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Short Communication

Molecular characterization of Indian Dolichos bean (*Lablab purpureus* L. var. typicus Prain) accessions using RAPD markers

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Abstract

In this study, 29 genotypes of (*Lablab purpureus*) Indian bean augmented from different geographical regions were analyzed for genetic diversity using 39 RAPDs. The binary matrix data revealed 97.4% polymorphism among genotypes, with 2322 amplification fragments. The PIC values ranged from 0.25 to 0.96 with an average of 0.70. Markers OPA05, OPB15, and OPV12 showed high discrimination among all the genotypes. A magnificent range of Jaccard's genetic similarity coefficient was evaluated and UPGMA based dendrogram classified genotypes into 4 major clusters. IC-384066 and NSJ/NAIP/ 192 were found to be unique and highly diversified. The same grouping patterns were also observed in the model-based STRUCTURE analysis (K=2). The study showed the degree and distribution of genetic diversity in Dolichos bean, which can be utilized to identify the parental lines, to develop mapping populations and breeding.

Key words: Dolichos bean, genetic diversity, RAPD, STRUCTURE, UPGMA,

Dolichos bean (lablab) is one of potential source of protein (20-25%) having high nutritional value. Lablab also is known as Indian bean, is a self-pollinated crop with varying chromosome numbers (2n=20, 22, 24). Assessment of genetic diversity in a crop species is a prerequisite for the enhancement of crop yield and desirable traits as a part of effective and scientific breeding. Numerous plant genetic diversity studies have been conducted using a wide range of molecular markers like RAPDs, SSRs, AFLPs, ISSRs, and SNPs in the last three decades (Mondini et al. 2009). Among these, Random Amplified Polymorphic DNAs (RAPDs) are widely used in genetic diversity studies due to their quick detection of polymorphisms at a number of loci using small amounts of genomic DNA (Rai et al. 2010 and Pidigam et al. 2019). Dolichos bean has been neglected in terms of research and development based on molecular markers and consequently limiting the genetic diversity studies (Maass et al. 2005; Islam 2008). Therefore, the present investigation was carried out to determine the extent of genetic diversity among Indian Dolichos bean accessions.

The plant genetic material consists of 29 Dolichos bean germplasm augmented from Chhattisgarh, Telangana, Andhra Pradesh, Tamilnadu, Bihar and Karnataka including 3 check varieties. Young leaves were collected from each Dolichos genotype at 20 days old seedling stage and genomic DNA was extracted following CTAB method (Doyle and Doyle, 1990) with minor modifications.

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The isolated DNA samples were run on 0.8% agarose gel to assess the quality and the quantity was checked by using Biophotometer (Eppendorf make). A total of 39 RAPD primers (Table 1) were evaluated using the genomic DNA of Dolichos germplasm, for their capability to amplify clear and polymorphic bands. The amplification was carried out in a total reaction volume of 20.00l mixture. PCR products were visualized on 1.5% agarose gel. The banding patterns of genotypes were scored manually. This binary data was used for further analysis. The PIC value was calculated as the measure of the effectiveness of genetic marker in diversity studies using the formula (Botstein et al. 1980). The genetic associations between genotypes were evaluated by calculating the jacquard's similarity coefficient using software NTSYSpc2.0 (Rohlf, 1998). The unweighted pair-group method with arithmetic averages (UPGMA) was used to construct the dendrogram.

The binary data obtained from the RAPD primers banding patterns has resulted in 97.4% polymorphism, whereas 100% polymorphism was observed at the allelic level, with a total number of 2322 amplicons. The number of alleles ranged from 2 to 8 with a mean value of 4.1 per marker. RAPDS1027 and OPD5 yielded the highest number of alleles (8) and OPA18 followed with 7 alleles. The calculated PIC value based on allelic frequency was in the range of 0.25 to 0.96, with the mean value of 0.70. Highest PIC value was showed with OPA5, OPB15, and OPV12. Nearly 60% of the primers yielded higher PIC values than the average value.

The cluster analysis using NTSYs software evaluated the Jaccard's genetic similarity coefficient in the range of 0.12 to 0.86. The UPGMA method-based dendrogram showed 4 major clusters. Cluster I and cluster II are again divided into two sub-clusters each. Cluster I consisting of 15 genotypes, and cluster II is with 12 genotypes. IC-446571 and IC-546387 show the highest similarity coefficient of 0.86, and one more pair SDG-136, RND-01 illustrated 0.83 of similarity coefficient are located in sub-cluster-A of cluster I. The two bush-type varieties Arka Jay and Arka Vijay used in this study placed in sub-cluster-A of cluster-II

Table 1. RAPD primers used in diversity analysis

S.No.	RAPD primers	Sequence	Alleles	PIC	S.No.	RAPD primers	Sequence	Alleles	PIC
1	OPA01	CAGGCCCTTC	2	0.73	21	OPL05	ACGCAGGCAC	4	0.29
2	OPA02	TGCCGAGCTG	4	0.51	22	OPL07	TTGGCACGGG	2	0.78
3	OPA03	AGTCAGCCAC	4	0.39	23	OPL08	AGCAGGTGGA	5	0.89
4	OPA04	AATCGGGCTG	3	0.84	24	OPL12	GGGCGGTACT	3	0.62
5	OPA05	AGGGGTCTTG	4	0.96	25	OPS03	CAGAGGTCCC	3	0.85
6	OPA06	GGTCCCTGAC	5	0.73	26	OPS07	TCCGATGCTG	4	0.80
7	OPA07	GAAACGGGTG	5	0.84	27	OPS10	ACCGTTCCAG	5	0.57
8	OPA09	GGGTAACGCC	6	0.55	28	OPS18	CTGGCGAACT	3	0.89
9	OPA13	CACCACCCAC	3	0.26	29	OPV12	ACCCCCCACT	3	0.92
10	OPA18	AGGTGACCGT	7	0.75	30	RAPD1063	GGTCCTACCA	4	0.74
11	OPB01	GTTTCGCTCC	2	0.68	31	RAPD1234	TCGCAGCGTT	5	0.88
12	OPB02	TGATCCCTGG	3	0.66	32	RAPDS1027	ACGAGCATGG	8	0.64
13	OPB03	CATCCCCCTG	3	0.73	33	RAPDS1136	GTGTCGAGTC	4	0.78
14	OPB15	GGAGGGTGTT	5	0.92	34	RAPDS1155	GAAGGCTCCC	3	0.81
15	OPC07	GTCCCGACGA	5	0.54	35	RAPDS1184	GACGGCTATC	3	0.82
16	OPD05	TGAGCGGACA	8	0.50	36	RAPDS1189	AGTCCCCCTC	6	0.86
17	OPD07	TTGGCACGGG	6	0.72	37	RAPDS1265	GAGCTACCGT	4	0.70
18	OPE01	CCCAAGGTCC	3	0.84	38	RAPDS1358	ACCCCAACCA	5	0.52
19	OPH01	GGTCGGAGAA	4	0.71	39	OPL12	GGGCGGTACT	1	0.70
20	OPH02	TCGGACGTGA	3	0.59					

showed 71% similarity. IC-384066 and NSJ/NAIP/192 are highly diversified with other genotypes with the least similarity of the coefficient of 0.12 and 0.17. Genotype SNJ11-068 also showed a diversifying nature with other genotypes of clusters I and II (Basu et al. 2002).

The model-based STRUCTURE analysis (Pritchard et al., 2000) was carried out to investigate the relationships between the Lablab accessions. Based on the ÄK method, we acquired the best K value at K=2 (Fig. 1). Whereas, we also found increased ÄK peak value at K=7, K=8, K=3 and K=4, when ÄK values compared with LnP (D) values increased by k from 2 to 10. All over, the highest ÄK peak value at K=2, suggesting that 29 Dolichos accessions can be grouped into two subpopulations with each population consisting of 14 and 15 accessions, respectively. In addition, these two populations (at K=2) were studied for the number of individuals and admixtures. As a result, based on probabilities (e”80%), out of 29 genotypes, 21 were assigned to two groups and 8 genotypes were retained in as admixed. Among these two groups, population 1 had 11 and population 2 had 10 genotypes (Fig. 1).

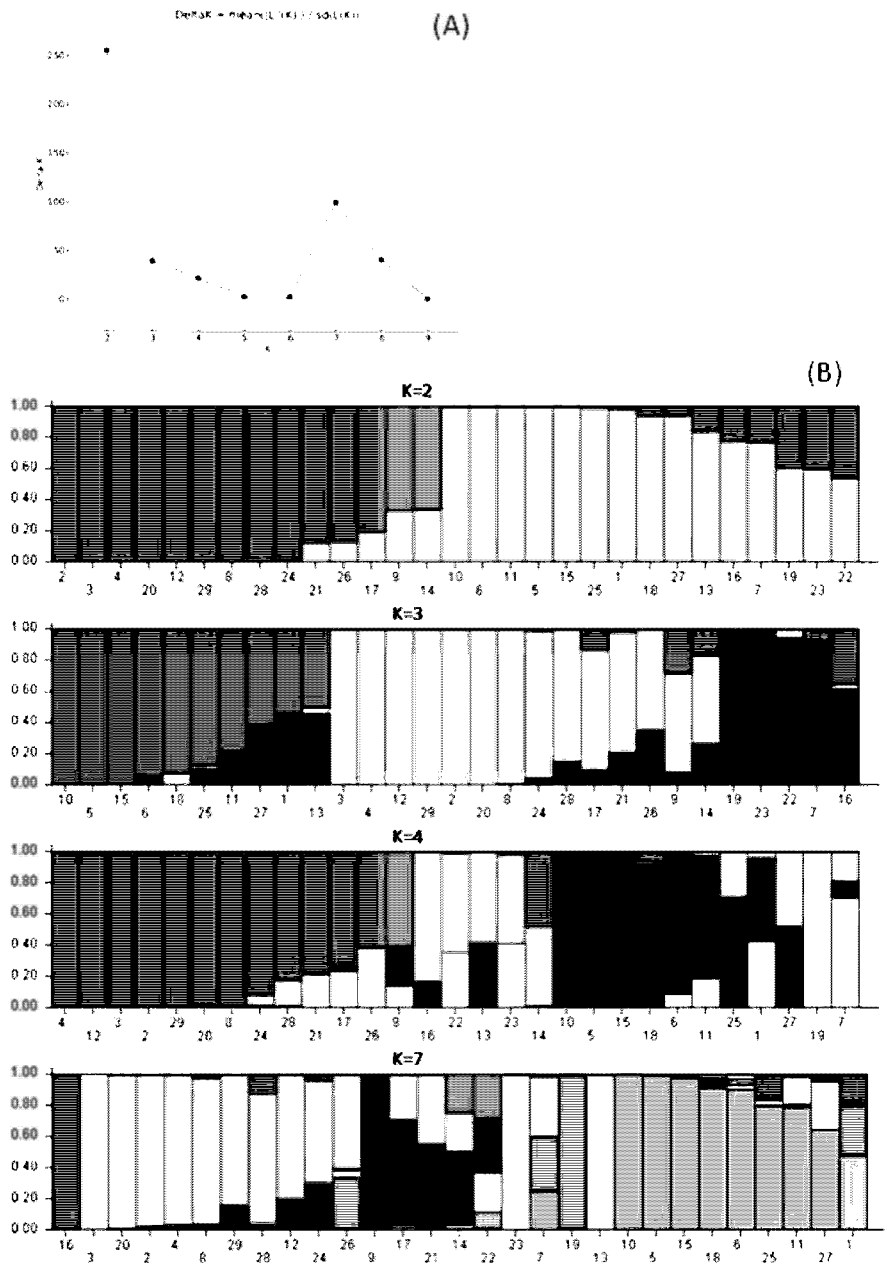

Fig. 1. A: Delta K value, B: Population structure inferred by model-based approach RAPDs of increased peak values of K

Genetic characterization of germplasm is an important element in the breeding program for an effective enhancement of crop and its utilization. Several studies have been conducted for genetic understanding of lablab using molecular markers *i.e*., Sujithra et al. (2009) and Rai et al. (2010). The present study, thus reported the utility of RAPD primers to provide insight into genetic diversity. Out of 39 primers, 38 (97.4%) generated clear polymorphic fragments. The binary matrix data retrieved from these markers produced a total number of 2322 amplicons with 100 % polymorphism. This level of polymorphism is higher than the previously reported by Rai et al. (2010), Gnanesh et al. (2006) and Saravanan et al. (2013), indicating a fair amount of variation at the DNA

level among these genotypes. A high degree of polymorphism was obtained with the primers OPD5, RAPDS1027 (8 bands), and OPA18 (7 bands). This variation in the number of amplified polymorphic bands with different primers is due to several factors like sensitivity of working environment, less number of annealing sites in the genome, primer, used DNA quantity and equipment. The highest PIC value of OPR15 observed by Saravanan et al. (2013), is very nearer to the highest PIC value observed in the present study with OPA5 (0.96), primers OPB15 (0.92) and OPV12 (0.92) followed with highest PIC value, suggesting the discrimination power of markers.

Cluster analysis was performed with the similarity coefficient, resulted in the range of 0.12 to 0.86 and very nearer to the other studies of lablab using RAPDs and AFLPs (Rai et al. (2010) and Maass et al. (2005)), which are within the range of 0.38 to 0.96 to 0.217 to 0.915, explaining the large genetic distances. UPGMA based dendrogram differentiated the 29 genotypes into four clusters, indicating a highly diversifying nature from the bulk of the materials. These genotypes may carry some particular characters or valuable genes for further improvement of the crop. Two genotypes *i.e.,* IC-446571 and IC-546387 showed the highest similarity (86%) among all the other genotypes indicating the close association between these two genotypes genetically. Moreover, the genotype IC-546387 also mentioned for its high number of pods and pod yield per plant. Overall, the wide range of Jaccard's similarity coefficient values suggests that a rich diversity exists between the genotypes from different geographical regions. As a result, it revealed that markers like RAPDs may accurately assay the degree of genetic change distinguishing two genomes in genetic diversity studies.

The model-based STRUCTURE analysis of the 29 Dolichos accessions revealed the highest likelihood, when the genotypes were grouped into two populations (K=2). This low value of K in the present investigation might be explaining the high amount of gene flow among genotypes (Henareh et al. 2016). Interestingly, the neighborhood of the genotypes in UPGMA based cluster analysis was similar to the results obtained through structure analysis at K=2 *i.e.,* cluster I placed in population2 and cluster 2, 3, 4 were placed in population1 and this type of similarity between UPGMA clustering and STRUCTURE analysis was also reported by different studies (Surapaneni et al. 2016). Veluru et al. (2019) carried out STRUCTURE analysis based on microsatellite allelic data also partitional 148 rose genotypes into different populations with some individual genotypes having genomic adminixture. Fst values obtained (population differentiation), could be considered as a strong population sub-division among the accessions. The differentiation of Fst values was used as a summary of genetic discrimination between two groups, which rely on the allele frequencies at a particular locus responsible for a variety of peculiar properties related to genetic diversity (Jakobsson et al. 2013). The admixture of genotypes in the populations could be due to the mixed ancestry from parents belonging to different gene pools.

We conclude that RAPD markers were effective in discriminating the different Dolichos bean genotypes for genetic diversity. Markers OPA5, OPB15 and OPV12 with the highest PIC value suggesting the high discrimination power in the diversity analysis. Three genotypes viz., SNJ11-068, NSJ/NAIP/192 and IC-384066 were identified as highly diversified. The STRUCTURE analysis also showed a similar vicinity of genotypes to the result obtained by the UPGMA cluster analysis. Thus, the present investigation results are useful to identify the parental lines in breeding programs, to develop mapping populations and hybrid development for exploiting natural genetic variation available in the accessions.

Authors' contribution

Conceptualization of research (SP, PS); Designing of the experiments (SP, PS, GA); Contribution of experimental materials (SM, SN); Execution of field/ lab experiments and data collection (SP, PS, GA); Analysis of data and interpretation (SP, GA, HY, PS); Preparation of manuscript (SP, GA, HS, PS, HY).

Conflict of interest

The authors declare that there is no conflict of interest.

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