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CHARACTERIZATION OF BREEDING LINES OF COMMON BEAN AS REVEALED BY RAPD AND RELATIONSHIP WITH MORPHOLOGICAL TRAITS

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Abstract

Different type of data can be used to estimate genetic diversity. In this work, genetic diversity among common bean breeding lines by random amplified polymorphic DNA (RAPD) markers, and morphological traits to analyze differences among common bean breeding lines were studied and usability of RAPD markers for estimation of genetic diversity among common bean breeding lines in comparison with morphological traits were evaluated. Eight RAPD markers generated polymorphic patterns, yielding a polymorphism rate of 80%. The average genetic similarity among the common bean breeding lines was 0.55 with values ranging from 0.19 between H-166 and H-212 and 0.67 between H-212 and H-128 breeding lines, having the highest genetic similarity. In the principal component analysis (PCA), the first three principal components explained about 81% of the variation in morphological traits. A Mantel's test showed low correlation between RAPD and morphological data distance matrices.

Introduction

Legumes play an important role in human nutrition. Edible bean (*Phaseolus vulgaris* L.) originate from Latin America and is one of the most important legumes in the World due to its high nutrient value (carbohydrates, protein, minerals and vitamins), extensive production, consumer use, and commercial value. It is traditionally a basic food crop in many developing countries and it serves as a major plant protein source for rural and urban areas.

Plant breeding is one of the oldest human activities. It dates back as early as agriculture itself (Dursun & Guleryuz, 2003). Therefore, plant breeding strategies have been developed for plant species in all around the world. Common bean breeding in Turkey was started by breeders in second terms of 20th century. Many common and snap bean cultivars were bred and approximately 250.000 ton common beans and 545.000 tons snaps bean were yearly produced in our country (Anon., 2005).

Increases in yield and quality in plant species is one of the aims of the plant breeders. For these reasons, many methods are used for breeding. Selection is invincible method used in plant breeding program and plants are selected in terms of their phenotypic characters in this breeding method. But, variation and similarity are determined at plant phenotypic characters which are influenced by environmental conditions. Lots of effort and time are needed for breeding plant in this situation.

For genetic improvement, the usefulness of molecular markers will increase as their capacity to predict the behavior of breeding lines in field experiments (also increases).

Such prediction is possible when there is an association between the loci controlling molecular traits and those controlling morpho-agronomical traits. Advances in molecular biology have allowed the development of rapid, sensitive and specific screening methods to study genetic diversity and relatedness between individuals. Over the last 10 years, polymerase chain reaction (PCR) technology has led to the development of simple and quick techniques called RAPD which detects PCR fragment polymorphisms, using a single primer of arbitrary nucleotide sequence (Williams *et al*., 1990).

In the presented study, genetic diversity of breeding lines derived by selection from breeding program originated from common beans grown in Erzincan region where micro-climate is suitable for common bean was determined by RAPD as well as morphological traits; to analyze differences among common bean ecotypes and to compare results based on RAPD markers and morphological traits.

Material and Method

RAPD: Bulk immature unifoliate leaves from each breeding line was used for extracting DNA as described by Dellaporta *et al*., (1983). The reactions were performed in 0.2 ml tubes in Mastercycler personel apparatus (Eppendorf, Germany) programmed to cycle 45 times under the following conditions: for the first two cycles, denaturation for 30 s at 94^{o}C, annealing for 60 s at 37^{o}C, and elongation for 2 min at 72^{o}C; second two cycles, denaturation for 30 s at 94^{o}C, annealing for 60 s at 35^{o}C, and elongation for 2 min., at 72^{o}C; the subsequent 41 cycles were run with the denaturation temperature reduced to 93^{o}C, followed by a 4-min hold at 72^{o}C. After amplification, the reaction products were separated by electrophoresis in 1.4% agarose gels, stained with Ethidium bromide, and photographed under ultraviolet light with Nikon Coolpix5000. A total of 10 primers were used based on the band resolution and polymorphism they provided (Table 1).

Data analysis: The DNA bands were scored as 0 (absence) or 1 (presence). Genetic similartiy between two cultivars i and j was estimated following the formula of Nei & Li (1979). Based on the genetic similarity matrix (denoted GS), UPGMA cluster analysis were used to assess pattern of diversity among the bean entries. Dendrograms were created with the TREE program of NTSYS. All calculations were performed using the NTSYS-pc version 2.1 software (Rohlf, 2000).

A principal component analysis (PCA) was performed on observed morphological traits after standardization. Based on standardized trait values, euclidian distances (mdij) between the lines were calculated. Morphological similarities (msij) were also calculated as (1-mdij). Matrix of these values is denoted MS. Using the matrix (denoted MD) of euclidian distances, an UPGMA cluster analysis was performed producing a second dendrogram depicting relationships among cultivars relative to their morphological characteristics.

Morphological and agronomic traits: Sixteen traits (Table 2) were scored on the 15 common bean breeding lines. All traits were evaluated on ten plants taken from a row plot of 2 m and two replicates as described by Dursun & Guleryuz (2003a and 2007b). Traits were morphological trait (LA) and yield components (Table 2). All traits were standardized before analysis by subtracting the mean value and dividing by the standard deviation; this allows to remove scale effects before calculating Euclidian distances.

Table 1. Nucleotide sequences of 8 polymorphic primers and total number of bands per primer.

Primers	Nucleotide sequences	Total number of RAPD bands
A-09	5'-GGGTAACGCC-3'	2
A-20	5'-GTTGCGATCC-3'	2
E-20	5'-AACGGTGACC-3'	2
A-19	5'-CAAACGTCGG-3'	2
Y-16	5'-GGGCCAATGT-3'	3
Q-04	5'-AGTGCGCTGA-3'	5
D-08	5'-GTGTGCCCCA-3'	4
V-10	5'-GGACCTGCTG-3'	2

Table 2. Sixteen morphological traits used to calculate morphological distances.

Code	Traits
SGD	Seed germination date
FD	Flowering date
TM	Time for maturity
PL	Pod length
PW	Pod width
PNP	Pod number per plant
FPW	Fresh pod weight
SNPP	Seed number per pod
SL	Seed length
SW	Seed weight
TSW	1000-seed weight
LA	Leaf area
TN	Total Nitrogen (N)
CP	Crude protein
YPD	Yield per dacar
SYP	Seed yield per plant

Comparison between RAPD markers and morphological traits: Simple (*r*) coefficients between genetic similarities (gsij) and morphological similarities (msij) were calculated. P-values for these coefficients were calculated based on their respective asymptotic distributions (Kendall & Stuart 1979). Correspondence between the two similarity matrices GS and MS (matrix of msij values) was tested with the Mantel Z statistic (Mantel, 1967). Significance of Z was determined by comparing the observed Z values with a critical Z value obtained by calculating Z for one matrix with 500 permuted variants of the second matrix. All computations were performed with appropriate procedures of the NTSYS-pc version 2.1 software (Rohlf, 2000). The purpose of these comparisons was to evaluate the usefulness of RAPD markers as predictors of morphological variability in the genotypes studied.

Results

RAPD polymorphism and genetic distance: Ten RAPD markers dispersed across the genome were used to test the genetic diversity of 15 breeding lines. Eight RAPD markers

generated polymorphic patterns and two did not give any bands, yielding a polymorphism rate of 80%. The 8 primers used generated 32 amplification products (bands), with an average of 2.75 bands per primer. Of these, 21 were polymorphic (2.62 bands per primer) and 11 were monomorphic (1.38 bands per primer). The number of polymorphic bands varied from two to five for the primer Q-04 (Table 1).

The average genetic similarity among the common bean breeding lines was 0.55 with values ranging from 0.19 between H-166 and H-212 and 0.67 between H-212 and H-128 breeding lines, having the highest genetic similarity.

UPGMA dendrogram (Fig. 1) was drawn to show a visual picture of the relationships among the breeding lines. The dendrogram based on the RAPD analysis (Fig. 1) showed that there are two main branches and one main cluster originated from a common branch, but are distinct from each other. Two main branches were breeding lines H-86 and H-85. Main cluster were cascaded into four subgroups. There were one branch, H-161 and one subgroup in the first cascade. In the second cascade, there were one branch which was breeding line H-98 and two subgroups including breeding lines H-71, H109, H-166 and H-211 in the first subgroup and H-68, H-139, H-210, H-83, H-212, H-128 and H-139 in the second subgroup.

Morphological analyses: In the principal component analysis (PCA), the first four principal components (having eigenvalues >1) explained about 81% of the variation. The first axis indicated about 31.2% of the variation. It was linked to variable related to seed number per pot (SNPP) which was positively correlated to PL (r = 0.60), PNP (r = 0.63), FPW (r = 0.59) and DHY2 (r = 0.69), and negatively correlated to SGD (r =0.91), FD (r =0.71) and TM (r =73). The second axis, explaining 28.8% of the variation was pod width (PW). It was highly correlated positively with SL (r = 0.73), SW (r =75), TSW (r =81) and LA (r =55). Third axis was crude protein (CP) which was explaining 12.1% of the variation. It was only correlated positively to TN (r = 64). There are one branch and one group based on the projection of breeding lines in the principal plan (Fig. 2). Branch was H-128 breeding line which was distinct than other breeding lines based on morphological data. Interestingly, main group was also subdivided into one branch breeding line H-210 and one subgroup, which was cascaded subgroups. H-86 and 139 was the closest and followed by pair of H-71 and H-211. It can be noted that these cascaded branch and groups are clearly distinguished in the principal plan. The morphological-based dendrogram (Fig. 3) has a good fit to the MD matrix and gave results in good agreement with the variety grouping obtained from the principal components analysis. There was low correlation between RAPD and morphological data distance matrices based on the result of Mantel's test.

Discussions

In this study, the utility of RAPD and morphological characters in the analysis of common bean breeding lines were compared. Both RAPD and morphological characters were sufficient to assess the variability in common bean genotypes. Main source of variation resulted from genetic source found in origin place where first selection was made. Selected and promising breeding lines were evaluated at molecular level as well as the morphological level. Existence of genetic variability among breeding lines were found at two levels.

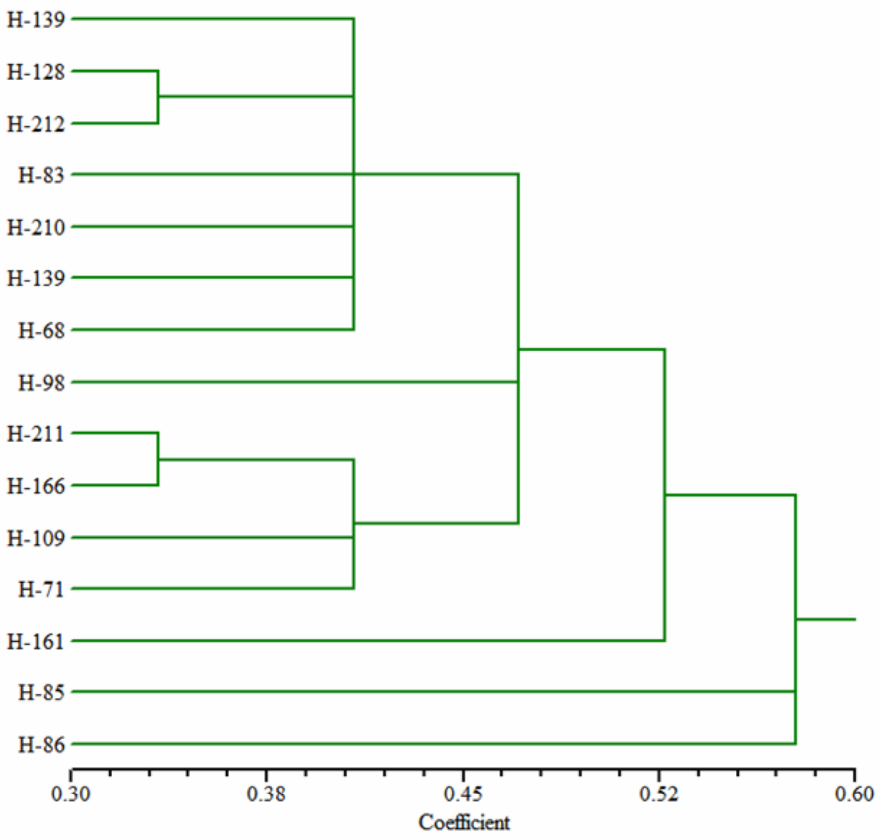

Fig. 1. Dendogram of common bean genotypes based on RAPD data using UPGMA.

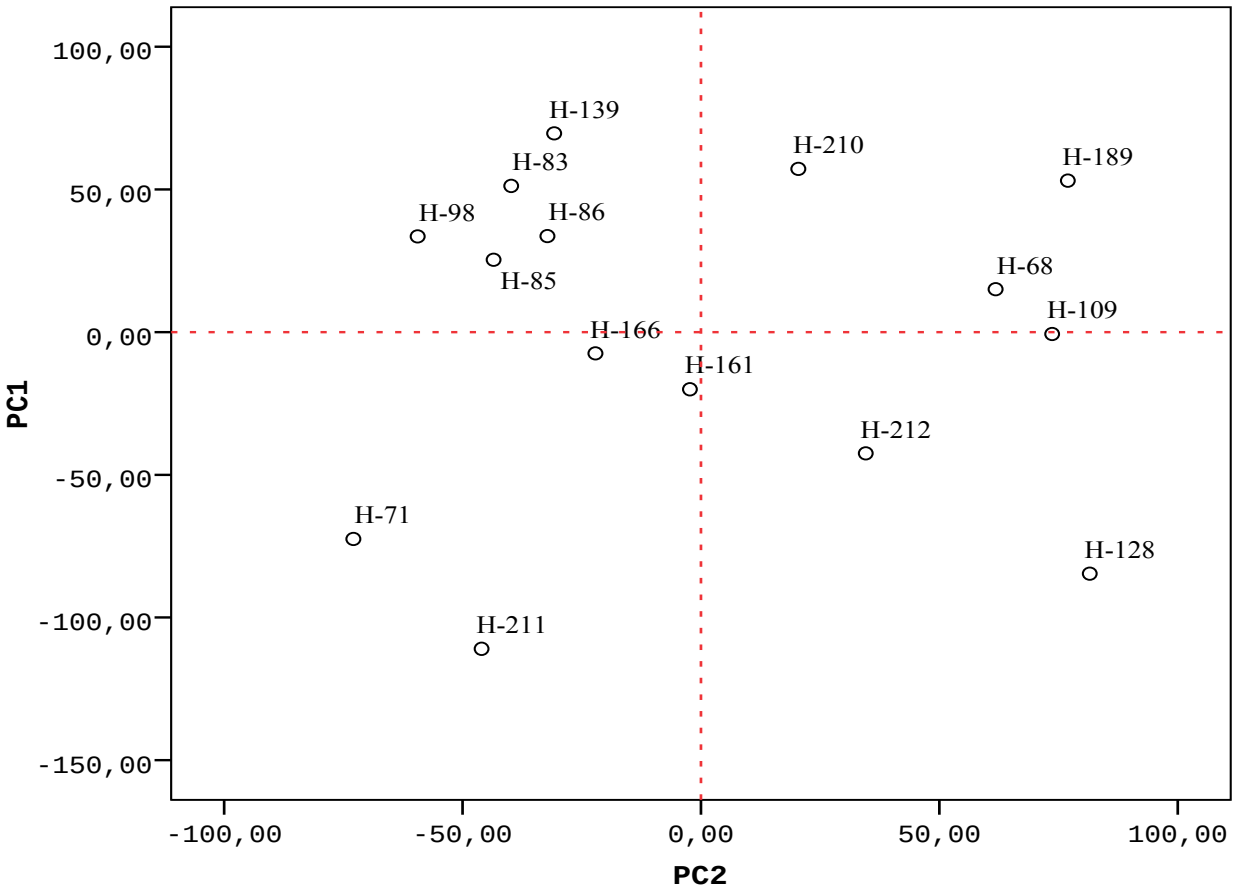

Fig. 2. Principle component analysis two-dimensional plots of 15 common bean genotypes based on morphological trait values.

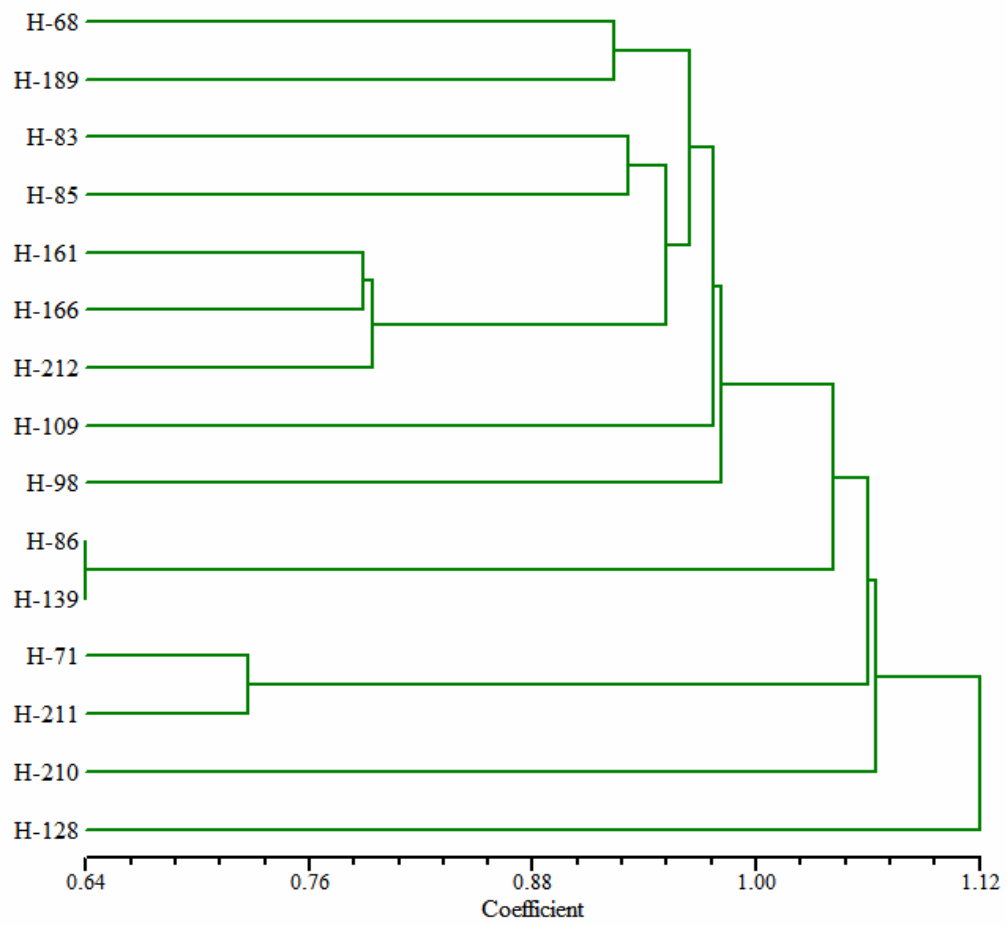

Fig. 3. Dendogram of common bean genotypes based on morphological data using UPGMA.

The UPGMA dendongrams confirmed the differences RAPD resulting from and morphological traits to find out genetic variability of common bean. No correlation was found between the GD matrices obtained by molecular and morphological data. Similar result was also obtained from other studies by Maric *et al*., (2004). This could be either related with the RAPD technique or some with the analysis of morphological traits. There is always influence of environment in morphological traits and they can show considerable variation. Also, some traits can be incorrectly measured and so cause problems in the estimation of genetic diversity. The number and choice of morphological traits and sample size can also affect the correlation. At the same time, number of primers can also influence the correlation. It is possible that if more RAPD primers had been used, a better correlation with morphological traits would have been obtained.

RAPD is also used for marker assisted selection studies in bean breeding. Marker-assisted selection can provide an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding disease resistance genes (Kelly & Miklas, 1998). For common bean, PCR-based RAPD markers linked with more than 20 disease resistance genes have been obtained to date (Park *et al*., 1998; Namanja *et al*., 2006; Mutlu *et al*., 2005).

The results of this study indicate that RAPD analysis could be successfully used for the estimation of genetic diversity among common bean genotypes. Further studies are needed to link the RAPD markers detected in this study to breeding traits for cultivar development.

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