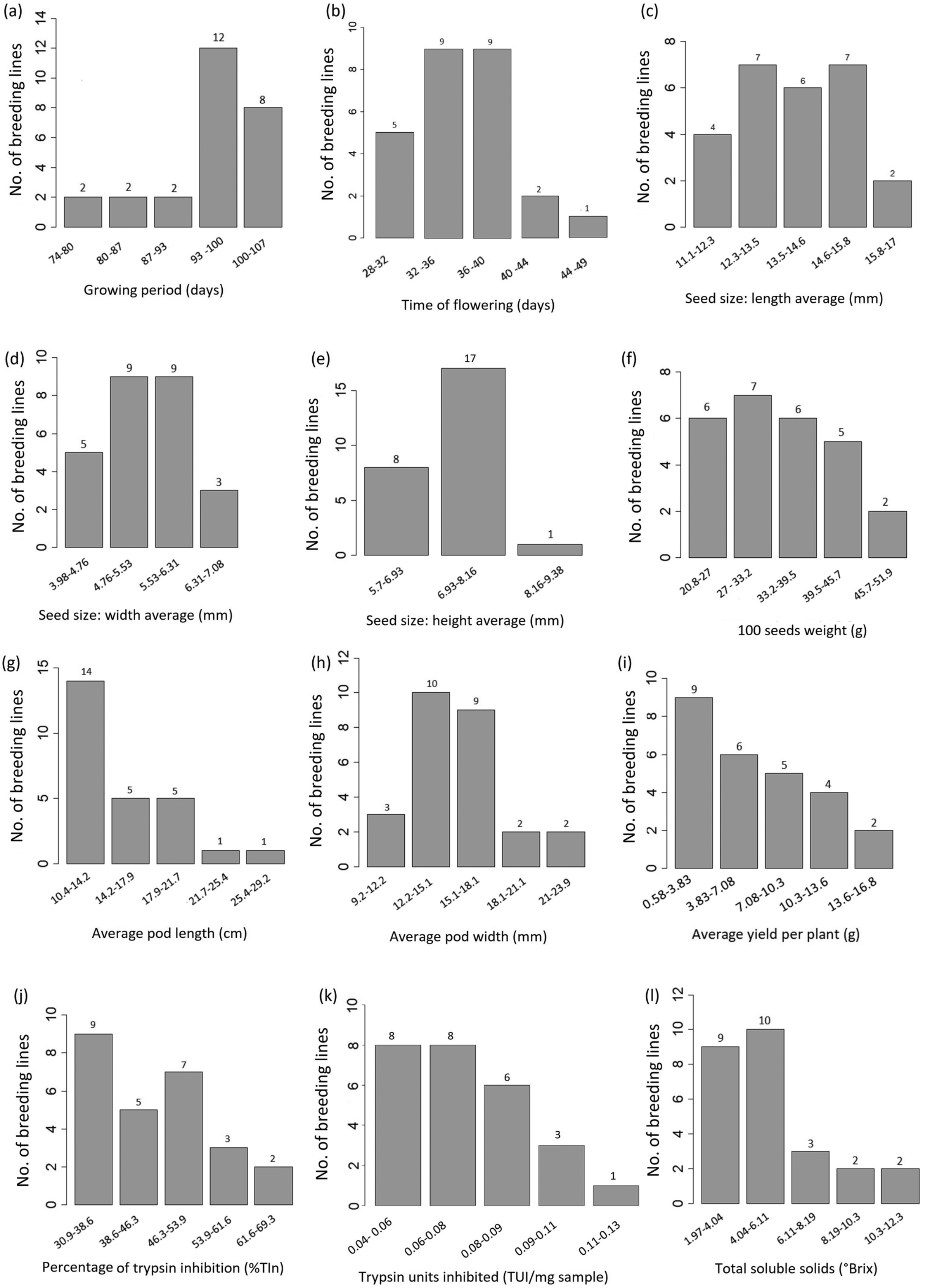


Figure 1. Frequency distribution of 26 common bean breeding materials for 12 quantitative traits related to agromorphological (a–i) and biochemical parameters (j–l).

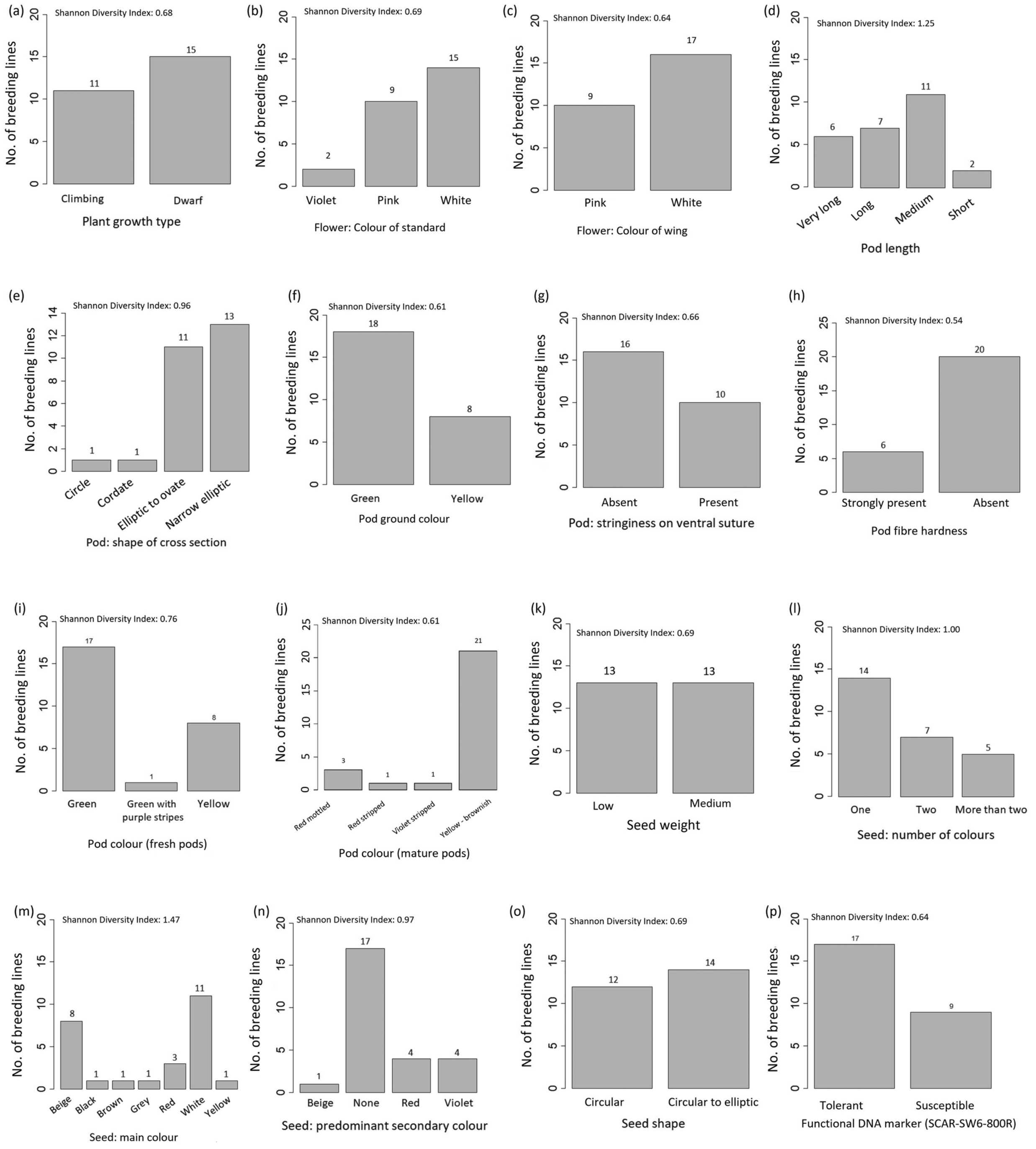


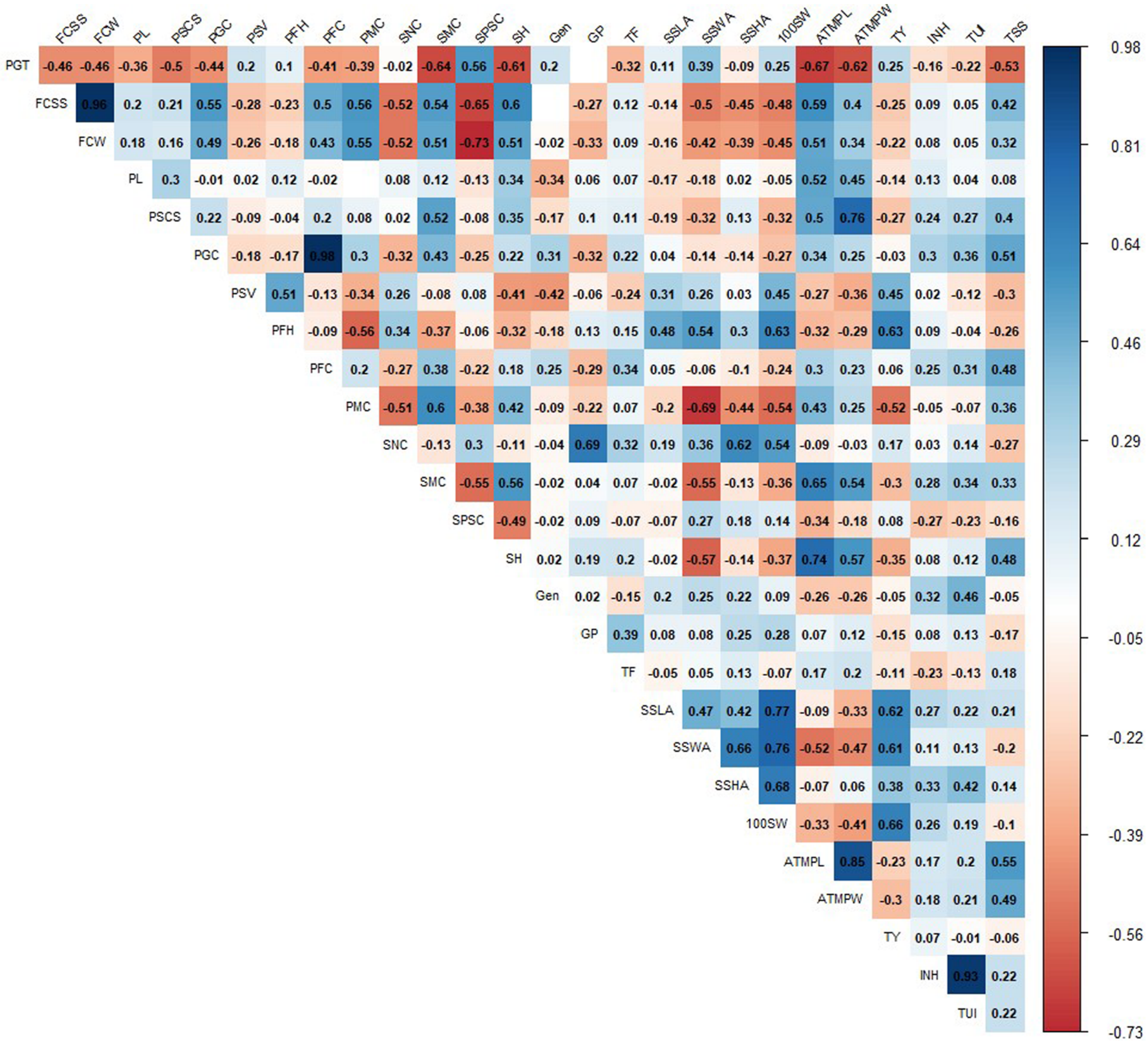
Figure 2. Frequency distribution of 26 common bean breeding materials for 15 quantitative traits related to agromorphological (a–o) and molecular parameters (p).

long in six breeding lines, long in seven, medium in 11, and short in two (H′=1.21). Regarding the shape of the pod in cross-section, 13 breeding lines had narrow elliptical-shaped pods, 11 had elliptical to ovate-shaped pods, one had a circular-shaped pod, and one had a cordate-shaped pod (H′=0.96). A total of 16 breeding lines did not show any pod stringiness on the ventral structure, whereas for 10 lines, pod stringiness on the ventral suture was present (H′=0.66). Pod fiber hardness was absent in 20 breeding lines, whereas it was strongly present in six (H′=0.54). As seen in Figure 2, among the 26 bean breeding lines, 17 had green pod ground color, while eight had yellow pod ground color (H′=0.61). One breeding line formed pods with a unique pod color, characterized as green with purple stripes (H′=0.76). The predominant color of mature pods was yellow-brownish in 21 breeding lines, while three had red mottled, one had red striped, and one had violet-striped mature pods (H′=0.61). Only two types of pod ground color were observed: 18 bean breeding lines had green pods and eight had yellow pods (Figure 2).

Seven main seed colors were observed, that is, 11 breeding lines had white seeds, eight lines had beige, three lines had red, and one line each had black, brown, gray, and yellow seeds, resulting in a high diversity (H'=1.47). The majority of breeding lines (14) had seeds with one color, while seven breeding lines developed seeds with two colors. In addition, five breeding lines developed seeds with more than two colors (H'=1.00). The predominant secondary seed colors were red (four breeding lines), violet (four breeding lines), and beige (four breeding lines) (H'=0.97). The shape of the seeds was oval/circular to elliptical in 14 breeding lines and round/circular in 12 breeding lines (H'=0.69). Based on the molecular SCAR marker, the majority of breeding lines (17) were resistant, and nine breeding lines were susceptible to bruchid infestation (Figure 2).

3.3. Correlation between traits of interests

The analysis of Spearman correlation coefficients with a significance of $p < 0.01$ revealed significant positive and negative associations between different variables within the common bean breeding materials, encompassing both qualitative and quantitative traits (Figure 3). In particular, very high positive correlations were found between pod ground color – PGC and fresh pod color – PFC (0.98), flower standard color – FCSS and flower wings color – FCW (0.96) and percentage of trypsin inhibition – INH and trypsin units inhibited – TUI (0.93). High positive correlations were found between pod-related traits,



	FCSS	FCW	PL	PSCS	PGC	PSV	PFH	PFC	PMC	SNC	SMC	SPSC	SH	Gen	GP	TF	SSLA	SSWA	SSHA	100SW	ATMPL	ATMPW	TY	INH	TUI	TSS
PGT	-0.46	-0.46	-0.36	-0.5	-0.44	0.2	0.1	-0.41	-0.39	-0.02	-0.64	0.56	-0.61	0.2		-0.32	0.11	0.39	-0.09	0.25	-0.67	-0.62	0.25	-0.16	-0.22	-0.53
FCSS		0.96	0.2	0.21	0.55	-0.28	-0.23	0.5	0.56	-0.52	0.54	-0.65	0.6		-0.27	0.12	-0.14	-0.5	-0.45	-0.48	0.59	0.4	-0.25	0.09	0.05	0.42
FCW			0.18	0.16	0.49	-0.26	-0.18	0.43	0.55	-0.52	0.51	-0.73	0.51	-0.02	-0.33	0.09	-0.16	-0.42	-0.39	-0.45	0.51	0.34	-0.22	0.08	0.05	0.32
PL				0.3	-0.01	0.02	0.12	-0.02		0.08	0.12	-0.13	0.34	-0.34	0.06	0.07	-0.17	-0.18	0.02	-0.05	0.52	0.45	-0.14	0.13	0.04	0.08
PSCS					0.22	-0.09	-0.04	0.2	0.08	0.02	0.52	-0.08	0.35	-0.17	0.1	0.11	-0.19	-0.32	0.13	-0.32	0.5	0.76	-0.27	0.24	0.27	0.4
PGC						-0.18	-0.17	0.98	0.3	-0.32	0.43	-0.25	0.22	0.31	-0.32	0.22	0.04	-0.14	-0.14	-0.27	0.34	0.25	-0.03	0.3	0.36	0.51
PSV							0.51	-0.13	-0.34	0.26	-0.08	0.08	-0.41	-0.42	-0.06	-0.24	0.31	0.26	0.03	0.45	-0.27	-0.36	0.45	0.02	-0.12	-0.3
PFH								-0.09	-0.56	0.34	-0.37	-0.06	-0.32	-0.18	0.13	0.15	0.48	0.54	0.3	0.63	-0.32	-0.29	0.63	0.09	-0.04	-0.26
PFC									0.2	-0.27	0.38	-0.22	0.18	0.25	-0.29	0.34	0.05	-0.06	-0.1	-0.24	0.3	0.23	0.06	0.25	0.31	0.48
PMC										-0.51	0.6	-0.38	0.42	-0.09	-0.22	0.07	-0.2	-0.69	-0.44	-0.54	0.43	0.25	-0.52	-0.05	-0.07	0.36
SNC											-0.13	0.3	-0.11	-0.04	0.69	0.32	0.19	0.36	0.62	0.54	-0.09	-0.03	0.17	0.03	0.14	-0.27
SMC												-0.55	0.56	-0.02	0.04	0.07	-0.02	-0.55	-0.13	-0.36	0.65	0.54	-0.3	0.28	0.34	0.33
SPSC													-0.49	-0.02	0.09	-0.07	-0.07	0.27	0.18	0.14	-0.34	-0.18	0.08	-0.27	-0.23	-0.16
SH														0.02	0.19	0.2	-0.02	-0.57	-0.14	-0.37	0.74	0.57	-0.35	0.08	0.12	0.48
Gen															0.02	-0.15	0.2	0.25	0.22	0.09	-0.26	-0.26	-0.05	0.32	0.46	-0.05
GP																0.39	0.08	0.08	0.25	0.28	0.07	0.12	-0.15	0.08	0.13	-0.17
TF																	-0.05	0.05	0.13	-0.07	0.17	0.2	-0.11	-0.23	-0.13	0.18
SSLA																		0.47	0.42	0.77	-0.09	-0.33	0.62	0.27	0.22	0.21
SSWA																			0.66	0.76	-0.52	-0.47	0.61	0.11	0.13	-0.2
SSHA																				0.68	-0.07	0.06	0.38	0.33	0.42	0.14
100SW																					-0.33	-0.41	0.66	0.26	0.19	-0.1
ATMPL																						0.85	-0.23	0.17	0.2	0.55
ATMPW																							-0.3	0.18	0.21	0.49
TY																								0.07	-0.01	-0.06
INH																									0.93	0.22
TUI																										0.22

Figure 3. Spearman correlation coefficients for each pairwise comparison are shown in each cell. Positive correlations (from 0 to 1) are displayed in blue, negative correlations in red (from 0 to -1). The color intensity (dark to light) indicates the strength and direction of the relationships between the traits. Empty cells indicate non-significant correlations at $p < 0.01$. The abbreviations are as described in *2.1. Experimental design and data collection.*

especially for pod shape of cross-section – PSCS (0.76) with technologically mature pod width – ATMPW and technologically mature pod length – ATMPL with seed shape (0.74). Furthermore, high positive correlations were found between seed-related traits, especially 100 seed weight – 100SW with seed length – SSLA and seed width – SSWA, 0.77 and 0.76, respectively. A high negative correlation was found for the predominant secondary seed color – SPSC with flower wings color – FCW (-0.73). In addition, a low positive correlation was found between the functional SCAR marker – Gen and the trypsin units inhibited – TUI and the percentage of trypsin inhibition – INH, 0.46 and 0.32, respectively (Figure 3).

3.4. Differentiation among breeding lines

The data were analyzed using hierarchical cluster analysis and factor analysis of mixed data (FAMD) to investigate the patterns of differentiation between the studied breeding lines. Cluster analysis revealed that the breeding lines were divided into two main groups (Figure 4A). The first group included nine breeding materials (KIS1B, KIS2B, and KIS5B – KIS11B) and the second group consisted of 17 breeding lines (Figure 4B). FAMD showed that the first two dimensions explained 24.4% and 11.4% of the total variance and differentiation between breeding lines, respectively (Figure 6A). Similar to the cluster analysis, the FAMD biplot formed by the first two dimensions divided the lines into two main groups along the first dimension (Figure 6A). The differentiation between breeding lines was mainly due to variations in the qualitative traits (Figure 5A). In particular, the number of seed colors (SNC) and the main seed color (SMC) contributed the most to the differentiation (>8% of the total variation) (Figure 5A & B). Overall, the contribution of the studied traits to these

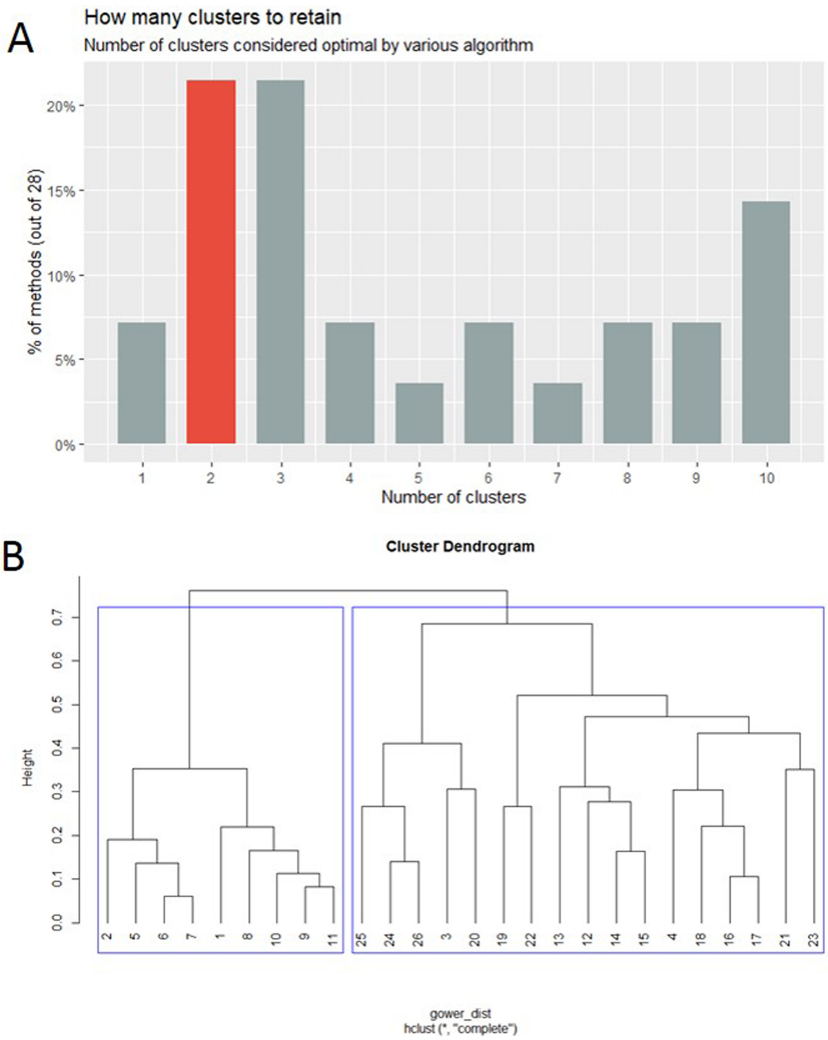


Figure 4. (A) The optimal number of clusters and (B) UPGMA dendrogram based on Gower distance showing the relationship between the studied breeding materials.

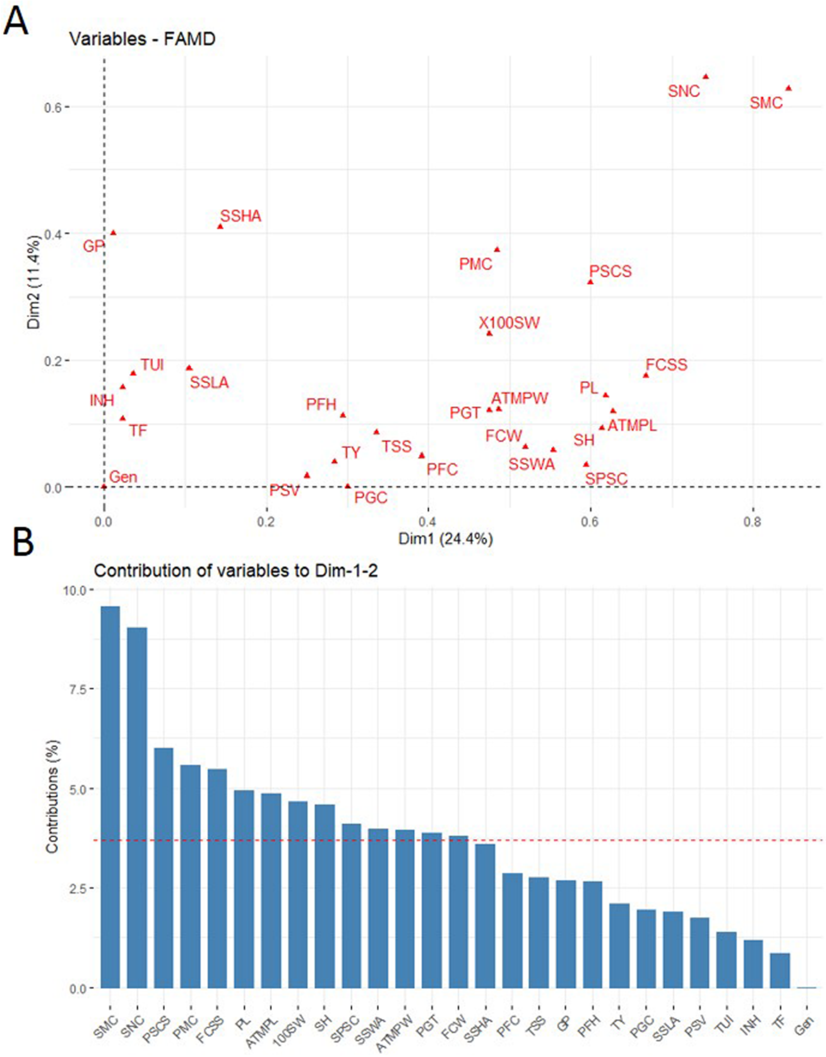


Figure 5. The contribution of qualitative and quantitative traits to the differentiation between the common bean breeding materials studied.

common bean breeding materials was mainly associated with pod- and seed-related qualitative traits. Among the quantitative traits, technologically mature pod length (ATMPL) and 100 seed weight (100SW) contributed the most to differentiation (Figure 5B). In contrast, the molecular trait functional SCAR marker (Gen) contributed the least to differentiation. Regarding the biochemical traits, the highest contribution was observed for total soluble solids (TSS), followed by Trypsin units inhibited (TUI) and percentage of trypsin inhibition (INH).

Similar to FAMD, the HCPC biplot divided the studied breeding materials into two major clusters, which could be further subdivided into various sub-clusters (Figure 6B, Tables S1 and S2). The first cluster comprised 11 breeding lines with two sub-clusters; the first comprised four breeding lines (KIS4B, KIS7B-9B) and the second seven lines (KIS1B, KIS3B-5B, KIS10B, KIS16B, and KIS17B) (Figure 6B). The breeding lines in the first sub-cluster were mainly characterized by yellow fresh pod color (PFC) and yellow pod ground color (PGC), followed by white main seed color (SMC), climbing growth type (PGT), round/circular seed shape (SH), and narrow elliptical cross-section of pod shape (PSCS). On the other hand, breeding lines in the second sub-cluster were mainly characterized by a very long pod length (PL), white main seed color (SMC), narrow elliptical pod shape of cross-section (PSCS), one seed color (SNC), and round/circular seed shape (SH) (Table S2). The second cluster comprised 15 breeding lines with five sub-clusters (Figure 6B). Here, the first sub-cluster included the two breeding lines KIS18B and KIS21B, which were mainly characterized by more than two seed colors (SNC). The second sub-cluster included six breeding lines (KIS2B, KIS11B-14B, KIS17B), mainly characterized by violet secondary

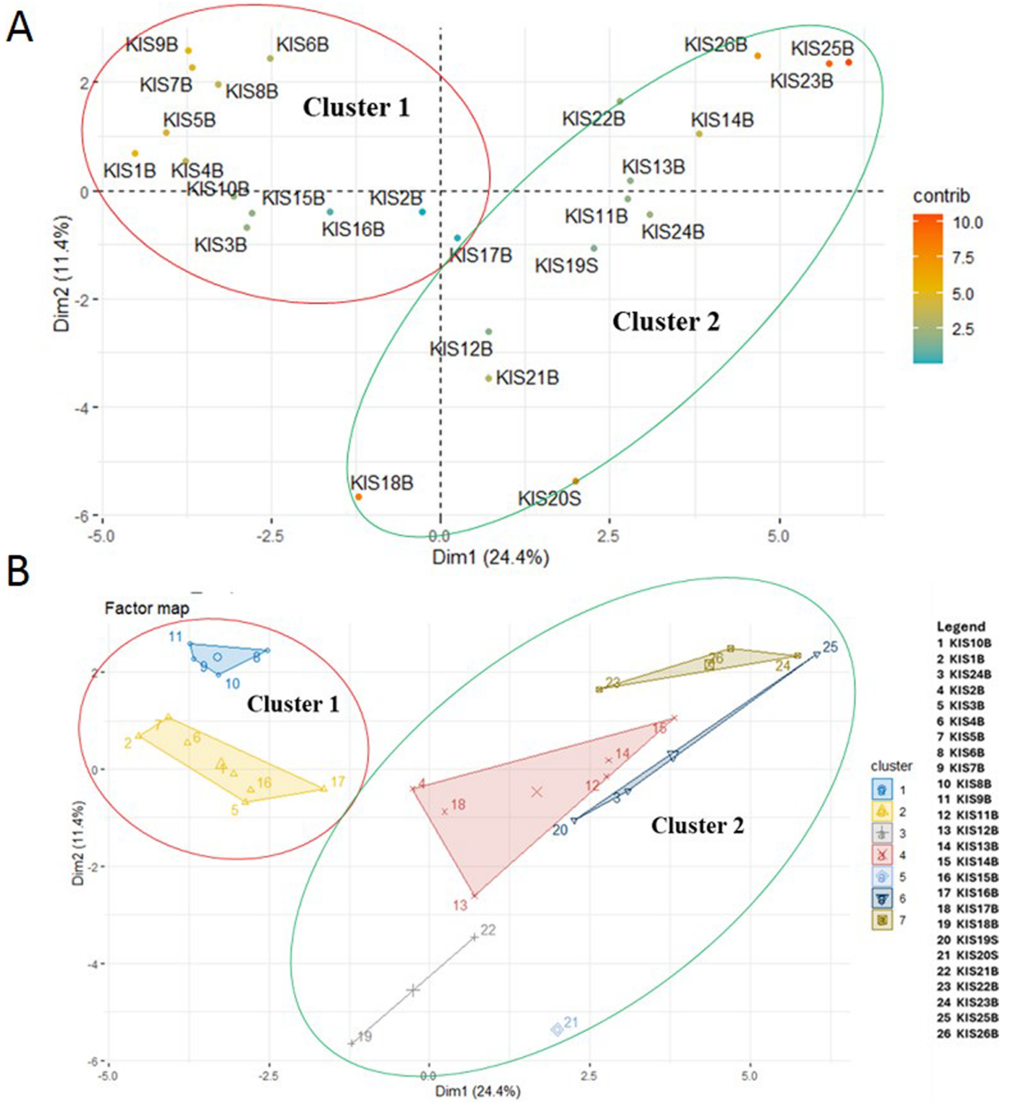


Figure 6. (A) Factor analysis of mixed data (FAMD) biplot and (B) subsequent hierarchical clustering of principal components (HCPC) biplot showing differentiation between common bean breeding materials based on 27 agromorphological-biochemical-molecular traits.

seed color (SPSC), pink flower wing color (FCW) and pink flower standard color (FCSS), white main seed color (SMC), one seed color (SNC), narrow elliptic pod shape in cross section (PSCS), climbing plant growth type (PGT), white FCSS, and low or medium seed weight (SW) (Figure 5a). The third sub-cluster was represented by a single breeding line, KIS20S, characterized by black main seed color (SMC) and violet-stripped mature pod color (PMB). The fourth sub-cluster comprised the three breeding lines KIS19S, KIS23B, and KIS24B, which were characterized by a strongly present pod fiber hardness (PFH), susceptibility to bruchids infestation (Gen), and present pod stringiness on ventral suture (PSV). Finally, the fifth sub-cluster also included three breeding lines (KIS22B, KIS23B, and KIS26B), which were mainly characterized by a red mottled color of the mature pods (PMC), a pink color of the flower wings (FCW), the flower standard (FCSS), and the present PSV (Table S2).

The application of the MCDA weighted average approach enabled the ranking of breeding materials according to their suitability for final selection and registration of new varieties. A composite score reflecting the overall performance of each breeding material formed the Composite Performance Index (CPI), which was ranked from best to worst, as summarized in Table 3. The results showed that the five breeding lines performed best in the following order: KIS6B (CPI = 53.1), KIS23B (CPI = 51.0), KIS22B (CPI = 46.2), KIS8B (CPI = 46.0), and KIS7B (CPI = 44.5). These breeding lines were selected as the most elite material and will be included in the registration of the new bean varieties. Lines KIS6B, KIS8B, and KIS7B have a climbing growth type and develop long/very long, narrow elliptical yellow pods (>18 cm) without

Table 3. Results of the multi-criteria decision-making (MCDM) analysis.

Line	CPI	Rank
KIS1B	33.157	15
KIS2B	25.273	19
KIS3B	19.757	23
KIS4B	28.532	18
KIS5B	31.584	17
KIS6B	53.052	1
KIS7B	44.543	5
KIS8B	45.965	4
KIS9B	41.457	8
KIS11B	42.227	7
KIS12B	16.038	25
KIS13B	33.750	14
KIS14B	35.170	11
KIS15B	34.311	13
KIS16B	24.862	21
KIS17B	34.936	12
KIS18B	16.316	24
KIS19S	42.276	6
KIS20S	25.043	20
KIS21B	12.756	26
KIS22B	46.155	3
KIS23B	50.995	2
KIS24B	31.766	16
KIS25B	37.342	10
KIS26B	39.009	9

CPI: composite performance index.

stringiness on the ventral suture, which is why they are intended for the cultivation of fresh pods (snaps). Lines KIS23B and KIS22B, on the other hand, are dwarf beans with green pods and present stringiness on the ventral suture, which makes them more suitable for the production of dry beans. These two lines develop medium-weight seeds (100 seed weight = 35.0–51.0 g), with the KIS23B line developing seeds with two colors (main beige, secondary red), while the KIS22B line has yellow seeds. In addition, dwarf lines do not require climbing support during plant growth and are suitable for the mechanical harvesting of seeds. All five top-ranked lines were resistant to bruchids according to the molecular marker SCAR-SW6-800R. Among the biochemical traits determined on the fresh pods, the percentage of trypsin inhibition in KIS6B, KIS23B, KIS22B and KIS7B was over 53%TIn and the trypsin units inhibited were over 0.08 TUI/mg sample except for KIS8B (38% TIn and 0.067 TUI/mg sample, respectively). The total soluble solids content was low in KIS23B and KIS22B (<5°Brix), medium in KIS6B and KIS7B medium (6 – 9°Brix) and KIS8B has high sugar content (>10°Brix).

4. Discussion

The present comprehensive analysis of 26 common bean breeding materials includes 12 quantitative and 15 qualitative agromorphological-biochemical-molecular traits. Assessment of phenotypic variation in common bean is crucial for understanding its adaptability, agronomic potential, and breeding value (Scarano et al., 2014). Our study revealed significant multitrait-related diversity among the breeding materials analyzed, providing valuable insights for the final selection of elite lines in a breeding program. Among the quantitative traits, the seed yield per plant (TY) showed the highest coefficient of variation (CV) of 67%. Moreover, frequency distribution analysis revealed a wide range of TY from 0.6 to 15.8 g, highlighting the considerable variability in yield potential between these breeding lines. Similarly, a wide range of biochemical traits determined in technologically mature pods, including total soluble solids (TSS), trypsin units inhibited (TUI) and percentage of trypsin inhibition (INH), showed significant variability, with CVs ranging from 24 to 42%. These traits showed significant diversity among breeding lines, suggesting that it is possible to identify promising genotypes for final selection in breeding programs aimed at improving traits related to plant architecture, seed yield, and nutritional quality (Nogueira et al., 2021).

The importance of trypsin inhibitors in improving resistance to bruchids has been well-documented (Ahmad et al., 2018; Appleby & Credland, 2003; Gatehouse & Boulter, 1983; Pradhan et al., 2020). This study suggests that some common breeding lines exhibit increased resistance to bruchids by upregulating trypsin inhibitor activity. A low positive correlation was found between a molecular SCAR marker (SCAR-SW6-800R) (Gen) and both trypsin units inhibited (TUI) (0.46) and the percentage of trypsin inhibition (INH) (0.32) determined in technologically mature pods (snaps). This is the first such study on bean pods; thus far, only studies on bean seeds have been conducted, which makes the positive correlation obtained interesting. The molecular marker SCAR-SW6-800R is a valuable indicator for identifying bean genotypes that are resistant to pod weevils (Blair et al., 2006). The identification of bean genotypes with desirable traits, such as disease resistance and specific pod and seed traits, provides opportunities for targeted selection and breeding initiatives and emphasizes the importance of these initiatives for the development of improved bean varieties tailored to different environmental conditions and market preferences (Pipan et al., 2013). The three-year study of several bean genotypes from Mesoamerican and Andean gene pools revealed significant differences in trypsin inhibitors in the seeds, ranging from 19.5 to 25.4 TIU/mg dry matter (Piergiovanni & Pignone, 2003). In addition, studies have shown that both biotic and abiotic stress factors, such as drought, heat, and salt stress, as well as insect herbivory, can lead to increased trypsin inhibitor activity (Piergiovanni & Pignone, 2003; Sánchez-Hernández et al., 2004). However, the extent of this increase varied from bean genotype to bean genotype. Understanding the relationship between protease inhibitor activity and allelic variation in the genes coding for such inhibitors is crucial. Based on this understanding, breeders can make informed decisions to develop new bean varieties with increased resistance to pests and diseases as well as improved nutritional profiles and agronomic traits. This approach ensures that bean varieties are suitable for different agroecological zones and meet consumer demand.

The evaluation of pod-related traits, including pod color, length, and width, of technologically mature pods that are typically consumed highlights the wide diversity of common bean breeding materials studied. Of the 26 breeding lines evaluated, 18 had a green pod color and eight had a yellow pod color. Pod morphology and color play a crucial role in common bean as they influence consumer preferences, especially for pods consumed as green beans in different genotypes (Khatun et al., 2022). The width of fully developed pods ranged from 9.2 to 23.0 mm in these breeding lines and the length of these pods ranged from 10.4 to 28.2 mm, indicating a wide range of pod colors, sizes and shapes. However, the recorded traits showed high heritability, and for most traits, the estimated Shannon diversity index (H′) was high, indicating that a few major genes were involved (Savić et al., 2020).

This study is consistent with previous research (Caproni et al., 2019) and highlights considerable variability in bean pod color, shape, and length. In particular, fresh pods with green hues, occasionally accompanied by characteristic colors, such as green with purple stripes, highlight the different phenotypic appearances within genotypes. Similarly, variation in mature pod color illustrates the range of pod phenotypes in the common bean gene pool (García-Fernández et al., 2021). A moderate positive correlation was observed between pod-related traits, especially pod length (PL), technologically mature pod length (ATMPL) and pod width (ATMPW), which is consistent with the results of previous studies (García-Fernández et al., 2021). In addition, the considerable variability in pod length, ranging from short to very long in different breeding lines, highlights the plasticity of pod morphology and its potential impact on agronomic traits, such as yield and harvest efficiency. Modern bean varieties usually have elongated cylindrical pods, which can also influence the seed shape (Wallace et al., 2018). The results showed that 20 breeding lines had no pod fiber hardness, whereas six had a strong presence of pod fiber hardness. Pods with low wall fiber hardness are characteristic of various types of string/snap beans, which often have thick and fleshy pod walls (Wallace et al., 2018). The low correlation between pod stringiness on the ventral suture and a molecular SCAR marker, particularly for resistance to the bruchid pest, highlights an important aspect of common bean biology and agronomy. The presence of pod fibers, which contribute to pod hardness, is thought to be responsible not only for structural support but also for defense against pathogens and pests. Wallace et al. (2018) suggested that a reduction in pod fibers could impair the plant's ability to defend against pathogens, highlighting the multifunctional role of this trait.

It is noteworthy that most of the quantitative traits analyzed in our study showed significant positive correlations. As suggested by Pipan et al. (2024), this positive correlation between quantitative traits may result from polygenic control, in which genes exert pleiotropic effects on multiple traits, or from linkage disequilibrium between different loci. Based on the Shannon-Weaver diversity index (H′), qualitative traits showed moderate to high diversity (0.54 to 1.47), with seed color showing the highest diversity and pod fiber hardness the lowest. Vidak et al. (2015) suggested that H′ is higher for seed color. Quantitative traits showed slightly higher diversity (H′=0.76–1.54), with seed length and 100 seed weight showing the highest diversity (H′≥1.53), while seed height was the lowest. Traits such as seed size and color contributed significantly to the overall diversity observed in common bean breeding lines. In addition, the analysis revealed that the variations in seed size dimensions, such as height, width, and length among breeding lines, further emphasized the diversity in seed morphology.

Seed color is one of the most important characteristics that guide breeders, farmers, processors, and consumers in variety selection (Ibrahim Bio Yerima et al., 2020). In the present study, the majority of breeding lines had white or beige-colored seeds, which were often correlated with several other traits, including 100 seed weight, seed width, seed shape, flower color of standard, flower color of wings, pod color (at maturity), and seed yield per plant. White-seeded genotypes are special because they occur in all Central American and Andean landraces of common bean, but have never been observed in the wild (McClean et al., 2018). The significant variability and strong positive correlations observed between seed yield per plant (TY) and various seed-related parameters such as 100 seed weight (SW100), seed length (SSLA) and seed width (SSWA) were the main findings of our study. Seed parameters play a crucial role in shaping the overall diversity and performance of plant populations (Sinkovič et al., 2019). They serve as fundamental components for characterizing genetic variation and for understanding the underlying mechanisms that control plant growth, development, and productivity.

Factor analysis of mixed data (FAMD) and hierarchical clustering based on principal components (HCPC) revealed different groupings among common bean breeding lines. According to FAMD, two groups with different numbers of breeding lines were formed. Traits such as the number of seed colors and the main seed color contributed the most to the differentiation. Overall, qualitative pod- and seed-related traits were the most influential. Furthermore, HCPC differentiation of breeding materials into seven subgroups showed that qualitative rather than quantitative traits contributed to the diversification of the studied breeding lines. This suggests that priority should be given in breeding programs to cross between groups that emphasize this qualitative aspect to increase genetic diversity. In particular, pod color at maturity, main seed color, and number of seed colors were found to be the most influential qualitative traits affecting diversity. The importance of pod and seed size and color for crop marketability has been highlighted in previous studies (Khatun et al., 2022). In addition, there appears to be a crucial relationship between seed and pod morphology, with the pod serving as a protective organ for seeds (García-Fernández et al., 2023). This relationship could explain the observed positive association between seed and pod color, further highlighting its importance for diversity analysis. To incorporate these results into the breeding decision-making process, the overall performance of each breeding material was evaluated using the Composite Performance Index (CPI). Using comprehensive multi-criteria decision analysis (MCDA), five elite breeding lines (Table 3, Rank 1–5) were selected for the final registration process.

5. Conclusions

This study revealed a considerable diversity of agro-morphological, biochemical, and molecular traits in promising common bean breeding materials, and provided insights that are crucial for final selection in breeding programs. Finally, based on the results of the multi-criteria decision analysis, five elite breeding lines (KIS6B, KIS23B, KIS22B, KIS8B and KIS7B) were identified as candidates for registering new bean varieties to meet the challenges of agriculture and improve food security.

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Authors' contributions

Conceptualization: Lovro Sinkovič, Hourieh Tavakoli Hasanaklou and Barbara Pipan; methodology: Hourieh Tavakoli Hasanaklou, Eva Plestenjak and Barbara Pipan; formal analysis: Lovro Sinkovič, Hourieh Tavakoli Hasanaklou and Mohamed Neji; resources: Barbara Pipan; writing – original draft: Hourieh Tavakoli Hasanaklou, Mohamed Neji, Eva Plestenjak and Peter Dolničar; writing – review and editing: Lovro Sinkovič, Vladimir Meglič and Barbara Pipan; project administration and funding acquisition: Lovro Sinkovič, Peter Dolničar, Vladimir Meglič and Barbara Pipan. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

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About the authors

Mr Lovro Sinkovič holds a PhD and is an associate researcher at the Crop Science Department of the Agricultural Institute of Slovenia. His research interests lie in the fields of food science and the nutritional quality of agricultural crops.

Mrs Hourieh Tavakoli Hasanaklou is a researcher with PhD in the Crop Science Department of the Agricultural Institute of Slovenia. Her research interests are centred on the areas of biochemical processes and plant physiology.

Mr Mohamed Neji holds a PhD and is an associate researcher at the Crop Science Department of the Agricultural Institute of Slovenia. His research activities are concentrated on the interdisciplinary fields of plant genomics and genetics.

Miss Eva Plestenjak holds a Master of Science degree and is currently a young researcher at the Crop Science Department of the Agricultural Institute of Slovenia. Her research interest is plant genetics.

Mr Peter Dolničar holds a PhD and is a senior research associate at the Crop Science Department of the Agricultural Institute of Slovenia. His research interests are in the fields of plant breeding and physiology.

Mr Vladimir Meglič holds a PhD and is a senior researcher at the Crop Science Department of the Agricultural Institute of Slovenia. His research activities are focused on the interdisciplinary areas of plant breeding and genetics.

Mrs Barbara Pipan holds a PhD and is an associate researcher at the Crop Science Department of the Agricultural Institute of Slovenia. Her research interests are in the fields of plant breeding, genetics and genomics.

ORCID

Lovro Sinkovič http://orcid.org/0000-0002-6123-8085
Hourieh Tavakoli Hasanaklou http://orcid.org/0000-0001-6600-9065
Mohamed Neji http://orcid.org/0000-0003-3847-2129
Eva Plestenjak http://orcid.org/0000-0002-9707-828X
Peter Dolničar http://orcid.org/0000-0002-3397-0009
Vladimir Meglič http://orcid.org/0000-0002-7739-0145
Barbara Pipan http://orcid.org/0000-0002-3860-119X

Data availability statement

All data generated or analyzed during this study are included in this published article.

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