Scientific Papers, UASVM Bucharest, Series A, Vol. LIV, 2011, ISSN 1222-5339

MOLECULAR DIVERSITY OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS

RALUCA FILIMON, B. NECHIFOR, LIZICA SZILAGYI

University of Agronomic Sciences and Veterinary Medicine of Bucharest

Keywords: *Phaseolus vulgaris, RAPD markers, genetic diversity*

Abstract

RAPD markers were used to evaluate genetic diversity among 8 common bean cultivars (Phaseolus vulgaris L.) that included six developed in Romania and two in CIAT Colombia. Only 4 of all the 8 random primers used in RAPD reactions showed polymorphism acceptable for an effective characterization of bean cultivars. These four primers (OPD-08, OPG-03, OPG-12, OPY-20) generated 33 DNA bands, of which 17 bands showing polymorphism (48.84%). On average, each primer generated 8.25 bands, of which 4.25 were polymorphic.

Genetic distances were calculated using Nei&Li (1979) similarity coefficient, displayed in a dendrogram (UPGMA method).Cluster analysis based on RAPD amplification products divided genotypes in two main groups, according to their geographical origin. Genetic similarity vary greatly (60% - 96%), depending on the pairs of genotypes and on the groups.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.; $2n = 2x = 22$) are New World crop with worldwide significance for human nutrition. Bean is a traditional grain legume cultivated and bred in Romania. The crop is consumed principally for its dry (mature) beans, shell beans (seeds at physiological maturity), and green pods. When consumed as seed, beans constitute an important source of dietary protein (22% of seed weight) that complements cereals.

Evidence based on allozymes [7], seed proteins [4]), morphological traits [7]) and DNA markers [6], [1] indicates that two major gene pools exist in cultivated common bean, one Middle American - MA (Mexico, Central America and Brazil) and one Andean South American - AA. Singh et al. (1991a) proposed that within each gene pool three races could be distinguished in plant and seed morphology and adaptation regimes [2]. Analysis of genetic relationships in crop species is an important component of crop improvement programs, as it serves to provide information about genetic diversity, and is a platform for stratified sampling of breeding populations. Polymorphisms represented by differences in DNA sequences. These methods are being used as complementary strategies to traditional approaches for assessment of genetic diversity, the major advantage

being that they analyze the variation at the DNA level itself, excluding all environmental influences. The analysis can be performed at any growth stage using any plant part and it requires only small amounts of material. PCR-based techniques such as Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs, microsatellites) have been used to characterize variability in *Phaseolus* spp.

The objective of this research was to analyze of eight bean cultivars by means of molecular markers (RAPD), in order to quantify and unveil the structure of their genetic diversity.

MATERIAL AND METHODS

Plant material

Eight commercial varieties of common bean (*Phaseolus vulgaris* L.): six developed in Romania and two in CIAT Colombia have been analyzed using RAPD markers (Table 1).

Molecular analysis

1. Isolation of plant DNA

The study was conducted in the greenhouse and Molecular Genetics laboratory from University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania in 2008. The genomic DNA was isolated from young leaves of greenhouse - grown plants according to the CTAB procedure, after [3]. Genomic DNA was isolated from approximately 1 g of fresh leaves of 10 plants of each variety taken for the study.

2. Random Amplified Polymorphic DNA (RAPD) analysis

The 11 primers were used for genetic diversity evaluation of the 8 bean cultivars, but only 4 showed polymorphism (OP-A - 12, OP-G - 03, OP-D-08, OP-Y-20). DNA obtained was amplified by the RAPD procedure with decamer random primers from "Operon Technologies" (California, USA), that identified polymorphisms. Polymerase Chain Reaction (PCR) were achieved in a final volume of 25 µl, containing: 25 ng DNA, 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.4 mM of one primer decamer (Operon Technologies, Alameda, CA, USA) and one unit of Taq DNA polymerase. The amplification was performed using a M.J. Research thermal cycler, programmed for 42 cycles, each consisted of: one denaturation step at 94 ^{0}C for 1 min., one annealing step at 36 ^{0}C for 1 min and one extension step at 72 ^{0}C for 2 min. The extension step in the last cycle was 7 min at 72 °C.

RAPD amplification products were evaluated by electrophoresis on 1.2% agarose gels in 0.5x TBE buffer stained with ethidium bromide.

Table 1

Name, origin, growth habit and seed morphological traits of eight bean commercial cultivars

No.	Cultivars	Origin*	Growth habit**	Seed size***	Seed color	Flower color
1.	**Delia**	Romania - NARDI Fundulea	II	Small	White	White
2.	**Lizica**	Romania - NARDI Fundulea	II	Small	White	White
3	**Florena**	Romania - NARDI Fundulea	II	Small	White	White
4.	**Avans**	Romania - NARDI Fundulea	III	Medium	White	White
5.	**Ami**	Romania - NARDI Fundulea	II	Medium	White	White
6.	**Starter**	Romania - NARDI Fundulea	II	Small	White	White
7	**SEA 5**	Colombia – CIAT	III	Small	Cream	Blue
8	**SEA 13**	Colombia – CIAT	III	Small	Cream	Blue

NARDI - National Agricultural Research and Development Institute; CIAT–International Centre of Agriculture Tropicale;II = indeterminate upright bush;III = indeterminate semi-viney prostrate; *** 100 seeds weight: small seeds, < 25 g; medium, 25-40 g; large, > 40 g.*

3. Statistical analysis

The genetic similarity (Sij) was estimated using the Nei & Li coefficient, by the expression: Sij = 2 Nij / (Ni + Nj), where Nij - the number of bands in common between accessions i and j; Ni and Nj - the number of bands for accession i and j, respectively.

Distance genetics was computed after Nei&Li (1979) formula, using TREECON 1.3 b software package. A cluster analysis was performed using the Unweighted Pair-Group Method using Arithmetic Average (UPGMA) and the dendrogram was obtained in order to visualize the relationship among common bean cultivars.

RESULTS AND DISCUSSION

RAPD analysis

Four decamer random primers were used to differentiate between the eight beans genotypes. A total of 33 bands were amplified in bean genotypes taken in study. Of the 33 total bands, 17 were polymorphic. On the average, each primer amplified 8.25 bands, of which 4.25 were polymorphic. The percentage polymorphic loci varied from 33.33% (OPY-20) to 63.63% (OPD-08) with an average of 48.84% bands/primer (Table 2).

Table 2

RAPD primers used to detect polymorphism, number of bands for polymorphism between bean genotypes per primer

RAPD Primer	Sequence	Number of bands	Number of polymorphic markers	Percentage polymorphism
OP-A – 12	5‘-TCGGCGATAG-3‘	9	5	55.55
OP-G – 03	5‘-GAGCCCTCCA-3‘	7	3	42.85
OP-D-08	5‘-GTGTGCCCCA-3‘	11	7	63.63
OP-Y-20	5‘-AAGCGGCCTC-3‘	6	2	33.33
	Total	33	17	
	Average per primer	8.25	4.25	48.84

Genetic distance and similarity

Pair–wise comparisons between the tested genotypes were used to calculate the genetic similarity.

The lowest value of genetic similarity was recorded among Romanian bean genotypes Lizica, Florena and SEA5 (60%), indicating that these genotypes are highly differentiated genetically (genetic distance = 0.40) (Table 3).

Table 3

Genetic similarity (below diagonal) and genetic distance values (above diagonal) in 8 beans genotypes

Genotype	Delia	Lizica	Florena	Avans	Ami	Starter	SEA 5	SEA 13
Delia	***	0.04	0.04	0.07	0.13	0.09	0.48	0.32
Lizica	96	***	0.04	0.10	0.10	0.12	0.40	0.28
Florena	96	96	***	0.10	0.16	0.06	0.40	0.34
Avans	93	90	90	***	0.07	0.15	0.38	0.32
Ami	87	90	84	93	***	0.15	0.38	0.32
Starter	91	88	94	85	85	***	0.38	0.32
SEA 5	62	60	60	62	62	62	***	0,11
SEA 13	68	72	66	68	68	68	89	***

On the other hand, the higher genetic similarity was recorded among Romanian bean genotypes, Delia and Lizica (96%), Delia and Florena (96%), Lizica and Florena (96%), which shows little genetic distance between these genotypes (0.04). The high similarity found among these bean genotypes indicates that genetic diversity between them is narrow and due to their common origin in the breeding program. Similar results were reported by Szilagyi al all. [5] concerning high similarity between Romanian genotypes.

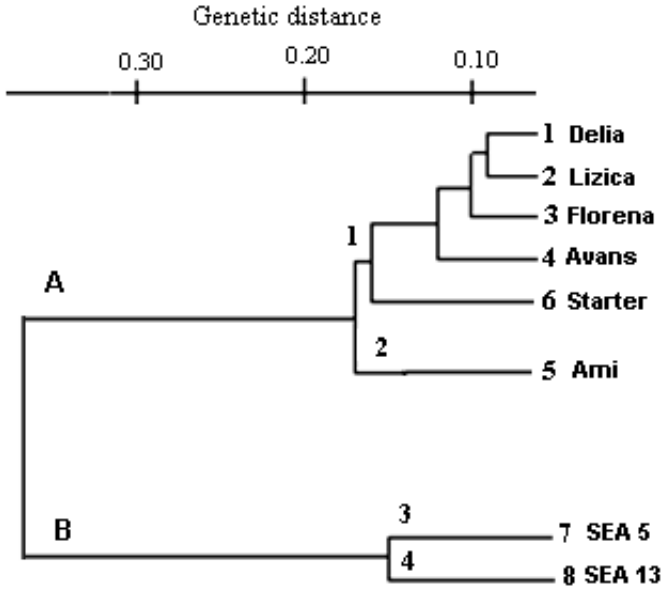

Fig. 1. Dendrogram of eight common bean genotypes based on RAPD data using UPGMA

Cluster analysis

The clustering obtained by UPGMA method is shown in Figure 1. The dendrogram divided the eight common bean cultivars in two **main clusters (A and B)**, according to their geographical origin.

Cluster A comprise all Romanian bean cultivars and cluster B contain all Columbian cultivars.

RAPD analysis detected in the branch **A** other two categories grouping five Romanian bean cultivars in the first subgroup: Delia, Lizica, Florena Avans, Starter and 1 Romanian bean cultivar – Ami in the second group.

Almost all cultivars included in cluster **A** have an indeterminate upright bush growth habit (type II), except Avans and Ami which have indeterminate semi-viney prostrate (type III) growth habit, seeds small to medium and white seeds color.

Cluster **B** includes 2 Columbian bean cultivar: SEA 5 and SEA 3 with indeterminate semi-viney prostrate growth habit (type III) with small beige-cream seeds.

CONCLUSIONS

1. The analyses performed in this study indicate that investigated common bean genotypes are genetically distinct. The eight bean genotypes formed two distinct groups according to their geographical origin.
2. Genetic similarity vary greatly (44%-96%), depending on the pairs of genotypes, on the groups and subgroups. It has lower values between genotypes from different clusters (A and B) and higher values between genotypes within each cluster. This suggests that there is a wide variation of DNA, larger between varieties from different groups and smaller between genotypes within groups.
3. Crosses between bean genotypes from these two major groups (A and B) might lead to high heterosis.

REFERENCES

1. Becerra Velasquez L., P. Gepts, 1994. *RFLP diversity of common bean (Phaseolus vulgaris) in its centers of origin*. Genome 37 (pp. 256-263).
2. Beebe S., P.H. Skrock, J. Tohme, M.C. Duque, F. Pedraza, J. Nienhuis, 2000. *Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD*. Crop Sci. 40 (pp. 264-273).
3. Doyle J.J., L.J. Doyle, 1990. *Isolation of plant DNA from fresh tissue*. Focus., 12 (pp. 13-15).
4. Gepts P, F.A. Bliss, 1986. *Phaseolin variability among wild and cultivated common bean (Phaseolus vulgaris) from Colombia*. Econ. Bot. 40 (4) (pp. 469-478).
5. Lizica Szilagyi, Şemun Tayyar, Matilda Ciucă, 2011. *Evaluation of genetic diversity in common bean (Phaseolus vulgaris l.) using RAPD markers and morpho-agronomic traits.* Romanian Biotechnological Letters (ISI, F.I - 0,152), Vol. 16, No. 1, 2011, Supplement (pp. 98-105).
6. Nodari R.O., E.M.K. Koinange, J.D. Kelly, P. Gepts, 1992. *Towards and integrated linkage map of common bean:1. Development of genomic DNA probes and levels of restriction fragment length polymorphism*. Theor. Appl. Genet. 84 (pp. 186-192).
7. Singh S.P., R. Nodari, P. Gepts, 1991c. *Genetic diversity in cultivated common bean: I. Allozymes*. Crop Sci. 31 (pp. 19-23).