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CHARACTERIZATION OF SELECTED SOYABEAN GERMPLASM THROUGH MOLECULAR MARKERS

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Abstract

Fifty genotypes of soybean were used for RAPD analysis. Thirty random primers were used for RAPD analysis in which 8 generated scorable amplification products across all the genotypes with high level of polymorphism, while the range of genetic distance was recorded from 0 to 96%. The cluster analysis revealed that genotype 017415 and 017461 were most distantly related to each other and hence it is suggested that in future breeding programs, these two should be crossed to create maximum genetic variability in local soybean breeding population.

Introduction

The assessment of the genetic relationships among the cultivated plants at molecular level is a fundamental component of crop improvement programmes. Randomly amplified polymorphic DNA markers (RAPD) along with the analysis of morpho-physiological traits in various crops are used to present the extent of genetic diversity (Iqbal *et al.* 2011). RAPD is a rapid, inexpensive and non-dependent on plant genome information. It has been extensively applied to ascertain genetic polymorphism in several plants (Iqbal *et al*. 2011). The RAPD marker system is a technology that requires relatively small investment and can be easily employed, and is widely used as efficient method for identification of genetic diversity at DNA level (Dos *et al.* 1994, Ferreira *et al.* 2000). It has been observed that RAPD markers are simple and useful instrument to assess diversity in crops. RAPDs are suitable for studying genetic diversity because they are easy to use, non-radioactive, quick, comparatively economical, largely automatable and require little quantity of DNA (Hedrick 1992). RAPD can provide valuable data in the analysis of population genetic structure including genetic diversity within and among populations, population subdivision, and degree of inbreeding and individual relatedness (Lunch and Milligan 1994, Laribi *et al.* 2011). Fifty genotypes of soybean were used to study DNA polymorphism for the estimation of genetic variability at molecular level. RAPD primers were used to identify differences at DNA level among the genotypes. Statistical analyses were carried out to estimate genetic diversity and phylogenetic relationship among the genotypes.

Materials and Methods

Fifty genotypes of soybean germplasm *viz.,* PK-017415, PK-017416, PK-017417, PK-017418, PK-017419, PK-017420, PK-017421, PK-017422, PK-017423, PK-017424, PK-017425, PK-017426, PK-017427, PK-017428, PK-017429, PK-017430, PK-017431, PK-017432, PK-017433, PK-017434, PK-017435, PK-017436, PK-017437, PK-017438, PK-017439, PK-017440, PK-017441, PK-017442, PK-017443, PK-017444, PK-017445, PK-017446, PK-017447, PK-017448, PK-017449, PK-017450, PK-017451, PK-017452, PK-017453, PK-017454, PK-017455, PK-017456, PK-017457, PK-017458, PK-017459, PK-017460, PK-017461, PK-017462, PK-017463 and PK-017464 obtained from Plant Genetic Resources Institute (PGRI), Islamabad were used for RAPD analysis.

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For good quality and purity, genomic DNA was isolated by following the method of (Kang *et al*. 1998) with some modifications.

RAPD analysis was performed on 50 soybean accessions. For PCR-RAPD analysis, 10 to 12 base oligonucleotide primers were used, obtained from Gene Link and Operon Technologies Inc. (USA). PCR was performed in a reaction volume of 25 µl including ddH_2O (14.5 µl), $MgCl_2$ (3.4 µl), Taq buffer (2.5 µl), dNTPs (1.6 µl), Primer (1 µl), Taq polymerase (0.5 µl) and genomic DNA (1.5 µl). A total of 30 primers were employed for screening of RAPD markers (Table 1). For optimization of RAPD primer test reaction was run at 28, 31, 32, 33, 34, 36 and 37ºC annealing temperature. The 10 bp oligonucleotide primers were mostly amplified at 34ºC. Out of 30 only eight primers were selected for further analysis. The optimized programming conditions of RAPD primers for PCR were: 94ºC for 4 min, 37 cycles of 94ºC - 1 min, 34ºC - 1 min, 72ºC - 2 min and final 72ºC - 7 min and 4ºC - hold. The amplification products were then analyzed for polymorphism after electrophoresis in 2% agarose gels.

Similarity coefficients were used to assess the relationship among accessions. All the scorable loci were considered for generation of bivariate 1 - 0 data matrix and genetic distances (GD) among the genotypes were estimated using Jaccards method and for estimation of genetic diversity dendrogram was constructed using the computer software NTSYS (Rohlf 1998).

Results and Discussion

Out of 30 primers, eight exhibited polymorphism and hence were used for DNA fingerprinting work. Some of the primers revealed characteristic fragments for some genotypes which were not produced in others. Only clearly scorable bands were included in the analyses. Minor bands which could not be scored reliably were not included in the analyses. Every single band was considered as a single allele/locus. The loci were scored as present (1) or absent (0) and missing (9) for analysis by NTSYS software (Fig. 1). Bi-variate data matrix was generated. Eight primers generated (571) reproducible and scorable amplification products across all the genotypes.

The data obtained from eight RAPD primers (AC-08, AC-05, AC-09, OPA-05, OPA-11, OPA-10, OPB-01, and AC-07) were used to analyze phylogenetic relationship among the genotypes using computer program "NTSYS". Fifty soybean genotypes were clustered into six main groups (Group A-F) based on the resulting dissimilarity matrix using cluster analysis. Group “F” was the largest group comprising 28 genotypes while clusters "A", "B" and "C" were smallest comprising two genotypes each. It is also evident from cluster analysis (Fig. 2) that genotype 1 (Acc # 017415) and genotype 47 (Acc # 017461) were most distantly related and are recommended for hybridization programs to create maximum genetic variability in local soybean breeding population.

Genetic distances (GD) among the genotypes were calculated using UPGMA procedure outlined by Nei and Li (1979). Range of genetic distance observed among the germplasm accessions was 0 - 96%. Minimum genetic distances (0%) were observed among 210 comparisons while maximum genetic distance (GD = 96%) was observed for only one comparison. Mean genetic distance of 50 *Glysine max* genotypes based on eight molecular markers studied showed that values for genetic distance among many genotypes ranged from 0 - 96%. Genotypes found at higher distances could be exploited for breeding purposes depending on their performance in respect to other trait.

Genetic distance provides us an assessment about how much a variety, or genotype is located close to other one. If the genetic distance is minimum that’s mean these have many similarities in

Table 1. Sequences of RAPD primers used.

Sl. No.	Primers	Sequence	PCR response	Sl. No.	Primers	Sequence	PCR response
1	GLB-17	5’-AGGGAACGAG-3’	-	16	AC-09	5’-AGAGCGTAC-3’	+
2	GLB-18	5’-CCACAGCAGT-3’	-	17	AC-04	5’-ACGGGACCTG-3’	-
3	GLB-13	5’-TTCCCCCGCT-3’	+	18	AC-11	5’-CCTGGGTCAG-3’	-
4	GLB-12	5’-CCTTGACGCA-3’	+/-	19	AC-12	5’-GGCGAGTGTG3’	-
5	OPA-5	5’-AGGGGTCTTG-3’	+	20	AC-13	5’-GACCCGATTG-3’	+/-
6	GLB-14	5’-TCCGCTCTGG-3’	-	21	AC-14	5’-GTCGGTTGTC-3’	-
7	GLB-15	5’-GGAGGGTGTT-3’	+	22	AC-15	5’-TGCCCGTGAGA-3’	-
8	OPA-10	5’-GTGATCGCAG-3’	+	23	AC-16	5’-CCTCCTACGG-3’	-
9	OPA-11	5’-CAATCGCCGT-3’	+	24	AC-17	5’-CCTGGAGCTT-3’	-
10	OPB-1	5’-GTTTCGCTCC-3’	+	25	AC-18	5’-TTGGGGGAGA-3’	-/+
11	OPB-4	5’-CGACTGGAGT-3’	+	26	AC-19	5’-AGTCCGCCTG-3’	-
12	GLB-09	5’-TGGGGGACTC-3’	-	27	AC-20	5’-ACGGAAGTGG-3’	-
13	GLB-19	5’-ACCCCCGAAG-3’	-	28	AC-07	5’ACGGAAGTGG-3’	+
14	GLB-16	5’-TTTGCCCGGA-3’	-	29	OPB-5	5’-TGCGCCCTTC-3’	-/+
15	AC-05	5’-GTTAGTGCGG-3’	-/+	30	OPA-2	5’-TGCCGAGCTG-3’	-

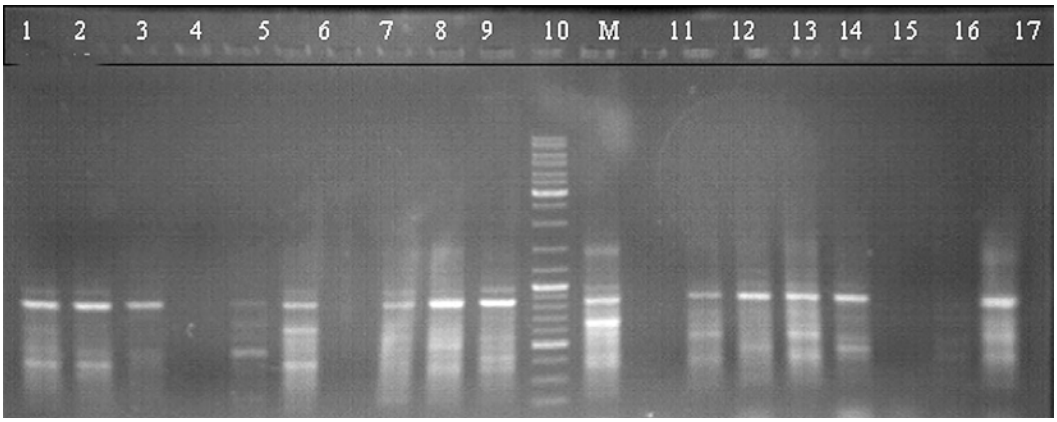

Fig. 1. PCR amplification profiles of 19 DNA samples using RAPD primer (AC-07).

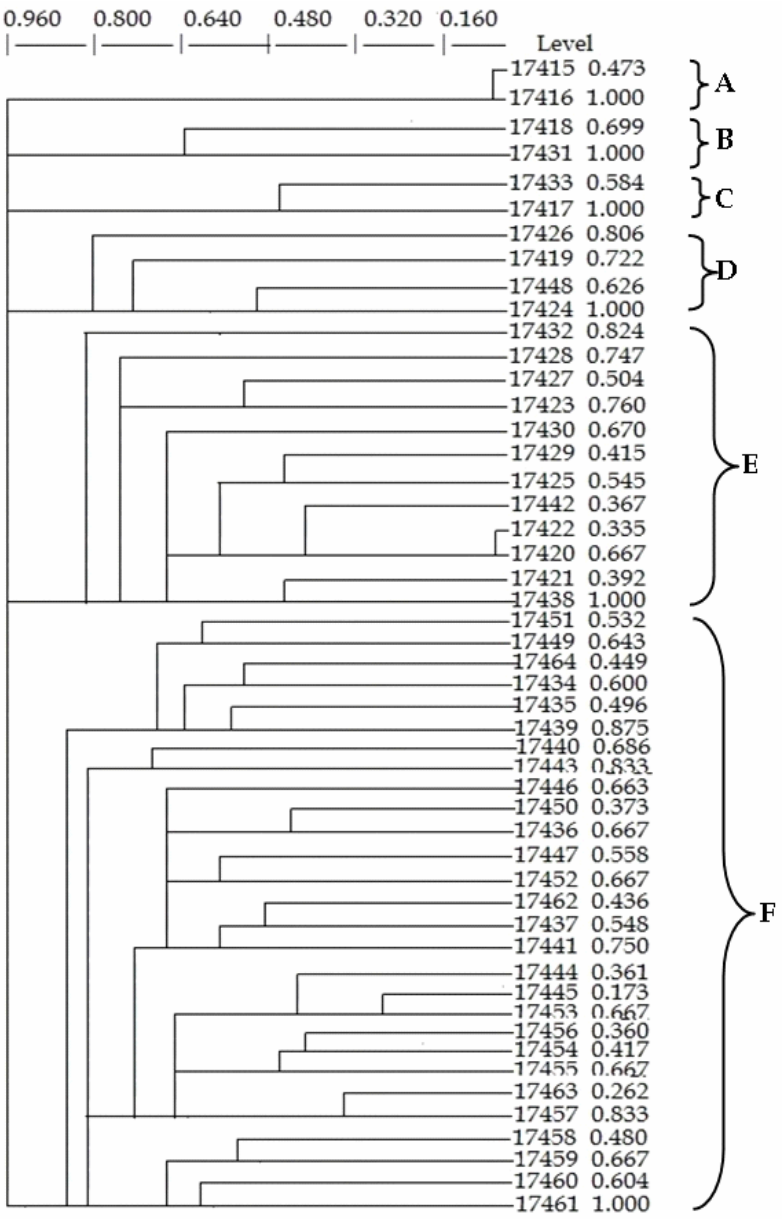

Fig. 2. Dendrogram of 50 genotypes of soybean.

common and if the genetic distance is maximum it shows that genetic variation is higher between different genotypes. Distance analysis is also used to establish polygenetic pattern and to describe evolutionary processes (Felsenstein 1982, 1984). Cluster analysis based on molecular markers has been reported in several studies, such as (Khan *et al.* 2013, Bibi *et al.* 2012). In soybean, similar studies on genetic diversity assessment and its utilization have been conducted previously by (Brown-Guedira *et al*. 2000, Singh *et al.* 2006, Thompson *et al.* 1998 and Kumawat *et al.* 2015).

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