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Simple Sequence Repeats Marker based Detection of Genetic Diversity of Indian Bean *Dolichos lablab* (L.) of Family Fabaceae

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ABSTRACT

Background: Assessment and molecular characterization of genetic diversity among the *Dolichos lablab* (L.) have huge implication in scheming strategies for breeding. In India, there is less comprehensive information on the potential genetic diversity of *lablab* bean and this is a major challenge for systematic use of *lablab* bean in genetic breeding programs. To exploit the available trait of interest, the genetic diversity of the locally available genotypes must be known. The PCR-based SSR approach requires just nanogram amounts of template DNA, subjected to quick detection and less influenced by environment. In this manner SSR have been utilizing broadly to study hereditary assorted qualities of yields.

Methods: In this study, genetic diversity on ten Indian *Dolichos lablab* (L.) genotypes were surveyed utilizing SSR markers at Department of Botany, Bundelkhand University, Jhansi during 2019-20 got from Indian gene bank. For SSR analysis, 5 primers were used for checking the diversity in *Dolichos lablab* (L.) and the performance of markers were measured by using 4 parameters (1) PIC, (2) Mean resolving Power, (3) Resolving Power and (4) MI.

Result: SSR (05) markers produced a total of 51 bands, out of which 45 bands (88.23%) were polymorphic which varies from 6 (Primer-111) to 15 (Primer-AGB-9) with mean of 10.5 bands per primer. The polymorphic information content value ranges from 0.265 to 0.488 with a mean value of 0.390. The value of Matrix index is 3.2. The value of resolving power ranges from 2.2 to 12.60 with a mean value of 7.40. In the UPGMA dendogram, the 10 genotypes were separated into two main clusters with all the primers used. Our present examination uncovered that genetic markers might be effectively used for deciding hereditary variety and connections in *Dolichos lablab* (L.) genotypes and could be utilized as a part of breeding programs.

Key words: *Dolichos lablab* (L.), Polymorphic information content, Resolving power, SSR.

INTRODUCTION

Grain legumes hold an important position in World's Agriculture due to their high protein content and their atmospheric nitrogen fixation capacity. The pulses represent the only concentrated type of dietary proteins for many developing countries. As far as the developed countries are concerned, legumes are key source of protein, being good biologically valuable animal feed. Indians generally favor vegetarian food and the pulses are the major source of protein. *Lablab* had a great number of qualities due to its adaptability, which can be used efficiently in different conditions. Grain yields are significantly higher than cowpeas (Adebisi and Bosch, 2004). It is not only resistant to drought, but it can also thrive in a variety of environmental conditions. It can be used as a cover crop for benefit, as its thick green cover protects the soil from desiccation and reduces wind or rain erosion. Up to six tons of dry matter / ha has been known to be given as green manure. It also restores nitrogen biologically into the soil, thus increasing the yields of subsequent crops in an economically and environmentally friendly way.

In *Dolichos lablab* (L.) there is an urgent need to establish a variety of *lablab* genotypes with intrinsic values such as highest yield, combined to their consistent production not confined to a specific area and appropriate for seasonal development from long ago. Hence, there is an urgent need to develop the all season *lablab* variety with determined early maturity, growth of plant and highest harvest index with excellent characteristic of pod, resistance to bean mosaic virus, pests and insects, superior storage and higher content of seed protein.

Evaluation of germplasm lines in any crop improvement program to evaluate the existing variability is a first step. The chances of evolving desired plant types would be

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greater the variability present in the breeding material. Clear understanding of the variability of the different quantitative characters of the breeding material is an asset for the breeder of plants to select superior genotypes phenotypic expression basis. Estimation of phenotypic and genotypic variances for different quantitative characters along with the heritability and genetic advance expected by yield selection and its components are useful in designing an effective breeding program in this regard. Yield is a complex character affected by diverse components that contribute to the crop's genetic potential.

It is also essential to understand the genetic variety and population structure of *Dolichos lablab* (L.) for its conservation, management, and exploitation of the variation for its improvement. Genotyping gives essential information on the genetic diversity of the materials under consideration. Genetic variation assessments at the DNA level became simple after the development of molecular markers (Reif *et al*. 2003). The quantity of molecular marker systems that are utilized for the genetic diversity study of *Dolichos lablab* (L.) include; random amplified polymorphic DNA (RAPD) (Williams *et al*. 1990; Sultana *et al*. 2000; Tian *et al*. 2005; Gnanesh *et al*. 2005), amplified fragment length polymorphism (AFLP) (Vos *et al*. 1995; Venkatesha *et al*. 2007; Tefera *et al*. 2006; Patil *et al*. 2009) and simple sequence repeats (SSRs) (Singh, 1999; Wang *et al*. 2004; Wang *et al*. 2007). Of these, SSR markers are provided with the number of benefits as compared to any other marker systems applied in plant breeding. These advantages include SSR polymorphisms of co-dominant nature (Slavov *et al*. 2005), easiness to assay, a great amount of reproducibility, which is the most significant in a genetic examination, high polymorphic genetic information contents (Powell *et al*. 1996), their huge quantity as well as a division in genomes (Varshney *et al*. 2005). They have been preferentially associated with non-repetitive DNA (Morgante *et al*. 2002). On account of the high reproducibility and polymorphic data substance of SSR marker, our investigation set to analyse genetic diversity in Indian *Dolichos lablab* (L.) bean genotypes by using SSR Marker.

Objectives of our study is to check the genetic diversity and genetic relatedness among *Dolichos lablab* (L.) bean genotype through SSR marker; to construct a Phylogenetic tree and a similarity matrix Data and generate polymorphism data by using SSR marker among *Dolichos lablab* (L.) from the state of UP. India.

MATERIAL AND METHODS

Genotypes used

For the present Investigation, ten genotypes (VRSEM- 847, VRSEM-739, VRSEM-799, VRSEM-757, VRSEM-776, VRSEM-733, VRSEM-730, VRSEM-746, VRSEM-843, VRSEM-702) procured from Indian Institute of Vegetable Research, Varanasi were used for studying the genetic diversity among them.

Extraction of DNA

Leaves were used by CTAB process for extraction of DNA, with little change in the procedure (Doyle and Doyle, 1987). Around 150 mg of leaves with extraction buffer (1 ml) were crushed by using mortar and pestle. Extraction buffer was then applied to tubes already having crushed leaf. With normal mixing, incubate the tubes for 45 minutes at 65°C. Isoamyl alcohol: Chloroform (1:24) has been added in equal volume and mixed for 5 minutes, following centrifugation for 15 minutes at 14000 rpm. 2/3 amount of isopropanol has been added to the supernatant. Centrifuged for 10 minutes at 14000 rpm and drained the supernatant and washed the pellet with 70% ethanol containing DNA and suspended in 100 µl sterile Distilled Water. 10-15 µl RNAse, incubated at 65°C for 30 minutes. Qualitative DNA assessment was conducted on 0.8 percent agarose gel by running sample and quantification was made by recording its absorbance at 260nm and 280 nm.

SSR analysis

The SSR amplification were carried out by the method given by Tautz, (1989) with minor modifications A total of five SSR primers were used for amplification of 10 genotypes. Each 50 µl PCR reaction consisted of 0.4 mM dNTPs, 1 X PCR buffer, 0.5 unit of Taq polymerase, 2.5 mM MgCl2, 0.5 µM of forward and reverse primer and 20 ng of DNA. The amplification conditions were 1 minutes at 94°C (Initial Denaturation), followed by Denaturation at 94°C for 1 min., 55°C for 55 sec. (Annealing), 72°C for 1 min. (Extension) and final extension of 10 minutes at 72°C. The samples of PCR were casted on 2.5% molecular grade Agarose gel (Hi-media) after amplification, which were ready in 0.5 X buffer (TBE) with 0.5 µg / ml EtBr. Finally, amplified products were resolved for 180 min on 2.5 percent Agarose gel at 50V and gel was visualized under UV light and the image was captured.

Data analysis and polymorphic information content

In Gel Documentation Unit, digital camera took a photograph of the gel to score banding results. Bands were shown according to their molecular size (length of polynucleotide amplified). To evaluate the molecular scale, each sample of primer was filled by 1 kb DNA Ladder. The distance covered by bands generated from the well has been converted into molecular sizes in relation to molecular marker weight. Absence of band was scored as '0' and existence of band as '1'. Lightly seen bands weren't scored however measured for scoring if found equivalent to major band. To check the existence of the bands and to assess the reproducibility, all the tested primers were repeated three times.

Genetic profile of *Dolichos lablab* (L.) were estimated by the performance of the primers which were calculated by means of following four parameters *i.e*. Polymorphic information content (Roldan-Ruiz *et al*. 2000), Mean

resolving power (Prevost and Wilkinson, 1999), Resolving power (Prevost and Wilkinson, 1999) and Matrix index (Varshney *et al*. 2007).

RESULTS AND DISCUSSION

SSR analysis

The bands of DNA were amplified from five SSR primers against *Dolichos lablab* (L.). From the molecular weight of 1636 base pair to 128 base pair, different primers revealed a total of fifty-one bands indicating 88.23 percent polymorphism (45 bands). The polymorphism varies between 90% and 100%. Banding arrangement with primer AGB 9 generates the largest no. of bands *viz*. 15 followed by 11 bands (VM 4); 10 bands (CEDG 198 and DMB SSR 182) while, primer (111) revealed a total of 6 bands which is least (Table 2, Fig 1).

The ten bands generated with primer DMB SSR 182 exhibited 90 percent of polymorphism. The uppermost band generated at about 1247 bp and the lowest at about 176 bp. Ten bands generated with primer CEDG 198 exhibited 90 percent of polymorphism. The uppermost band generated at about 822 bp whereas the lowest at about 190 bp. Against primer VM 4, eleven bands were generated; of which polymorphic bands are nine thus revealing 90.90 percent polymorphism. The uppermost band generated at about 562 bp whereas the lowest at about 128 bp. The pattern of banding generated by primer AGB 9 revealed 15 bands having 80% percent polymorphism among the accessions considered. The uppermost band generated at about 738 bp and the lowest at 178 bp. Finally, the pattern of banding generated with primer 111 intensified six bands with 100 percent polymorphism rate. The uppermost band generated at about 1636 bp and the lowest at 152 bp (Table 1).

SSR Markers are co-dominant (Narshimulu *et al*. 2011) and locus-specific and generally amplify one locus (Gupta and Varshney, 2000). These markers evaluate the variability and the identification of connections between accessions

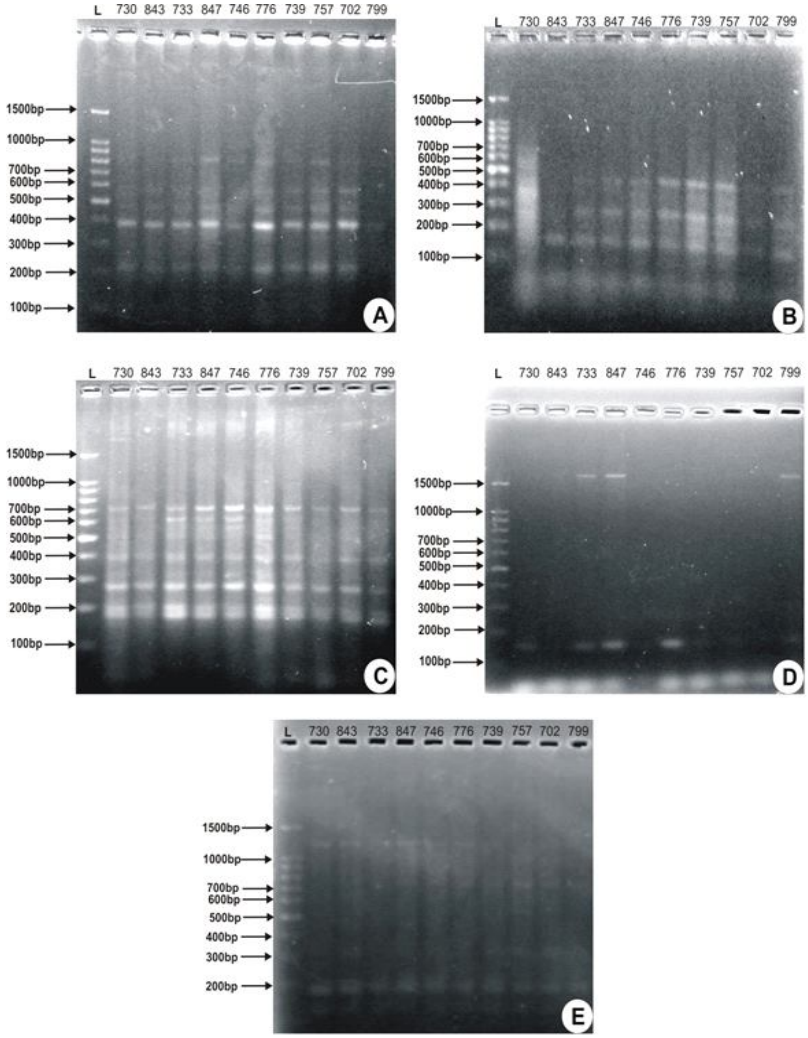

Fig 1: (A-E) Banding patterns formed from *Dolichos lablab* (L.) genotypes by the use of five SSR primers (A) VM 4, (B) CEDG 198, (C) AGB 9, (D) 111, (E) DMB-SSR 182.

Table 1: Band Matrix of 10 *Dolichos lablab* (L.) genotypes with all SSR primers.

Details	M.W.	Rf scores	730	843	733	847	746	776	739	757	702	799	Remark
	1636	0.254	0	0	0	0	0	0	0	0	0	1	Poly
	1611	0.259	0	0	1	1	0	0	0	0	0	0	Poly
111	211	0.781	0	0	0	0	0	0	1	0	0	0	Poly
	176	0.815	0	0	0	0	0	0	0	0	1	1	Poly
	166	0.828	0	0	0	0	0	1	0	1	0	0	Poly
	152	0.843	1	0	1	1	0	0	0	0	0	0	Poly
	738	0.427	1	1	1	1	1	1	1	1	1	1	Mono
	700	0.440	0	0	0	0	0	1	0	0	0	1	Poly
	633	0.464	0	0	1	1	1	1	0	0	0	0	Poly
	541	0.506	0	0	0	0	1	0	0	0	0	0	Poly
	520	0.519	0	0	0	1	0	0	0	0	0	0	Poly
	513	0.524	1	1	1	0	0	0	0	0	1	1	Poly
	400	0.595	1	1	1	1	0	0	0	0	0	0	Poly
AGB 9	368	0.616	0	0	0	0	0	0	0	0	1	1	Poly
	392	0.600	0	0	0	0	1	1	1	1	0	0	Poly
	335	0.640	0	0	1	0	0	0	0	0	0	0	Poly
	315	0.655	0	0	0	0	0	0	1	0	0	0	Poly
	266	0.700	1	1	1	1	1	1	1	1	1	1	Mono
	210	0.766	0	0	1	0	1	0	0	0	0	0	Poly
	200	0.779	1	1	0	1	0	1	1	0	1	1	Poly
	178	0.806	1	1	1	1	1	1	1	1	1	1	Mono
	822	0.449	0	0	0	1	0	0	0	0	0	0	Poly
	786	0.462	0	0	0	0	0	1	0	1	0	0	Poly
	553	0.557	0	0	0	0	0	1	0	1	1	1	Poly
	543	0.563	0	0	0	0	0	0	1	0	0	0	Poly
CEDG	432	0.624	0	0	0	0	0	1	0	0	0	0	Poly
198	451	0.614	0	0	0	1	0	0	1	1	1	0	Poly
	371	0.662	1	1	1	1	1	1	1	1	1	1	Mono
	207	0.808	1	1	0	0	0	0	0	0	0	0	Poly
	200	0.814	0	0	1	1	0	0	1	0	1	0	Poly
	190	0.822	0	0	0	0	1	1	0	1	0	1	Poly
	1247	0.365	0	0	1	1	1	1	0	0	0	0	Poly
DMB	1223	0.370	1	1	0	0	0	0	1	1	0	0	Poly
SSR	1034	0.413	0	0	0	0	0	0	0	0	0	1	Poly
182	978	0.426	0	0	0	0	0	0	0	0	1	0	Poly
	946	0.434	0	0	0	0	0	0	0	1	0	0	Poly
	793	0.491	0	0	0	0	1	1	1	0	1	1	Poly
	758	0.504	0	0	1	0	0	0	0	1	0	0	Poly
	314	0.746	0	1	0	0	0	0	0	0	0	0	Poly
	294	0.762	0	0	0	0	0	0	0	1	1	1	Poly
	176	0.877	1	1	1	1	1	1	1	1	1	1	Mono
	562	0.391	1	0	0	0	0	0	0	0	0	0	Poly
	450	0.432	1	0	1	1	1	0	0	0	0	0	Poly
	400	0.454	0	0	0	0	0	1	1	1	1	0	Poly
	365	0.472	0	0	0	0	0	0	0	0	0	1	Poly
	305	0.511	1	0	0	0	0	0	0	1	0	0	Poly
VM 4	274	0.529	1	0	0	0	0	0	0	0	0	0	Poly
	256	0.540	0	0	0	1	1	1	0	0	0	0	Poly
	225	0.558	0	1	0	0	0	0	1	1	0	0	Poly
	183	0.584	1	0	0	0	0	0	1	0	1	1	Poly
	151	0.607	0	1	0	0	0	1	1	1	0	0	Poly
	128	0.625	1	1	1	1	1	1	1	1	1	1	Mono

for breeders (Li *et al*. 2011). From the molecular weight of 1636 base pair to 128 base pair, different primers revealed a total of fifty-one bands indicating 88.23 percent polymorphism. The polymorphism varies between 90% and 100%. Banding arrangement with primer AGB 9 generated the largest no. of bands *viz*. 15 while, primer (111) revealed a total of 6 bands which is the least. In evaluating *Dolichos lablab* (L.), SSR markers have proven a more accurate variety in genes. In 143 germplasm of bean (Indian) with one hundred and thirty-four SSR markers (*Lablab*

Table 2: Polymorphism detected and Parameters by the use of 5 SSR primers.

Name of Primer	Total no. of Bands	No. of polymorphic Band	% of Polymorphic Bands	PIC	MRP	RP	MI
DMB SSR 182	9	8	88.88	0.435	0.67	6.00	3.09
CEDG198	10	9	90.00	0.321	0.66	6.60	2.60
VM4	11	10	90.90	0.445	0.65	7.20	4.04
AGB9	15	12	80.00	0.488	0.84	12.6	4.68
111	6	6	100.00	0.265	0.37	2.20	1.59

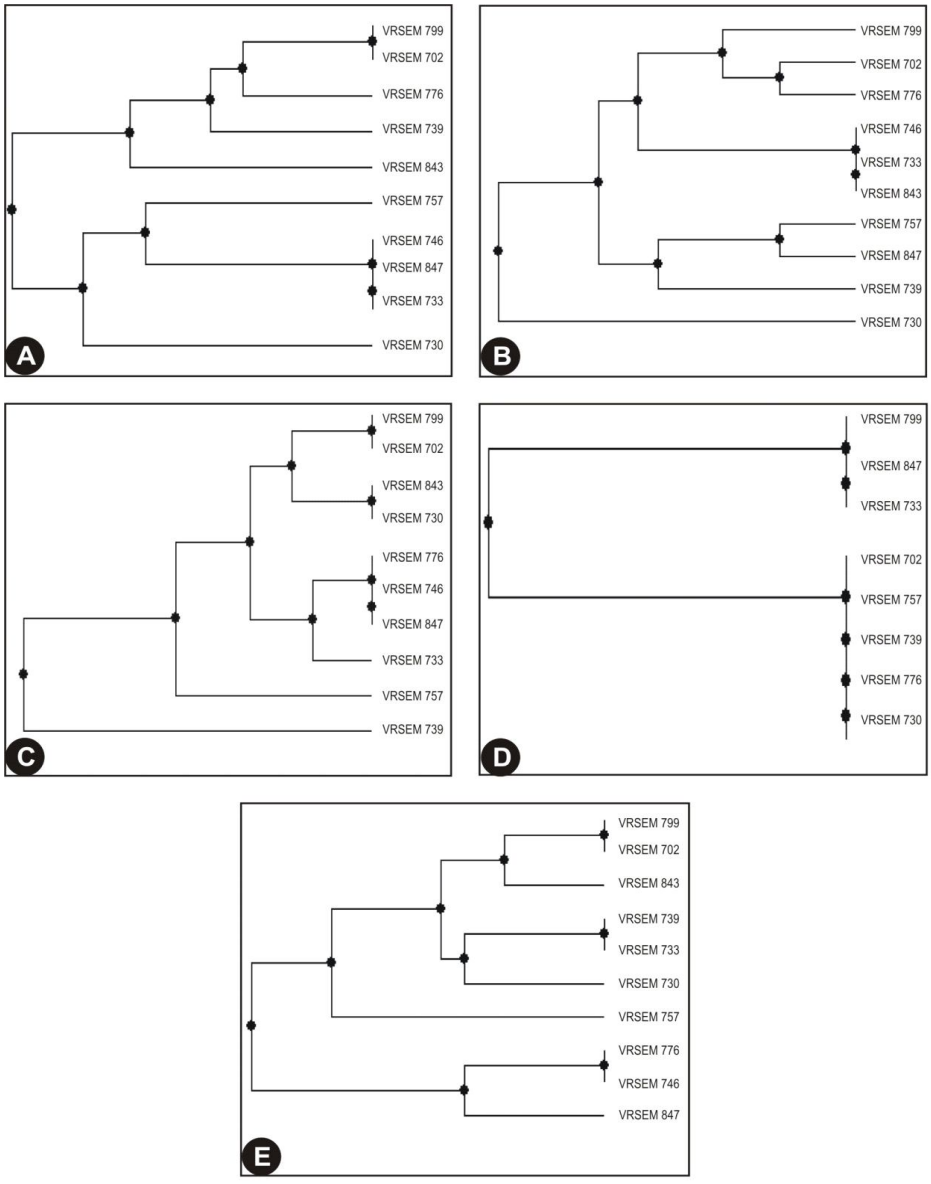

Fig 2: (A-E) Dendogram obtained from five SSR primers based on genetic distance (A) VM 4, (B) CEDG 198, (C) AGB 9, (D) 111, (E) DMB-SSR 182.

purpureus), genetic diversity is reported by Rai *et al*. (2011). With the 5 RAPD primers, ten *Dolichos lablab* (L.) accessions demonstrated major genetic variation. As a result, primer AGB 9 produced max. numbers of bands (15) and bands with a minimum number were revealed by primer 111 *i.e*. 6. Our findings support Zhong *et al*. (2008) and Zhang *et al*. (2013) who investigated various markers and identified a large degree of variability between the twenty-four *Dolichos lablab* (L.) accessions studied (Keerthi *et al*. 2018). The accession classification based on the EST-SSRs was similar to the genomic AFLP and SSR work by Woodhead *et al*. (2005). The variation in the band number, governed by various markers (Kernodle *et al*. 1993) is induced by the various variable factors like the structure of primer, some annealing site, *etc*. The revealed bands also had specific molecular weight to check the distinctions in length between primary connections and the DNA that resulted in band changes (Devos and Gale, 1992).

Data analysis and polymorphic information content

Performance of marker was checked by the use of different parameters *i.e*. polymorphic Information Content, Resolving Power, Mean Resolving Power and Matrix Index. Primer 111 (0.265) has lowest PIC value whereas primer AGB 9 had highest (0.488) with a mean PIC value of 0.390. In compare, Asare *et al*. (2010) imply low value of PIC about 0.38 at an average of 3. 8 alleles for every loci in 141 cowpea genotype gathered starting with nine geographic locales for Ghana. In 92 common bean PIC value detected is 0.54 (Díaz and Buendía, 2011). Similarly, Benchimol *et al*. (2007) found a PIC value varied from 0.05 - 0.83 while studying genetic dry beans diversity with 87 SSR loci the average value of PIC was 0.45. PIC values range from one (very high discriminative power with many alleles in equal frequencies) to zero (which is an indicative of monomorphism) and the higher the PIC value, the more informative is the SSR marker (Nagy *et al*. 2012). The value of Matrix Index ranges from 4.68 (AGB 9) to 1.59 (111) with a mean of 3.2. Higher the MI, better the method is. The value of resolving power ranges from 2.2 to 12.60. The highest value of RP and MRP was recorded for the primer AGB 9 and lowest for primer 111 (Table 2).

Genetic relationship and phylogenetic study

For every Marker, Genetic Relationship among the Genotypes was determine based on Jaccard's Pairwise similarity coefficient. Genetic Relationships and pooled diversity data by using marker grouped each genotype into its clusters. According to Beaumont *et al*. (1998) the genetic gap is any quantitative evaluation of genetic difference at the level of the sequence or the level of allele frequency, measured between persons, populations or species. Pairwise similarity among the genotypes ranged from 0.55 to 0.93 (Primer-AGB-9), followed by 0.33 to 0.89 (Primer-CEDG 198), 0.40 to 0.86 (Primer- VM4), 1 to 0.67 (Primer 111) and 0.33 to 0.86 (Primer DMB-SSR182).

Dendrogram formed was derived from the matrix similarity analysis by UPGMA (Unweighted pair group method with arithmetic averages) cluster analysis by using GenAlEx (Genetic Analysis in Excel, version 6.2) software (Peakall and Smouse, 2006). Based on the genetic distances with all primers, the UPGMA neighbour-joining tree method generated two distinct clusters for the 10 *Dolichos lablab* (L.) genotypes (Hair *et al*. 1995) (Fig 2). Thus, Cluster analysis groups individuals or objects based on characteristics they possess so that individuals with similar descriptions are mathematically gathered into the same cluster (Atienza *et al*. 2005).

CONCLUSION

It is conclude that SSR markers are helpful in the evaluation of *lablab* diversity and the choice of a collection to make strides the proficiency of genotypes for utilizing in *lablab* conservation and breeding. Studies shows the presence of moderate genetic variability among the elite *Dolichos lablab* (L.) genotypes. It confirmed that the potential efficiency of SSR molecular markers in cultivars of *lablab* and landraces classification and indicate the feasibility of comprehensive attempt in determining the relationships among *lablab* landraces by the use of some molecular markers. The success were improved by using SSRs based EST-SSRs on (expressed sequence tags-SSR) instead of using genomic SSR (g-SSRs) because EST-SSRs comes from genome transcribed regions and are expected to be conserved across a larger taxonomic range. It is studied that EST-SSRs were three times more transferable across genus *Helianthus* species as compared to g-SSRs.

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