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Assessing landraces and varieties of beans (*Phaseolus spp* L.) using molecular markers and agronomical traits as a source of new germplasm

Оценка на местни форми и сортове фасул (*Phaseolus spp* L.) с помощта на молекулярни маркери, и по агрономически признаци, като източник на нова зародишна плазма

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ABSTRACT

The conservation and research of genetic resources of key crops and their use in the global farming system present the main trends for developing sustainable agriculture and producing healthy food products. This study aims to investigate the available genetic resources of (*Phaseolus* spp. L.) landraces and breeding lines/varieties to identify promising genotypes with a view to their further use for breeding as a source of new germplasm. Investigation and evaluation of 18 accessions of *Phaseolus* spp. L. from Bulgaria for economically important agronomic traits and molecular approaches using ISSR and SCAR marker systems were carried out. The genetic characterization by seven SCAR markers was applied to the selected genotypes to determine the genes associated with the resistance to economically important diseases. The population structure of *Phaseolus* spp. L. was determined using the grouping method based on Bayesian models based on six ISSR markers and STRUCTURE software. Based on a comparison between the ISSR molecular marker and descriptors results and the higher Q value, the no admixture ancestry model and correlated allele frequencies were the parameters combination selected for describing the population structure of the selected genotypes. The correspondence between the ISSR-based analysis and the descriptors showed that 14 (77.78%) of the 18 *Phaseolus* accessions groups were in the same cluster in both methods. Promising genotypes have been identified based on the applied integrated research approach - phenotypic and genotypic with the potential for integrating into breeding programs as a source of new germplasm of *Phaseolus* spp. L.

Keywords: bean varieties, SCAR, ISSR marker, population structure, integrated approach

АБСТРАКТ

Съхраняването и изследването на генетичните ресурси на основните култури и използването им в световната система на земеделие са основните насоки за развитие на устойчиво земеделие и производство на здравословни хранителни продукти. Целта на това проучване е да се изследват наличните генетични ресурси на местни сортове и селекционни линии/сортове (*Phaseolus* spp. L.), за да се идентифицират обещаващи генотипове с оглед на по-нататъшното им използване за селекция като източник на нова зародишна плазма. Проведено е изследване и оценка на 18 образци *Phaseolus* spp. L. от България за икономически важни агрономически признаци и

молекулярни подходи с използване на ISSR и SCAR маркерни системи. Генетичното характеризиране чрез седем SCAR маркера беше приложено към избраните генотипи, за да се определят гените, свързани с устойчивостта към икономически важни болести. Структурата на популациите на *Phaseolus* spp. L. беше определена с помощта на метода на групиране, въз основа на шест ISSR маркера и софтуера STRUCTURE. Въз основа на сравнението между резултатите от ISSR молекулярните маркери и дескрипторите и по-високата стойност на Q, моделът без примеси на предците и корелираните алелни честоти бяха комбинацията от параметри, избрана за описване на популационната структура на избраните генотипове. Съответствието между анализа, базиран на ISSR, и дескрипторите показва, че 14 (77,78 %) от 18-те групи *Phaseolus* са в един и същ клъстер и при двата метода. Въз основа на приложения интегриран изследователски подход - фенотипен и генотипен, са идентифицирани обещаващи генотипове с потенциал за включване в селекционни програми като източник на нова зародишна плазма от *Phaseolus* spp. L.

Ключови думи: сортове/селекционни линии фасул, SCAR, ISSR маркери, структура на популацията, интегриран подход

INTRODUCTION

The genus *Phaseolus* is diverse, with over 80 cultivated and wild species, but *P. vulgaris* is the most common (Porch et al., 2013). The closely related species of *P. vulgaris* are *P. albescens*, *P. coccineus*, *P. costaricensis*, *P. dumosus*, *P. parvifolius* and *P. persistentus* (Bitocchi et al., 2017). In addition to *P. vulgaris*, four other species of the genus *Phaseolus* are cultivated: *P. dumosus* (perennial bean), *P. coccineus* (multi-colored bean), *P. acutifolius* (acute bean) and *P. lunatus* (lima bean) (Serrano-Serrano et al., 2010).

It was reported for European *P. vulgaris* germplasm that the main group of European accessions were of Andean origin (~ 68%), with fewer of Mesoamerican origin (~ 27%). The rest of the European accessions represented putative hybrids between these two gene pools (Sinkovič et al., 2019). Nowadays, common beans are showing increasing potential as a source of protein, not only in developing countries but throughout the world. According to a study by Xu et al. (2021), the total amount of CO_2 and equivalent greenhouse gases emitted from global food production is million tons annually. Of this amount, 57% is emitted from animal food production, including crops grown for animal feed. A solution to substantially reduce the impact of the food sector on climate change is to switch to plant protein sources. Common beans and legumes, in general, are highly promising for the necessary changes in humanity's dietary habits to mitigate increasing climate change and avoid mass starvation due to insufficient availability and accessibility of essential nutrients (Duc et al., 2015).

The effectiveness of the acquisition of new genetic variability can be determined by molecular tools, such as microsatellite markers (Šajgalík et al. 2019). DNA-based marker systems have many advantages (Sinha et al. 2023), over traditional plant phenotypic and biochemical markers. The main applications of DNA markers in agronomic research are cultivar identification, genetic purity assessment (Younis et al. 2022), hybrid testing, genetic diversity analysis (Özkan et al., 2022), species genetic map construction, Quantitative Trait Loci (QTL) mapping (Asins, 2002), genetic map-based gene cloning, mutation mapping, Marker Assisted Selection (MAS), marker-assisted backcross selection, marker-assisted pyramiding, major gene mapping, transformant characterization (Lateef, 2015). DNA markers are widely accepted as a tool with great potential for crop improvement (Amiteye, 2021; Hasan et al., 2021; Kage et al., 2016).

The main advantage of ISSRs is that no sequence data for primer construction are needed, low quantities of template DNA are required and furthermore, ISSRs are randomly distributed throughout the genome. Inter Simple Sequence Repeats (ISSR) as a multi locus molecular markers can be used for diverse plant species without the need for genetic sequencing and can be indicated for studies of diversity and genetic mapping of populations (Sehgal et al., 2008).

The Sequence Characterized Amplified Region (SCAR) are locus specific markers and have been applied in gene mapping studies and marker assisted selection. They are highly reproducible and by obtaining a codominant marker may be an additional advantage of converting RAPDs into SCARs, although SCARs may exhibit dominance when one or both primers partially overlap the site of sequence variation (Geetha et al., 2013).The present study assessed the population structure of 18 genotypes of *Phaseolus* spp. from Bulgaria based on important agronomical traits and molecular characterization via Inter Simple Sequence Repeats (ISSR) and Sequence Characterized Amplified Region (SCAR) marker systems.

MATERIAL AND METHODS

Plant material

In the present study, 18 genotypes, part of the available collection of the ethyl methane sulphonate (EMS) mutant lines from the Agricultural University-Plovdiv of *Phaseolus vulgaris* seed collection (Svetleva et al., 2012), and local *Phaseolus* spp. accessions from Bulgaria were selected based on morphological field observations and preliminary physiological and molecular data (Table 1). Seeds of landraces of *Phaseolus* spp. were collected from small gardens of local growers in rural areas and with more than five years of individual selection from the country's central, southeastern, and western parts. Plovdiv 10, Abritus, and Skitiya were included as reference cultivars for distinctness, uniformity, and stability test. Plants were grown on alluvium soil, with an altitude of 165 m without supplemental irrigation conditions on the University of Agriculture - Plovdiv experimental field.

DNA isolation

Genomic DNA was isolated from the last young, fully developed leaf of the plants selected and tagged for the study. Three hundred milligrams of leaf material from each genotype were sheared after freezing in liquid nitrogen to obtain a fine light green powder. The innuPREP plant DNA extraction kit (Analytik Jena AG, Jena, Germany) was chosen for DNA extraction (Morgenstern et al., 2020). The manufacturer's recommendations for protocol implementation were followed. The final volume of diluted DNA was 50 µl and was stored at -20 °C until use. The Epoch microplate spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used to examine the quality and quantity of DNA.

ISSR amplification

The primers used to perform the ISSR analysis (Table 2) were selected from a set of primers that have shown high levels of reproducibility and potential for polymorphism identification in previous studies (Muthusamy et al., 2008). ISSR PCR reactions were performed in a reaction volume of 25 µl, using for each reaction: ISSR primer - 1.5 µl; 12.5 µl Master Mix My Taq Red Mix (Bio line), H_2O -10 µl; 1 µl genomic DNA. ISSR PCR reactions were run under the following amplification regime: denaturation at 94 °C for 3 min, 40 cycles at 94 °C - 1 min, primer melting temperature - 45 sec, extension 72 at °C - 45 sec, followed by a final extension at 72 °C - 5 min.

Table 2 presents ISSR polymorphic primer sequences used to analyze 18 accessions of *Phaseolus* spp. with primer annealing temperatures, amplified bands, and amplified polymorphic bands. Only distinct, reproducible, well-resolved fragments, ranging from 200 to 3000 bp, were scored as present (1) or absent (0) for each ISSR marker with the 18 analyzed samples. The number of amplified bands, number, percentage and size range of polymorphic bands was evaluated.

SCAR analysis

A set of SCAR markers (Table 3) was used to identify the potential presence of a locus for tolerance to certain pathogens in the samples tested. By using specific primers, PCR amplification, and subsequent visualization of the results, observations were made by which we could assess whether a genotype tested had the required locus in its genome.

Population structure based on Bayesian models with ISSR

The population structure of 18 selected genotypes and landraces of *Phaseolus* spp. from Bulgaria (1, 8, 11, 15, 17, 18, 22, 23, 26, 29, 30, 32, 33, 34, 38, 39, 55a and

Table 1. List of *Phaseolus* spp. Bulgarian landraces and cultivars selected for analysis

Accssesion number	Origin	Name (local) Growth habit	Seed type, color (pattern)
1	CV (Cultivar)	Plovdiv 10 (P10) – B[1]	Round to elliptical. One color - white. Slight to medium veining.
8	CV Individual selection from the local population	Dobrudzhansky 2 - (0,025) - B	Elliptical. One color - white. Strongly pronounced veining.
11	CV Individual selection from local population	Dobrudzhansky 2 - (0,0062) - B	Round to elliptic. One color - white. Slight to medium veining.
15	CV Individual selection from local population	Dobrudzhansky 7 - (0,025) - B	Round to elliptical. One color - white. Slight to moderate veining
17	Cultivar	Abritus - B	Round to elliptical. One color - white. Weakly expressed veining.
18	Cultivar	Skitiya – B	Round to elliptical. One color - white. Weakly expressed veining.
22	Landrace	Granit Village – B	Elliptical. More than two colors- white, black, red, and violet. Varied distribution. Faint veining.
23	Landrace	Granit Village – C	Flat – Purple-white variegated
26	Landrace	Granit Village – B	Elliptical. One color - white. Weakly expressed veining.
29	Landrace	Raikin(yellow) –B	Elliptical. One color - white. Slight to medium veining.
30	Landrace	Redbelly - B	Elliptical. Main color - white. Secondary color - red around the hilum. Slight to medium veining.
32	Landrace	Mastilen – Vetren dol -B	Round to elliptical. Main color - beige. Secondary color – violet all over the seed. Weakly veined.
33	Landrace	Mastilev Kremenski – B	Round. Two colors. Main color - beige. Secondary color - brown.
34	Landrace	Eliderski – Vetren dol - B	Elliptical. Main colour - beige. Secondary colour - black. Slightly marked veining.
38	Landrace	Tsarski – Dolnoslav – B	Elliptical. One color - greenish. Slight to medium veining.
39	Landrace	Zaharski – Vetren dol - B	Elliptical. One color - white. Slight to medium veining.
55a	Landrace	Smilyanski – C1	Oval- flat. Two colors. Violet –black Slight to medium veining.
55b	Landrace	Smilyanski – C	Oval- flat. One color – white. Slight to medium veining.

[1] Growth habit – B – bush; C – climbing.

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Table 2. ISSR polymorphism information content (PIC) values of ISSR primers used for analysis of *Phaseolus* spp. with primer annealing temperatures, amplified bands, and polymorphic profiles

ISSR primer	Primer sequence 5' - 3'	Annealing temperature (°C)	Bands amplified	Polymorphic bands	Polymorphic band (%)
ISSR Ph7	AC(8)YG	53.9	8	5	62.5
ISSR Ph14	AG(8)YT	51.4	8	6	75
ISSR Ph11	GA(8)YC	53.9	11	8	73
ISSR Ph8	AC(8)G	52	7	5	71.4
ISSR PhE7	AC(8)CTG	55.4	10	7	70
ISSR PhE9	AG(8)C	55.4	9	6	67

* Cytosine / Thymine (pyrimidine).

Table 3. SCAR markers linked with disease resistance traits in common bean (*Phaseolus vulgaris*) and PCR condition

SCAR name	Pathogen	Product\ orientation	PCR protocol	Locus	Sequence of SCAR (5' -3')	Reference
SAP6	Common Bacterial Blight (CBB)	820 cis	34 cycles of 10s at 94 °C , 40s at 55 °C , and 120s at 72 °C; followed by one cycle of 5 minutes at 72 °C	QTL (GN#1 27)	GTC ACG TCT CCT TAA TAG TA GTC ACG TCT CAA TAG GCA AA	Miklas et al., 2000
KB126	Rust	405 / 430 Codomiant	1 cycle 94 °C for 5 min; 35 cycles at 94 °C 1 min, 45 °C 1 min and 72 °C for 1 min, final elongation step of 5 min at 72 °C	Ur-13	GAA TTC AAC CTC GGC CAC TAC C TTA AAC CTT CCG GAG GAT TC	Mienie et al., 2005
SF18R7	White mold	410/41 Codmiant	94 °C for 2 min, 35 cycles of 94 °C for 60s, 45 °C for 40s, and 72 °C for 40s followed by 1 cycle at 72 °C for 2 min	QTL WM7.3I9365-31	ACC GTA CGA ATT TGC TTA AGT G GAT CCA GTT ACC GGA AT	Soule et al., 2011
SQ4	Anthracnose & Rust	1440	34 cycles of 10s at 94 °C; 40s at 59 °C, 2 min at 72 °C, followed by one cycle of 5 min at 72 °C	*Co-2, Ur-11*	CCT TAG GTA TGG TGG GAA ACG A TGA GGG CGA GGA TTT CAG CAA GTT	Awale et al., 2008; Young and Kelly, 1996
SU20	Fusarium wilt	750	30 cycles of 60s at 94 °C, 30s at 70 °C and 60s at 72 °C	A55	ACA GCC CCC ATT GTG AAT TGT AT ACA GCC CCC ACA CTT ATG GCA	Brick et al., 2006; Fall et al., 2001
SH13	Angular leaf spot ALS (*Phaeoisariopsi*)	520 cis	35 cycles of 30s at 94 °C, 60s at 59 °C, and 90s at 72 °C	*Phg-1*	GAC GCC ACA CCC ATT ATG TT GCC ACA CAG ATG GAG CTT TA	Queiroz et al., 2004
SR2	Bean Golden Yellow Mosaic Virus (BGYMV)	530 / 570 Codominant	34 cycles of 10s at 94 °C, 40s at 60 °C, and 120s at 72 °C; one cycle of 5 minutes at 72 °C, 60 °C codominant; 65 °C = dominant	*bgm-1*	CAC AGC TGC CCT AAC AAA AT CAC AGC TGC CAC AGG TGG GA	Blair et al., 2007

55b) was determined by utilizing the grouping method based on Bayesian models implemented in the software STRUCTURE version 2.3.4 (Pritchard et al., 2000), based on data from six ISSR markers (Ph11, Ph8, Ph14, Ph7, Ph9E, and Ph7E). Each individual was assigned to the group with the highest membership coefficient (Q). This coefficient represents for each genetic group the proportion of the molecular markers analyzed inherited from their ancestors. The sum in each plant of the coefficient of belonging to all the groups equals one (Rosenberg, 2004).

Performed 10 independent runs with 100 000 iterations for the initial burn-in followed by 200 000 iterations. The run with the highest estimated Ln Pr(X/K) was selected. Four combinations of parameters for the software STRUCTURE were tested (Table 4).The most probable number of groups (K) was determined using the graphical methods of Evanno, Regnaut, Goudet (2005).

Table 4. Combinations of parameters in the software STRUCTURE

Number	Ancestry model	Alleles frequencies
1	Admixture	Correlated
2	Admixture	Independent
3	No admixture	Correlated
4	No admixture	Independent

Cluster analysis based on morphological descriptors

To compare Bayesian model results based on ISSR with morphological data, a distance matrix based on the Gower similarity coefficient (Gower, 1971) was calculated with R package StatMatch 1.4.1 (D'Orazio, 2012). This distance matrix was used to generate a dendrogram based on Ward method as implemented on R statistical language (R Core Team, 2023).

RESULTS

Throughout the vegetation and development of the plants, direct visual observations during the different growing stages and biometric, protein and fat determination analyses were performed to obtain an accurate phenotypic picture (Table 5). An officially approved descriptive protocol from the European Plant Variety Office - CPVO (Protocol for Distinctness, Uniformity and Stability Tests, *Phaseolus vulgaris* L., valid from 12/03/2009) was used to evaluate the characteristics of the studied forms.

The six ISSR primers amplified a total of 53 bands in the set of 18 bean genotypes, of which 37 bands (70%) were polymorphic. PCR amplification performed with ISSR markers showed a high percentage of polymorphic fragments. The size range of the amplified products was between 300 and 4000 bp.

The 7 SCAR primers were used to evaluate the *Phaseolus* spp. L. germplasm accessions for some economically important diseases: SAP6 (Common Bacterial Blight (CBB), KB126 (Rust), SF18R7 (White mold), SQ4 (Anthracnose & Rust), SU20 (Fusarium wilt), SH13 (Angular leaf spot ALS (*Phaeoisariopsi*)), and SR2 (Bean Golden Yellow Mosaic Virus (BGYMV)) via PCR. The presence of a band indicates the amplification of the corresponding resistance gene.

Selection of the most probable number of clusters for Bayesian clustering with software STRUCTURE version 2.3.4 with no admixture ancestry model and independent alleles frequencies of 18 *Phaseolus* spp. landraces from Bulgaria based on six ISSR markers, using the graphical methods of Evanno, Regnaut, and Goudet (2005) were applied.

Table 6 presents the positive (+) or negative (-) results obtained when testing 18 genotypes and describes the presence or absence of the locus of interest. The most probable number of clusters for the four combinations of parameters in the software STRUCTURE is 2.

Table 5. Analyzed 18 *Phaseolus* spp. genotypes by biometric data, protein and fat content

Landrace	W 100 seeds (g)	First pod position (sm)	Pod length (sm)	Protein content %	Fat content (%)
1	39	15	8.4	23.42	1
8	38	6.9	9	25.66	1.2
11	36	6.7	9.6	26.59	1.1
15	35	6.2	9	23.55	0.8
17	20	15	9	27.96	1.2
18	35	32	13	20	0.9
22	37	23	9	23.55	1
23	140	12	12.5	23.17	1.1
26	36	23	9.5	25.66	1.4
29	33	7.1	10.5	24.75	0.6
30	40	21	11.5	25	0.4
32	41	12	12	24.37	0.4
33	21	14	8.5	29.08	1.8
34	24	17	15	28.27	0.5
38	30	22	12	24.61	0.5
39	33.5	9.8	9.7	29.76	1.1
55a	98	23	13.3	24.54	1
55b	81	23	13.8	22.75	1.1

Table 6. Analyzed 18 *Phaseolus* spp. genotypes by SCAR markers linked with disease resistance traits in common bean (*Phaseolus vulgaris*)

№	SCAR						
	SAP6	KB126	SF18R7	SQ4	SU20	SH13	SR2
1	-	+	+	+	+	+	+
8	-	+	+	+	-	+	+
11	-	+	+	-	-	+	+
15	-	-	+	-	+	+	+
17	-	+	+	+	+	+	+
18	-	+	+	+	-	+	+
22	-	+	+	+	-	+	+
23	-	+	-	+	-	+	-
26	+	+	+	+	+	+	+
29	-	+	+	+	+	+	+
30	+	+	+	+	+	+	+
32	+	+	+	+	-	-	+
33	-	+	+	+	-	+	+
34	+	+	+	+	-	+	+
38	+	+	+	+	-	+	+
39	-	+	+	+	-	+	-
55a	+	-	-	-	-	-	+
55b	+	+	-	+	-	+	+

In each figure, the upper and left plot was the proposed method by Pritchard, Stephens and Donnelly (2000) based on the selection of the K when the curve was more or less plateau. An important difference between K = 1 and K = 2 indicates that K = 2 was the more probable number of clusters. The second and third plots were steps to the last plot (bottom right), which is the most important for the Evanno, Regnaut y Goudet (2005) method. In this plot, there was a difference between K = 2 and the other K, indicating that this was the most probable number of clusters. This difference was weaker for the admixture ancestry and correlated alleles frequency models (Figure 1).

Based on these results, the 18 *Phaseolus* spp. genotypes were assigned to the two clusters (Figure 2).

There were two assignments for *Phaseolus vulgaris* genotypes:

- For the correlated allele frequencies (11 accessions for cluster 2 and 7 accessions for cluster 1),
- For the independent allele frequencies (5 accessions for cluster 2 and 13 accessions for cluster 1).

The value of Q was higher than 0.9 in 64 (88.88 %) of the combinations of landraces and parameters for the software STRUCTURE. The higher Q values were in the no admixture ancestry model, and correlated allele frequencies varied from 0.9900 to 1.000. Selecting one of the combinations of parameters of STRUCTURE software depends on the biological, geographical, or other characteristics of *Phaseolus* spp. genotypes.

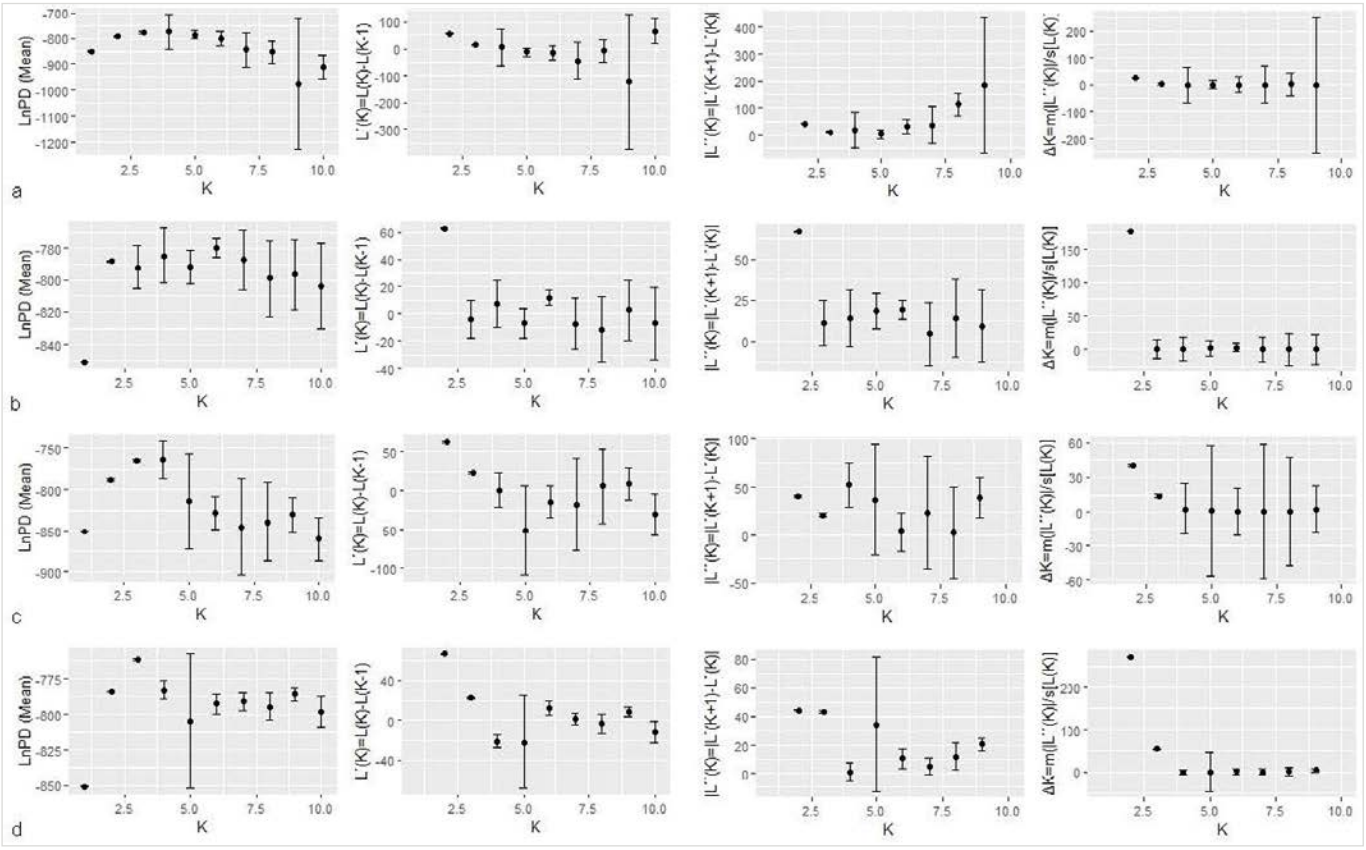

Figure 1. Selection of the most probable number of clusters for Bayesian clustering with software STRUCTURE version 2.3.4 by Evanno, Regnaut y Goudet (2005). Combination of parameters in software STRUCTURE a: admixture ancestry model and correlated alleles frequencies, b: admixture ancestry model and independent alleles frequencies, c: no admixture ancestry model and correlated alleles frequencies, d: no admixture ancestry model and independent alleles frequencies.

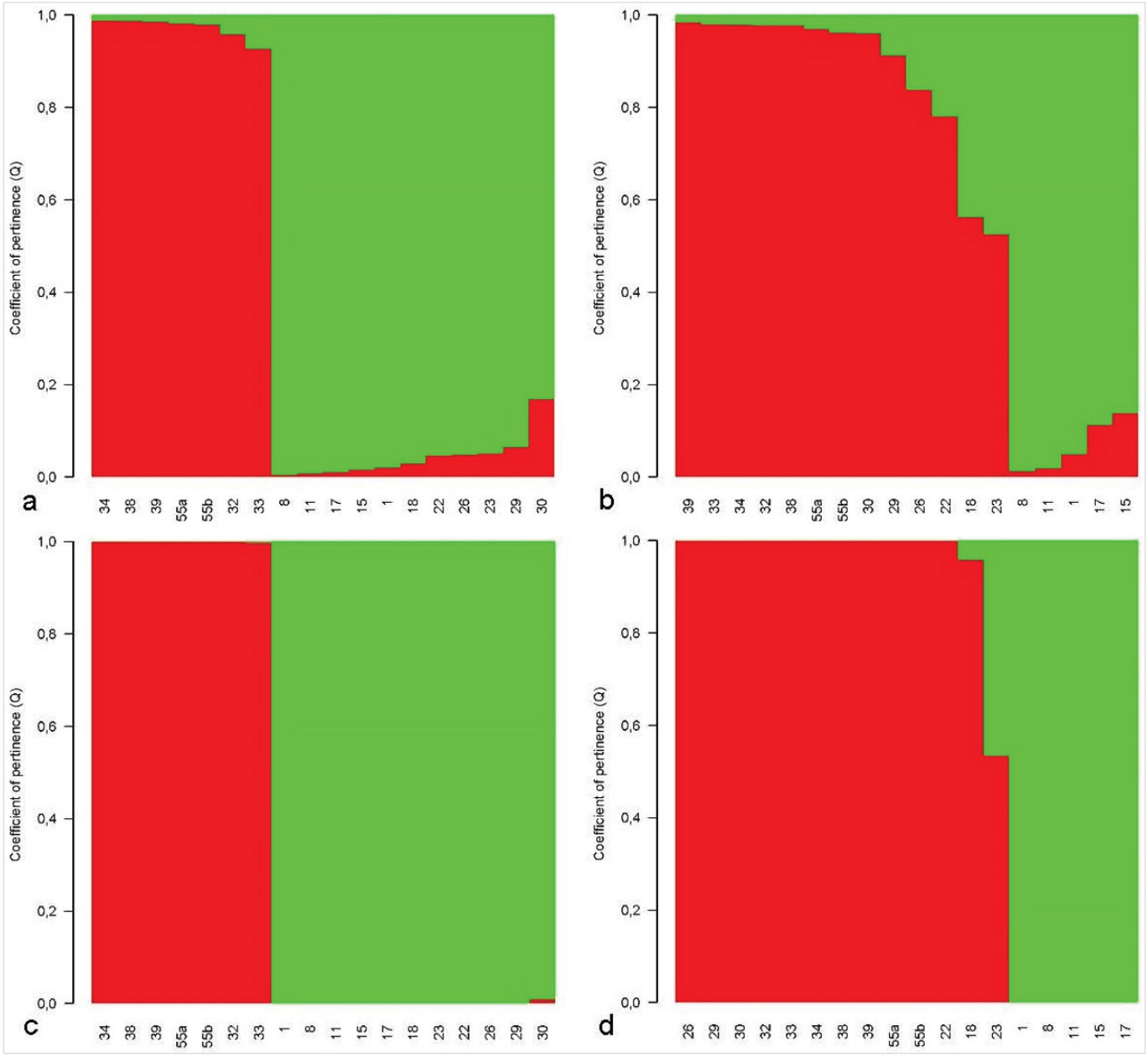

Figure 2. Population structure of 18 *Phaseolus vulgaris* landraces from Bulgari based on six ISSR markers. Combination of parameters in software STRUCTURE a: admixture ancestry model and correlated alleles frequencies, b: admixture ancestry model and independent alleles frequencies, c: no admixture ancestry model and correlated alleles frequencies, d: no admixture ancestry model and independent alleles frequencies.

The morphological descriptors were organized as shown in Table 7. The dendrogram in Figure 3 indicates the presence of two groups for the 18 *Phaseolus* spp. genotypes from Bulgaria.

Table 7 shows 14 (77.78 %) of the 18 *Phaseolus* landrace groups in the same cluster for both methods. This result indicates the correspondence between the analysis based on ISSR and morphological descriptors.

Table 7. Comparison of pertinence to groups of the 18 *Phaseous landraces* from Bulgaria based on Bayesian implemented in software STRUCTURE and dendrogram based on Ward method and Gower distance cluster methods

Landrace	Bayesian cluster (Correlated frequencies)	Dendrogram cluster	Different cluster
1	2	2	
8	2	2	
11	2	2	
15	2	2	
17	2	2	
18	2	2	
22	2	1	*
23	2	2	
26	2	2	
29	2	2	
30	2	2	
32	1	1	
33	1	1	
34	1	1	
38	1	2	*
39	1	2	*
55a	1	1	
55b	1	2	*

Nowadays, common beans are showing increasing potential as a source of protein, not only in developing countries but throughout the world. According to a study by Xu et al., 2021, the total amount of CO_2 and equivalent greenhouse gases emitted from food production worldwide is 17,318 ± 1,675 million tons annually. Of this amount, 57% is estimated from animal food production, including crops grown for animal feed. A solution to substantially reduce the impact of the food sector on climate change is to switch to plant protein sources. In this context, common beans and legumes, in general, are highly promising for the necessary changes in humanity's dietary habits to mitigate increasing climate change and avoid mass starvation due to insufficient availability and accessibility of essential nutrients. Thanks to intensive breeding efforts, common bean varieties with a wide range of morphological and agronomic characteristics have been developed.

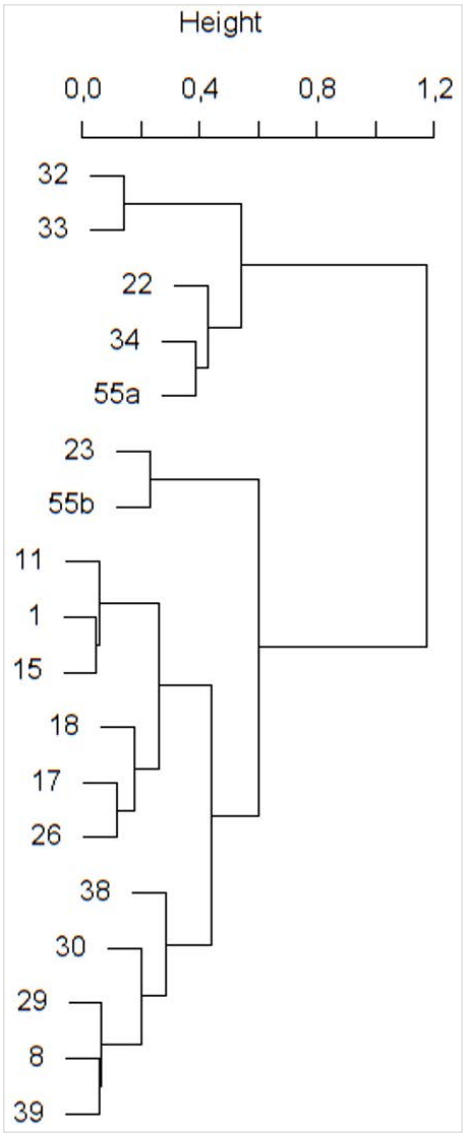

Figure 3. Dendrogram based on Ward method and Gower distance of 18 *Phaseolus* spp. genotypes from Bulgaria based on morphological descriptors

DISCUSSION

The better varieties resulting from successful breeding have gradually replaced the old populations of primitive forms and landraces in the regions where diversity was higher. Farmers are adopting these new varieties because of their higher yields and or their resistance to economically important pathogens, and therefore the exceptional diversity of the various ancient landraces is gradually diminishing, and genetic resources are becoming limited. With a wide range of genetically diverse plants, breeders can hope to solve the problems of increased yield, higher nutritional value, better adaptability, and better disease resistance. The need for new and better varieties can only be met with a large genetic base of plant diversity. Continued reduction in crop genetic diversity disrupts agroecosystem functions and the conduct and flow of various activities and processes (disease and pest control, pollination, soil processes, total biomass cover, carbon sequestration, and soil erosion prevention) as well as potential innovations in sustainable (ecological, organic) agriculture (Ulukan, 2011).

Molecular marker-based polymorphisms found among cultivated accessions suggest that there may have been at least two independent centers of origin in Central and South America (Bitocchi et al., 2013), resulting in the distinct centers of genetic diversity of Central America and the Andes (Angioi et al., 2010). Some evidence suggests that these two centers had already diverged before cultivation attempts began (Salinas et al., 1988). South American bean types tend to have larger seeds and leaves than those from Central America (Wortmann, 2006).

A cluster analysis outlined the relationships among the genotypes studied. Lei et al. (2020) investigated the genetic structure of four seed traits of common beans in China (weight per 100 seeds, seed length, and seed width and height). They performed marker-trait association in a national population of 395 common bean accessions using 116 polymorphic SSR markers. Lei et al. (2020) estimated population structure based on SSR markers and phaseolin, dividing the samples into two major subpopulations representing the two known gene pools. Seed weight and seed size were found to correlate strongly with population clustering. Cabral et al., (2018) evaluated the genetic diversity of 57 common bean genotypes using ISSR markers. They used 11 primers that generated 51 fragments, of which 76% were polymorphic. The data obtained indicate a high genetic variability. Cluster analysis showed the formation of 11 groups, with a tendency to cluster genotypes by region of origin and growth habit. The results also suggest that ISSR markers are efficient for quantifying the genetic

diversity of genotypes, and polymorphic markers can help select candidates for storage in global gene banks (Aziz et al., 2022).

To assess the heterogeneity of each genotype studied, a set of ISSR primers was used and all 18 genotypes were analyzed. Individual plants of each genotype were examined to confirm the ability of the selected marker system to detect a sufficient number of polymorphisms. After initial screening of the primers, it was found that some of them did not result in amplified fragments. These results may be due to the absence or low number of the corresponding microsatellite sequence in the genome of the test samples. Running the reactions with the selected primers resulted in one or more polymorphic fragments for each sample. Six ISSR markers were selected as highly reproducible, detecting high levels of polymorphism with up to 75% polymorphic bands among the selected genotypes.

The results showed that the ISSR markers tested provided adequate polymorphism and reproducible fingerprinting profiles to evaluate the genetic diversity of bean genotypes. The molecular diversity evaluated in this study, in combination with the agronomic and morphological characteristics of *Phaseolus* spp. genotypes may be valuable in conventional and molecular breeding programs.

SCAR markers, are also widely used in MAS (Park et al., 2008; Subbareddy et al., 2012). Mienie et al. (2005) developed three potentially useful SCAR markers for the Ur-13 locus associated with rust tolerance (*Uromyces appendiculatus*): KB126, KB85, and KB4 Hha I. KB126 and KB85 are codominant markers of insertion and deletion, and the Cleaved amplified polymorphic sequence (CAPS) marker KB4 Hha I can also distinguish between homo- and heterozygotes. O'Boyle et al. (2007) used the SCAR markers SU91 and BC420 for indirect selection of resistance to bacterial blight of beans (*Xanthomonas axonopodis pv. phaseoli* - Xap). Their results indicate that the use of MAS will not eliminate the need for direct phenotypic selection for Xap resistance, but on the other hand, MAS can be used to reduce the number of lines to be monitored for resistance. In the present work, the genotypes tested describe a mixed picture of positive and negative results, like some of them give positive results for all loci of interest, such as 26 and 30, which are distinguished for inclusion in breeding programs. The contribution of QTL associated with these molecular markers has been confirmed in a breeding population with desirable agronomic traits (Diaz et al., 2018). A major problem for the cultivation of common beans is the rapidly changing climatic conditions, which seriously compromise yields. A possible solution will be increasing breeding and improvement activities to create high-yielding bean varieties resistant to the main diseases and pests and well adapted to drought and high day and night temperatures. This goal can only be achieved through the integration of molecular methods in breeding - marker-assisted selection (MAS) (Martínez-Nieto et al., 2020). Finding specific loci associated with potential tolerance to different biotic and abiotic stresses is the main method allowing accelerating the selection of improved bean forms.

Considering all this information, beans are a promising grain legume that can satisfy the protein needs of a large part of the population. In this relation, reducing the production and consumption of many animal products, becoming now the largest source of greenhouse gases in the food industry, will be possible.

CONCLUSION

Landraces and obsolete varieties not yet included in collections or breeding programmes account for a significant proportion of crop genetic diversity. The collection, study, systematization and establishment of germplasm for breeding purposes is particularly important. Advances in bean breeding and improvement are mainly due to the use of proper approaches and methods in studying collections of plant genetic resources. Based on this comparison between ISSR results of molecular markers and morphological descriptors and the higher value of Q, the ancestral admixture-free model and correlated allele frequencies are the combination of parameters chosen to describe the population structure

of 18 selected genotypes of *Phaseolus* spp. from Bulgaria. The results of this study indicate that the landraces are significant sources of new germplasm and will serve as a basis for future research related to the conservation of genetic diversity of landraces and introduced *Phaseolus* spp.

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