

ASSESSMENT OF GENETIC DIVERSITY OF PHASEOLUS GERMPLASM BY MORPHOLOGICAL QUANTITATIVE CHARACTERS

Valbona HOB DARI, Belul GIXHARI, Fetah ELEZI, Doriana BODE and Hajdar KUÇI
Institute of Plant Genetic Resources, Agricultural University of Tirana, Albania

ABSTRACT

A study was carried out for the characterisation and pre-evaluation of genetic diversity present in the *Phaseolus* germplasm stored in Albanian Gene Bank and 50 local common bean accessions of different origins were involved to identify and select the most important accessions with favourable characteristics for use in breeding programs. The study was carried out at the Experimental Field of Agriculture University of Tirana during two subsequent growing seasons, 2017 and 2018. ANOVA analysis and Tukey-Kramer test found significant differences for most of the quantitative morphological characters. PCA results showed that the first three PCs contribute for a substantial proportion of total variation, 77.8%. The percentages of total variation accounted for by each of the first three PCs were 54.2%; 13.0% and 10.6%, respectively. PCA and ordination analysis identified the quantitative morphological characters with more significant contribution on respective PC1 and PC2 variances (PoL, SeL, SeY, PLH, PoW and SeW characters). Principal Component Analysis (PCA) and Dimensional Ordination method revealed considerable amount of diversity between common beans, identified the variances of the first three principal components (77.8%) and the proportion of the total variance each factor contributes for. Principal Component Analysis, in respect of genetic variability among 50 common bean accessions of different origin, divide these accessions into four groups. The amount of genetic variability found between common bean genotypes is enough for the selection of desirable characters valuable for common bean breeding programs and facilitates the maintenance of its germplasm in Gene Bank.

Keywords: Genetic diversity, *Phaseolus* germplasm, morphological characters

1. INTRODUCTION

Common beans are the main source of protein in human nutrition in many parts of the world. Thousands of legume species exist, but common beans (*Phaseolus vulgaris* L.) are from far the most consumed. Common bean (*Phaseolus vulgaris* L.) is a widely cultivated crop due to its good nutritional composition (high protein content in dry seed and a good source of fiber) besides its high market value. This vegetable is consumed either as a dry bean (pulse) or as fresh bean (fresh vegetable) and it is considered one of the most important grains for human alimentation. In some countries, beans are the primary source of protein in human diet.

Common bean (*Phaseolus vulgaris* L.) originates in Latin America and was domesticated in Mexico more than 6000 years ago. It spread from Latin America to Europe, Africa and other parts of the world. It grows well in areas of medium rainfall from the tropics to the temperate regions. Genus *Phaseolus* counts a total of 56 species. All species of this genus are with $2n = 22$ chromosomes, except for a single species which possesses $2n = 20$ chromosomes.

The conservation, study and uses of local plant resources is a basic problem for breeding programs in many countries. During conservation of germplasm the seeds undergo quantitative and qualitative changes. For preserving genetic diversity, characterization of bean accessions allows the identification of genetic variability present on bean germplasm, as it helps describe the differences between accessions and address breeding programs (Gixhari *et al.*, 2016). This is particularly crucial today, due to the increasing influence of different anthropogenic factors that can affect the genetic diversity of various valuable species (Krasteva *et al.*, 2002).

In Albania, the bean (*Phaseolus vulgaris*) is one of the most important crops. Actually, over 90% of bean cultivation area is cultivated with native bean landraces (Canko *et al.*, 2008). In Albania, beans germplasm represents the important genetic resources used directly by farmers and consumers.

The Albanian Gene Bank, part of the Agriculture University of Tirana, holds approximately 307 accessions of common bean germplasm. Common bean germplasm is composed especially by local genotypes or landraces. Landraces harbour amount of diversity that is of interest for future breeding work,

as well as for developing new varieties and consequently need to be preserved for future generations (Negri and Tosti 2002; Stoilova *et al.*, 2004; Stoilova *et al.*, 2005). Despite its economic importance, bean germplasm has not been characterized very well genetically, and little is known about the nature of the variability of this germplasm. Therefore, the information about extent of uses of various gene pools are extremely valuable for the rational planning of the use of bean germplasm in breeding programs (Gixhari *et al.*, 2016).

Traditionally, germplasm diversity is evaluated by morphological descriptors (Hobdari *et al.*, 2012; Gixhari *et al.*, 2016), which remain the only legitimate marker types accepted by the International Union for the Protection of New Varieties of Plants (UPOV 1990; 2009). Characterization of germplasm collections, based on morphological and agronomic traits, is of high interest for breeding work (Stoilova 2007; Gixhari *et al.*, 2013; Hobdari and Gixhari 2017). In some cases, other traits important for producing typical and high-quality products are also taken in consideration (Pereira *et al.*, 2005; Stoilova 2007). Singh *et al.*, (1991a) and Gixhari *et al.*, (2014) suggested the combination of agro-morphological, biochemical and molecular data for diversity studies since this combination offers complementary results. Albanian Gene Bank has carried out several studies using morphological and agronomic traits, during regeneration and characterization procedures (Hobdari *et al.*, 2012; 2017; Bode *et al.*, 2013; Gixhari *et al.*, 2013; Gixhari *et al.*, 2014), but bean germplasm stored in GenBank has still a considerable number of accessions that need to be analysed and characterized.

The present study aims to analyse the genetic diversity among fifty common bean accessions (genotypes) and the identification of the most important morphological characters for use in breeding programs of bean germplasm.

2. MATERIALS AND METHODS

Plant material: The analysis was performed on fifty common bean accessions (49 bean accessions stored in Gene Bank and one is local bean named “DIBRA” and the data are in Table 1 reported.

Table 1. Accessions of common bean germplasm stored in Gene Bank and included in the study

AGB1284	AGB1298	AGB1315	AGB1323	AGB1338	AGB1345	AGB1366
AGB1291	AGB1302	AGB1316	AGB1324	AGB1339	AGB1346	AGB1367
AGB1293	AGB1304	AGB1318	AGB1325	AGB1340	AGB1347	AGB1368
AGB1294	AGB1305	AGB1319	AGB1326	AGB1341	AGB1348	AGB1369
AGB1295	AGB1308	AGB1320	AGB1327	AGB1342	AGB1349	AGB1370
AGB1296	AGB1310	AGB1321	AGB1329	AGB1343	AGB1361	AGB1379
AGB1297	AGB1314	AGB1322	AGB1337	AGB1344	AGB1365	AGB1386

Morphological characters: Leaflet Length (LL), Plant Height (PLH) (cm), Pod Length (PoL), Pod Width (PoW), Number of Pod per Plant (PoPL), Number of Seeds per Pod (SPo), Seed Length (SeL), Seed Width (SeW) and Seed Yield (SeY).

The experimental site: The study for the assessment of common bean accessions diversity was carried out at the experimental field of Agriculture University of Tirana (latitude: 40°24'05"N; longitude: 019°41'08"E; elevation: 40m) during two subsequent growing seasons; 2017 and 2018.

Field observations: The measurements and field observations were carried out based on Gene Bank standards (FAO 2014), Recognized Reconstruction Guidelines (Salcedo 2008) and Descriptors (IBPGR 1982) for characterization and evaluation of genetic material.

Cultural practices: Sowing date and growing conditions as the distance between plants in a row and between rows, fertilizer application, number of plants established, plant protection, harvest date, etc. were the same for each bean accession and consistent with established farming practices of the area and with the variety used.

Statistical Analysis: The differences between common bean accessions and comparison for all the means of observed and evaluated characters were carried out using ANOVA analysis and Tukey-Kramer test. Principal Components Analysis (PCA) on correlation method was used to analyse relationships between bean genotypes and to identify the most important morphological characters that influence

highly on the total variability. All statistic data were calculated employing the SAS JMP Statistical Discovery (2012), and a dendrogram (ward method) and two-dimensional relationship diagram (phaseolus genotypes x morphological characters) were carried out.

3. RESULTS AND DISCUSSIONS

Analysis of morphological quantitative characters: ANOVA analysis found the presence of significant differences between common bean accessions for the greatest number of morphological characters at the probability $P_{0.05}$ and $P_{0.01}$. High degree of variation was observed for PLH, PoPL, PoL and especially for SeY characters. Low differences were found for PoW, SePo and SeW morphological characters (Table 2).

Table 2. Analysis of variance for all chamomile traits

Source of Variance. Traits	Sum of Squares (population) (df=49)	Sum of Squares (Error) (df= 50)	C. Total (df = 99)	Mean Square (population)	Mean Square (Error)	F' Ratio	Prob>F
LL	151.94392	3.78305	155.72697	3.10090	0.07566	40.9841	<.0001**
PLH	2622.6833	7.3854	2630.0687	53.5241	0.1477	362.3670	<.0001**
PoL	336.94194	5.07720	342.01914	6.87637	0.10154	67.7181	<.0001**
PoW	1.292301	0.22475	1.517051	0.026373	0.004495	5.8673	<.0001**
PoPL	2789.6720	8.2308	2797.9028	56.9321	0.1646	345.8490	<.0001**
SePo	26.402400	3.360000	29.762400	0.538824	0.067200	8.0182	<.0001**
SeL	211.43348	6.30185	217.73533	4.31497	0.12604	34.2357	<.0001**
SeW	33.178524	3.672700	36.851224	0.677113	0.073454	9.2182	<.0001**
SeY	9752489	1049258	10801747	199030	20985	9.4843	<.0001**

F ratio values significant at the $P_{0.05}$ level of the probability (*) and $P_{0.01}$ level of the probability (**)

Principal Components Analysis: Principal Components Analysis on correlations identified the variances of the principal components and the proportion of the total variance each factor contributes for. Eigenvalues and percent of variances each factor accounts for are in the Table 3 reported. According to the MINEIGEN criterion (Kaiser 1960) three principal components that account for 77. 8% of the total variation are retained for further analysis.

PCA results show that the major sources of variation in the total variance is given by the first two PCs. All quantitative variables contribute to 100% of total variation. The percentages of total variation accounted for by each of the first three PCs are 54.2%; 13.0% and 10.6%, respectively (Table 3). The first three PCs explain 77.8% of the original variation, and the variation >75% or near to 80.0% is acceptable for characterization and evaluation of plant collections in a Gene Bank (Cadima *et al.*, 2001; Jolliffe 2002).

Table 3. Eigenvalues matrix of principal components (common bean genotypes x morphological characters)

Principal Components/factor analysis						
PC No.	Eigenvalue	Percent variance	Cumulative Percent	χ^2	df	Prob. > χ^2
1	4.8785	54.205	54.205	315.787	36.304	<.0001*
2	1.1710	13.012	67.217	141.925	35.409	<.0001*
3	0.9548	10.608	77.825	113.664	28.468	<.0001*
4	0.7731	8.590	86.415	85.332	22.033	<.0001*

χ^2 – Chi Square, df– degree of freedom; Prob. – probability; *significance level equal to the 0.05 of probability

Complete information was received applying ordination analysis method (Jolliffe 2002). Dimensional scaling of relationships (common bean genotypes x morphological characters) that contributes for the larger proportion of the total variance in PC1, PC2 and PC3 revealed by PCA showed that the

contribution of each bean genotypes and of each quantitative morphological character was found in equal on the total of variation.

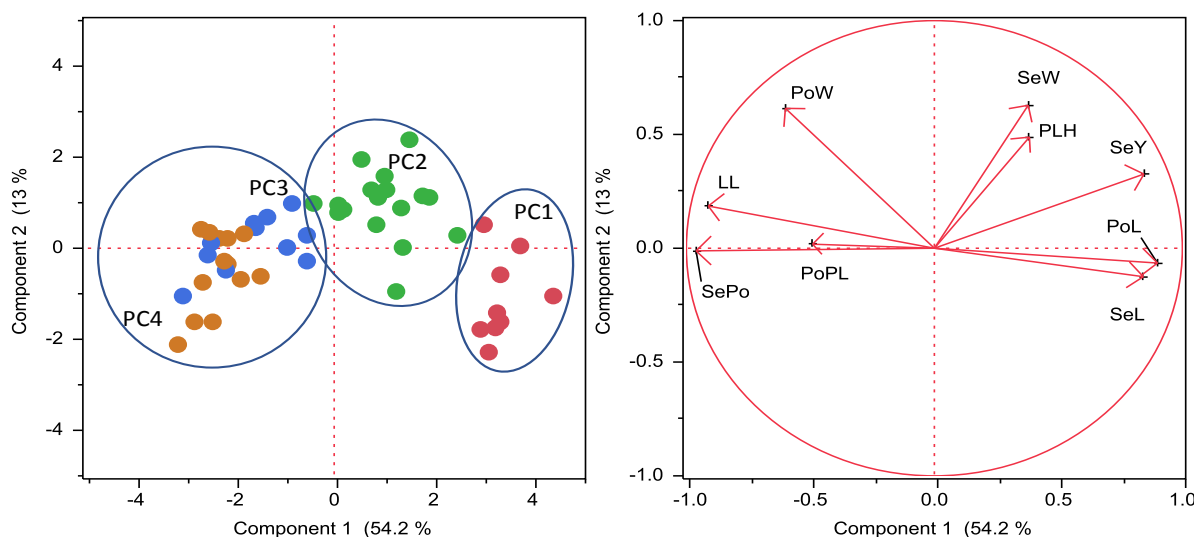


Fig. 1: Relationships among common bean genotypes based on morphological characters revealed by PCA.

PC analysis found 9 and 17 common bean accessions included in PC1 and PC2 that contribute for 67.2% of total variation, and contribution for 19.2% on the total variation was attribute of 24 bean accessions included in PC3 and PC4 (Table 3; Figure 1).

For PC1 (with 54.2% contribute on the total variation) characters as PoL, SeL and SeY were the most important source for the variation of PC1 (Figure 1). Two morphological characters as LL and SePo showed important **negative influence** on the PC1 variance (Table 4, Figure 1).

Variation in PC2 (13.0% of total variation) was mainly the result of differences in PLH, PoW, SeW and SeY morphological characters. SeY characters contributes the part of variation not expressed on PC1 (Table 4, Figure 1).

For PC3 (with only 10.6% contribute on the total variation) characters as PLH and PoPL were the most important source for the variation on the PC3 variance. PLH character contributes the part of variation not expressed on PC1 and PC2 and SeW showed important **negative influence** on the PC3 variance (Table 4, Figure 1).

Table 4. Matrix of vectors of three PC for common bean genotypes x morphological characters

No	Quantitative morphological characters		PC1	PC2	PC3
1	Leaflet Length	LL	-0.41268	0.17135	-0.05907
2	Plant height	PLH	0.17348	0.45097	0.64573
3	Pod Length	PoL	0.40725	-0.06017	0.09272
4	Pod Width	PoW	-0.27196	0.56788	0.00802
5	Pods per Plant	PoPL	-0.22226	0.01640	0.51430
6	Seeds per Pod	SePo	-0.43501	-0.01069	-0.00608
7	Seed Length	SeL	0.38151	-0.11562	-0.00501
8	Seed Width	SeW	0.17122	0.58029	-0.54582
9	Seed Yield	SeY	0.38247	0.30111	0.09165

In bold all eigenvectors > 0.30

The data about genetic relationships between bean genotypes and the most important morphological characters provides better understanding of germplasm sampling. Separation of common bean accessions

(genotypes) into four different PCs, and where the division among bean genotypes included on the PC1 and PC2 was very clear, is useful for the choice of parents for further crosses in breeding programs. This is not simple for the bean genotypes of PC3 and PC4 that both have similar most of the morphological characteristics.

The study identified the morphological characters with more significant weighting on respective PC1 and PC2 variances (PoL, SeL, SeY, PLH, PoW and SeW characters), which can be used successfully as morphological quantitative markers for evaluation and characterization of the bean germplasm.

The amount of genetic variability found in this study is available to the breeders for selection of desirable characters. This variability is a valuable source for creation of new gene combinations in bean breeding programs.

4. CONCLUSIONS

The field trials carried out in the present study helped characterize for the first time fifty common bean genotypes and identify the most important morphological diversity within and between bean genotypes, very useful for bean breeding programs.

PCA results showed that the first three PCs contribute for a substantial proportion of total variation, 77.8%.

The percentages of total variation accounted for by each of the first three PCs were 54.2%; 13.0% and 10.6% respectively.

PCA and ordination analysis identified the quantitative morphological characters with more significant contribution on respective PC1 and PC2 variances (PoL, SeL, SeY, PLH, PoW and SeW characters), which can be used successfully as morphological quantitative markers for evaluation and characterization of the bean germplasm.

The amount of genetic variability found between bean genotypes is sufficient for selection of desirable characters and for creation of new gene combinations to sustain common bean breeding programs.

REFERENCES

- Bode D, Elezi F, Gixhari B. 2013.** Morphological characterization and interrelationships among descriptions in *Phaseolus vulgaris* accessions. *Agriculture and Forestry*. **59(2)**: 175-185, Podgorica.
- Cadima JFCL, Jolliffe IT. 2001.** Variable selection and the interpretation of principal subspaces. *Journal of Agricultural, Biological, and Environmental Statistics*. **6**: 62–79.
- Canko A, Celami A, Shehu R. 2008.** Teknologjia e kultivimit të kultivarëve autokton të fasules. Project: Strengthening sustainable use of plant genetic resources for food and agriculture in Albania. TCP/ALB/3102 (D).
- FAO. 2014.** *Gene bank Standards for Plant Genetic Resources for Food and Agriculture*. Rev. ed. Rome.
- Gixhari B, Doko A, Hobdari V, Vrapı H. 2016.** Diversity of Grass Pea (*L. sativum*) landraces for sustainable field Grass Pea breeding in Albania. *International Journal of Ecosystems and Ecology Sciences (IJEES)*. **6 (1)**: 81-88.
- Gixhari B, Ismaili H, Lashi F, Ibraliu A, Dias S. 2013.** Diversity of Albanian plant genetic resources inventory assessed by eurisco passport descriptors. *Albanian Journal of Agriculture Sciences*. **12 (4)**: 741-746.
- Gixhari B, Pavelkova M, Ismaili H, Vrapı H, Jaupi A, Smýkal P. 2014.** Genetic diversity of Albanian Pea (*Pisum sativum* L.) landraces assessed by morphological traits and molecular markers. *Czech Journal of Genetics and Plant Breeding*. **50 (2)**:177–184.
- Gixhari B, Vrapı H, Hobdari V. 2014.** Morphological characterization of pea (*P. sativum* L.) genotypes stored in Albanian Gene Bank. *Albanian Journal of Agriculture Sciences*. 169-173
- Hobdari V, Bode D, Gixhari B, Elezi E, Doko A. 2017** Morphological characterization of durum wheat (T.durum Desf.) germplasm stored in Albanian Gene Bank. *Albanian Journal of Agricultural Sciences (AJAS), Albanian Journal of Agriculture Sciences. ISSN: 2218-2020. 16. Special issue.* University of Tirana Research paper. 609-615.

- Hobdari V, Gixhari B. 2017.** Analysis of variability in qualitative traits of *L. sativum* accessions in Albanian Gene Bank. *Albanian Journal of Agriculture Sciences*. 565-569.
- Hobdari V, Gixhari B, Elezi E, Bode D, Dias S, Libohova Z. 2012.** Characterization of Grass Pea (*Lathyrus sativum*) Genotypes Stored in Albanian Gene Bank. *Journal of International Environmental Application & Science (JIEAS)*. **7 (4)**: 709-714.
- IBPGR. 1982.** Descriptors for *Phaseolus vulgaris*. IBPGR, International Plant Genetic Resources Institute, Rome.
- Jolliffe IT. 2002.** Principal Component Analysis, Second edition, p.cm-Springer Series in Statistics. UK.143-180.
- Kaiser HF. 1960.** The application of electronic computers to factor analysis. *Educational and Psychological Measurement*. **20**:141-151.
- Krasteva L, Sevov V, Kicheva P, Shamov D, Sabeva M, Neykov S, Popova Z, Lozanov I. 2002.** Local plant genetic resources in Bulgaria *ON-Farm* conservation. Scientific Session of Jubilee, 120 years. Agricultural Science in Sadovo. Institute of Plant Genetic Resources. **I**: 57-63.
- Negri V, Tosti N. 2002.** *Phaseolus* genetic diversity maintained on-farm in Central Italy. *Genetic Resources and Crop Evolution*. **49**: 511-520.
- Salcedo JM. 2008.** Regeneration guidelines: common bean. In: Dulloo ME, Thormann I, Jorge MA. and Hanson J, editors. Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme. Rome, Italy. 9.
- SAS. JMP Statistical Discovery 2012. 10.** SAS Institute Inc.
- Singh SP, Gepts P, Debouck DG. 1991a.** Races of common bean (*Phaseolus vulgaris*, *Fabaceae*). *Economic Botany*. **45**:379-396.
- Stoilova T. 2007.** The collection of dry beans as initial material for breeding. International Research Conference-125 Years Agricultural Research in Sadovo, Plovdiv, June 13-14. Institute of Plant Genetic Resources, Sadovo.**1**: 53-58.11.
- Stoilova T, Pereira G, Tavares-de-Sousa MM, Carnide V. 2005.** Diversity in common bean landraces (*Phaseolus vulgaris* L.) from Bulgaria and Portugal. *Journal of Central European Agriculture*. **6 (4)**: 443-448.
- Stoilova T, Pereira G, Sabeva M, Chavdarov P. 2004.** Study on the phenotypic variability in landraces of dry beans (*Phaseolus vulgaris* L.). *Field Crops Studies*. **I (2)**: 226-233.
- UPOV. 1990.** *Guidelines for the conduct of tests for distinctness, homogeneity, and stability*. Document UPOV TG/4/7. Geneva, Switzerland.
- UPOV. 2009.** International Union for the Protection of New Varieties of Plants. *Guidelines for the conduct of tests for distinctness, uniformity and stability*. (UPOV Code: PISUM_SAT) TG/7/10.