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ASSESSMENTS OF GENETIC DIVERSITY IN COUNTRY BEAN (*Lablab purpureus* L.) USING RAPD MARKER AGAINST PHOTO-INSENSITIVITY

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Abstract: RAPD marker was used to evaluate genetic relationships among 11 genotypes of country bean, including first three genotypes were photo-insensitive and the rests were sensitive. The genotypes were grouped into two major clusters where photo-insensitive genotypes remain in cluster I and sensitive genotypes remain in cluster II. A total of 26 bands were detected, of which 57.69% were polymorphic and the remaining were monomorphic across all genotypes. A highest level of genetic distance was observed between CB04 and CB06 while the lowest level of genetic distance showed between CB01 and CB03. The highest similarity index between the genotypes CB01 and CB03 indicated less divergence between them. Low similarity indices were observed between CB04 and CB06, which indicated more divergence. Crossing between the genotypes with low similarity coefficient will manifest high heterosis. The identified genetically distinct cultivars could be potentially important source of germplasm for further improvement of country bean.

Key words: country bean, RAPD marker, polymorphism, genetic diversity

Introduction

Country bean (CB) (*Lablab purpureus* (L.) Sweet, Syn. *Dolichos lablab* L.) belongs to the family Leguminosae (Fabaceae) and genus *Lablab* with varying chromosome number of 2n = 20, 22, 24. It originated from India [DEKA & SARKAR, 1990] and is a good source of minerals and vitamins [BASU & al. 2002] with antioxidants [BRADLEY, 1999]. Lablab bean (*Lablab purpureus* L.) is one of the major winter vegetables of Bangladesh. It is commonly known as country bean in Bangladesh and is widespread throughout the country.

Assessment of genetic diversity based on phenotype has limitations, since most of the morphological characters are greatly influenced by environmental factors and the development stage of the plant. In contrast, molecular markers based on DNA sequence polymorphism, are independent of environmental conditions and show a higher level of polymorphism. This necessitates the assessment of genetic diversity present in country bean using the modern molecular approaches. The immense genetic diversity of landraces of crops is the most directly useful and economically valuable part of biodiversity. Unlike high yielding varieties, the landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period. Because of the limitations of morphological and biochemical markers, efforts are being directed to use molecular markers for characterizing germplasm diversity against high yield, year round, earliness and tolerant to high temperature and long day length. Molecular markers have demonstrated a potential to detect genetic diversity and to aid in the management of

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plant genetic resources [VIRK & al. 2000, SONG & al. 2003]. In contrast to morphological traits, molecular markers can reveal differences among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management. Several types of molecular markers are available today, including those based on restriction fragment length polymorphism (RFLP) [BOTSTEIN & al. 1980], random amplified polymorphic DNA (RAPD) [WELSH & al. 1990, WILLIAMS & al. 1990], amplified fragment length polymorphism (AFLP) [VOS & al. 1995] and simple sequence repeats (SSRs) [SINGH, 1999].

It is particularly useful for characterizing individual genotypes and selection of the parents for successful hybridization. Among the molecular markers, in the present study, we have used RAPD method of DNA fingerprinting, which is widely used in conservation biology because of quick results, cost-effectiveness and reproducibility.

The PCR-based RAPD approach using arbitrary primers requires only nanogram quantities of template DNA, no radioactive probes, and is relatively simple compared to other techniques [WILLIAMS & al. 1993]. However, morphological traits have certain limitations such as easily available of scorable markers, difficulty in scoring homozygous from heterozygous individuals, influence of environment in equating phenotypes with genotypes etc. On the other hand, molecular markers have many advantages such as abundance in polymorphism, no pleiotropic effect, less affected by environment and subjected to rapid detection [SINGH & al. 2005]. Therefore, RAPD has been using extensively for studying genetic diversity of crops in different parts of the world. However, no report on RAPD is available regarding the diversity of country bean in Bangladesh.

Materials and methods

Source of the genotypes

In the present investigation eleven genotypes (CB01 to CB11) of the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, were used for genetic diversity study against photo-sensitivity. Previously the genotypes were tested in the department using temporal planting and morphological markers and found that CB01, CB02 and CB03 genotypes were pho-insensitive and the rest were photosensitive but never tried with molecular markers.

DNA Extraction

Young leaves were used for extracting DNA by CTAB (Cetyl trimethyl ammonium bromide) with some modifications in protocol [DOYLE & DOYLE, 1990]. Fresh leaves from polybag raised plants were collected and immediately stored at 4 °C. One hundred and fifty mg of leaf tissue ground with 1 ml DNA extraction buffer using mortar and pestle. Extraction buffer (100 mM Tris HCl (pH 8.0), 20 mM EDTA, 1.4 mM NaCl and 2% CTAB per liter) was added in 2 ml tubes filled with ground leaf and mixed with vortex mixture. The tubes were incubated at 65 °C for 45 minutes with repeated shaking. Equal volume of chloroform : isoamylalcohol mix (24:1) was added and mixed thoroughly for 5 minutes, followed by centrifugation at 14000 rpm for 15 minutes. Two third volume of isopropanol was added to the supernatant. DNA was precipitated by centrifugation at 14000 rpm for 10 minutes and the pellet was washed in 70% ethanol and suspended in 100 µl of sterile distilled water with 10-15 µl (10 µg/µl concentrations) of RNAse and incubated at 65 °C for 30 minutes. The quality and quantity were checked through 1% agarose gel by electrophoresis. DNA concentration for PCR reaction was determined at 260 nm

wavelength with Spectrophotometer and the quality verified by electrophoresis on 1% agarose gel in TAE (Tris-acetate-EDTA) buffer.

RAPD analysis

Genomic DNA was used as template for PCR amplification as described by WILLIAMS & al. (1990). Several number of primers were used to get reproducible bands among them 4 arbitrary primers (OPERON Technologies, Inc. California, USA) were used to produce distinct marker profiles for 11 genotypes. The selected 4 primers generated in a much higher number of polymorphic bands. Amplification reactions were in the volumes of 25 µl volumes containing 10 µl of 5X Flexi Taq buffer, 4.0 µl $MgCl_2$ (25 mM), 2.5 µl dNTPs (4mM/µl), 2.5 µl random primer (10 pmol/µl), 0.25 µl Taq DNA polymerase (5 U/µl) and 4 µl of the extracted DNA (25 ng). Amplification was performed with Thermal cycler (Eppendorf) programmed for 45 cycles. After initial denaturation for two minutes at 94 °C, each cycle consisted of one minute at 94 °C, one minute at 42 °C and two minutes at 72 °C. The 40 cycles were followed by seven minutes of final extension at 72 °C.

The PCR product was mixed with 6 µl of loading dye (0.25% bromophenol blue, 0.25% Xylene Cyanol and 40% Sucrose, w/v) and spun briefly in a micro centrifuge before loading. The PCR products and 100 bp DNA ladder were electrophoresed using 1% agarose gel at 80 volts followed by staining with ethidium bromide then separated fragments and were visualized with an ultraviolet (UV) transilluminator (Biometra gel documentation system).

Data analysis

Data generated from the polymorphic fragments was scored as present (1) or absent (0) for each of the 11 genotypes. The diversity among the lines was worked out by subjecting the RAPD scores to cluster analysis. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The scores obtained using all the primers in the RAPD analysis were then pooled to create a single data matrix, to estimate polymorphic loci, gene diversity, genetic distance (D) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31) [YEH & al. 1999]. Genetic similarity values defined as the fraction of shared bands between the RAPD profiles of any two individuals on the same gel were calculated manually from RAPD markers of the same weight on the data matrix. Similarity index (SI) was calculated from 2nxy/nx+ny, when nxy is the number of the common DNA bands in x and y plants, nx and ny are the total DNA bands of X and Y plant respectively [HILL & al. 1996].

Results and discussions

Four random primers were used in PCR reaction to amplify DNA fragments from 11 genotypes because these primers generated higher number of polymorphic bands. A total of 26 bands were detected, of which 42.31% (11 bands) was monomorphic across all genotypes (Tab. 1). The remaining 15 bands (57.69%) were polymorphic among the varieties tested. This accounted to an average of 3.75 polymorphic bands per primer indicating the medium level of polymorphism expressed by arbitrary primers. Among the Ten oligonucleotide primers, TIWARI & al. (2003) found all the primers produced polymorphic amplicons though the extent of polymorphism varied with each primer. KUMAR & al. (2008) found 95% percent polymorphism of the total 124 amplified products in twenty-six common bean using 15 primers and RAI & al. (2010) found 70.27%

polymorphism, indicating fair amount of variation at the DNA level among these accessions.

Tab. 1. Polymorphism detected by the use of 4 random RAPD primers on 11 country bean genotypes.

Name of primers	Sequence of primer (5'-3')	Total number of bands	Number of polymorphic bands	% of polymorphic bands
OPA 04	AATCGGGCTG	4	3	75
OPD 03	GTCGCCGTGA	10	6	60
OPD 20	ACCCGGTCAC	7	3	43
OPF 02	GAGGATCCCT	5	3	60
Total		**26**	**15**	
Average		**6.5**	**3.75**	

The primers OPD 03 produced maximum number of fragments (10) followed by OPD 20 and OPF 02 produced 7 and 5 fragments respectively. The primer OPA 04 recorded minimum number of fragment (4). The RAPD profiles for two primers viz., OPA 04 and OPD 03, which produced diagnostic markers are given in Fig. 1 (A&B). RAI & al. (2010) observed the number of amplification products produced by each primer varied from 4 to 9 with an average of 5.69 bands per primer. The simple matching similarity was calculated using RAPD score and dendrogram was constructed using unweighted pair group method with arithmetic averages (UPGMA).

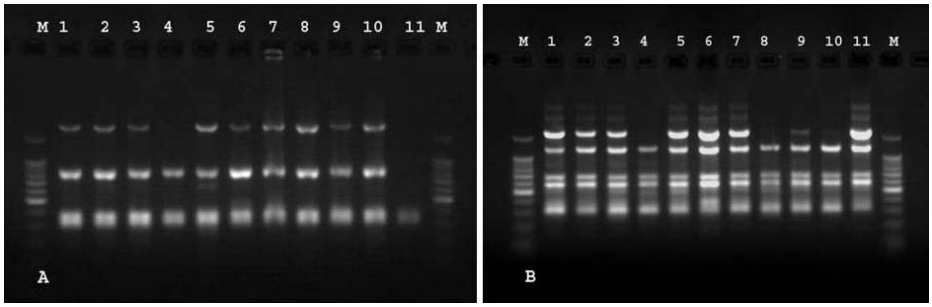

Fig. 1. RAPD profile of eleven genotypes of country bean produced using the random decamer primers OPA 04 (A) and OPD 03 (B). M, 100 bp DNA ladder and lane numbers correspond to the genotypes CB01 to CB11.

Cluster I consisted of two sub clusters, which included most of the testers. The sub cluster A of cluster I consisted of CB01, CB02, CB03, CB05, CB07 and CB11, while sub cluster B consists only one genotype, CB06. The sub cluster A of cluster II included CB04, CB08 and CB10 while sub cluster B of cluster II included only CB09 genotype (Fig. 2). The genotypes CB01, CB02 and CB03 were belong to the same cluster because they are photo-insensitive which is morphologically and temporally tested. BISWAS & al. (2010) found two major clusters based on Jaccard's similarity coefficient using UPGMA grouped among 14 genotypes of French bean.

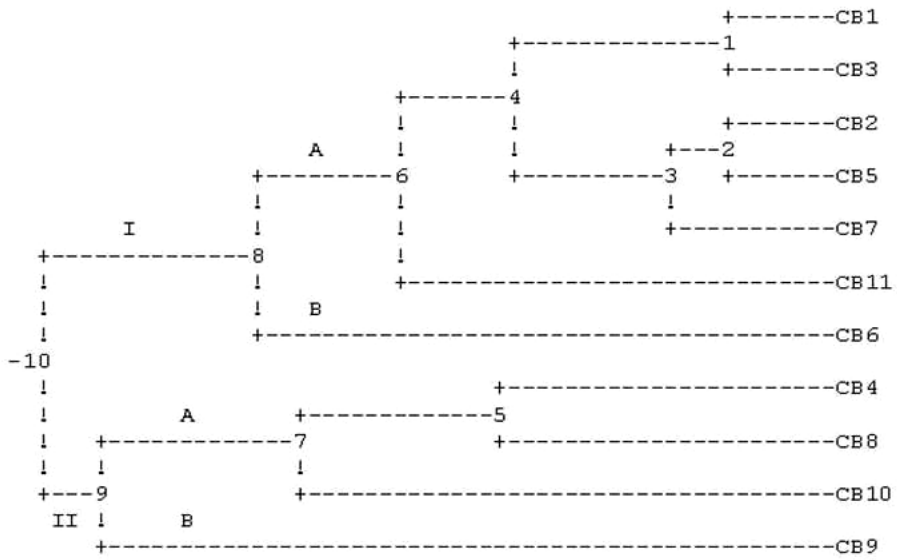

Fig. 2. UPGMA cluster analysis-based dendrogram showing genetic relationship among eleven genotypes of country bean.

Molecular markers analysis of the sixteen genotypes using 4 random RAPD primers produced polymorphism for most of the studied loci. As per the similarity index, the genotypes were grouped into two major clusters four sub clusters. A highest level of genetic distance (0.425) was observed between CB04 and CB06 while the lowest level of genetic distance (0.039) showed between CB01 and CB03 (Tab. 2). The highest similarity index between the genotypes CB01 and CB03 (0.97) indicated less divergence between them. Low similarity indices were observed between CB04 and CB06 (0.74) which indicated more divergence. RAI & al. (2010) showed RAPD based dendrogram showed similarity ranged from 0.38 to 0.96.

Tab. 2. Nei's genetic distance among eleven genotypes of country bean

genotypes ID	CB 01	CB 02	CB 03	CB 04	CB 05	CB 06	CB 07	CB 08	CB 09	CB 10	CB 11
CB 01	****										
CB 02	0.080	****									
CB 03	0.039	0.123	****								
CB 04	0.368	0.262	0.314	****							
CB 05	0.123	0.039	0.167	0.314	****						
CB 06	0.214	0.123	0.262	0.425	0.167	****					
CB 07	0.123	0.039	0.080	0.214	0.080	0.167	****				
CB 08	0.314	0.314	0.262	0.123	0.368	0.368	0.262	****			
CB 09	0.214	0.214	0.262	0.314	0.262	0.368	0.262	0.262	****		
CB 10	0.262	0.262	0.214	0.262	0.314	0.314	0.214	0.123	0.214	****	
CB 11	0.167	0.167	0.123	0.262	0.214	0.314	0.123	0.314	0.314	0.167	****

Crossing between the genotypes with low similarity coefficient will manifest high heterosis. The identified genetically distinct cultivars could be potentially important source of germplasm for further crop improvement programme in the country bean genotypes.

Conclusion

Country bean is an important vegetable crop in Bangladesh in terms of production and consumption. The cultivation of country bean in our country largely centralized in winter due to lack of high yielding and quality day neutral variety. The previous morphological characterization was not sufficient for the selection of parents to develop day neutral high yielding variety. In our study, RAPD marker was used to evaluate genetic relationships among 11 genotypes of country bean in our country. Therefore, the finding of the paper could help to develop high yielding variety designing appropriate breeding approach. The identified genetically distinct cultivars could be potentially important source for further improvement of country bean.

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