African Journal of Biotechnology Vol. 9 (2), pp. 137-144, 11 January, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Genetic relationship among Hyacinth bean (*Lablab purpureus*) genotypes cultivars from different races based on quantitative traits and random amplified polymorphic DNA marker

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Accepted 2 October, 2009

Genetic improvement of the thirty Hyacinth bean cultivars from different races were evaluated using RAPD markers which is essential to enhance the crop for economically and agriculturally important traits. RAPD markers were efficient in separating cultivars according to different races. Twenty six decamer primers could generate a total number of 148 bands out which 70.27% (104) were polymorphic. The number of amplification products produced by each primer varied from 4 to 9 with an average of 5.69 bands per primer. The size of amplified fragments ranged from 250 to 3000 bp. RAPD based dendrogram showed similarity ranged from 0.38 to 0.96. Genetic distances were concluded using Sorenson Dices similarity coefficient. The genetic relationship obtained with these markers was dissimilar to that provided by morpho-agronomical trait. Genetically distinct cultivars were identified that could be potentially important source of germplasm for further crop improvement programme in the country.

Key words: *Lablab purpureus*, Hyacinth bean, genotypes, genetic variation, dendrogram, RAPD marker.

INTRODUCTION

Hyacinth bean (*Lablab purpureus*) belongs to the family Leguminosae (Fabaceae), sub-family Papilionacae and genus Lablab with varying chromosome number of 2n = 20, 22, 24. It originated from India (Deka and Sarkar, 1990) and is a good source of minerals and vitamins (Basu et al., 2002) with antioxidants (Bradley, 1999). 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 Indian bean using the modern molecular approaches.

This would allow a more efficient utilization of plant characters in developing suitable varieties for yield and stability. In addition, an unambiguous, reliable, fast and cost-effective assessment of genetic diversity is important for determining the uniqueness and distinctiveness of the phenotypic and genetic constitution of genotypes to protect breeder's intellectual property rights (Franco et al., 2001). The cultivars however are characterized by extensive genetic variation; currently these selected germplasm are of two varieties that is, *Dolichos lablab* variety typicus and legnosus. These groups have been subdivided into races based mainly on morphological, agronomical and evaluation of the corresponding germplasm considering the importance of these races for germplasm management and genetic improvement and the hypothesis that a greater combination ability is expected in inter racial crossing (Singh et al., 1991). Among the different types of molecular markers, randomly amplified polymorphic DNAs (RAPDs) are useful for the assessment of genetic diversity (Williams et al., 1990)

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Table1. Description of Hyacinth bean germplasms/cultivars used in present study.

Genotypes	Varieties	Source (Place of collection)	Agro-climatic zone in India
VR SEM-97	Typicus	Raigarh, Chhattisgarh	Zone-VII
VR SEM-145	Typicus	Raipur, Chhattisgarh	Zone-VII
VR SEM-100	Typicus	Ambikapur, Chhattisgarh	Zone-VII
VR SEM-125	Typicus	Balaghat, Madhya Pradesh	Zone-VII
VR SEM-18	Typicus	Bastar, Chhattisgarh	Zone -VII
VR SEM-45	Lignosus	Bastar, Chhattisgarh	Zone -VII
VR SEM-930	Lignosus	Dewaria, Uttar Pradesh	Zone- IV
VR SEM-804	Typicus	Dewaria, Uttar Pradesh	Zone- IV
VR SEM-938	Typicus	Dewaria, Uttar Pradesh	Zone- IV
VR SEM-924	Typicus	Kanpur, Uttar Pradesh	Zone -IV
VR SEM-923	Lignosus	Kanpur, Uttar Pradesh	Zone -IV
VR SEM-934	Typicus	Bhadohi, Uttar Pradesh	Zone -IV
VR SEM-946	Typicus	Bhadohi, Uttar Pradesh	Zone -IV
VR SEM-752	Typicus	Varanasi, Uttar Pradesh	Zone -IV
VR SEM-764	Lignosus	Varanasi, Uttar Pradesh	Zone -IV
VR SEM-720	Typicus	Varanasi, Uttar Pradesh	Zone -IV
VR SEM-704	Lignosus	Varanasi, Uttar Pradesh	Zone- IV
VR SEM-949	Typicus	Jaunpur, Uttar Pradesh	Zone -IV
VR SEM-933	Typicus	Jaunpur, Uttar Pradesh	Zone- IV
VR SEM-772	Typicus	Samastipur, Bihar	Zone -IV
VR SEM-802	Lignosus	Samastipur, Bihar	Zone- IV
VR SEM-948	Typicus	Haridvar, Uttaranchal	Zone-I
VR SEM-501	Lignosus	Muchakova, Assam	Zone- II
VR SEM-201	Lignosus	East Khasi Hills, Meghalaya	Zone -III
VR SEM-101	Lignosus	East Khasi Hills, Meghalaya	Zone -III
VR SEM-186	Typicus	West Tripura, Tripura	Zone- III
VR SEM-7	Typicus	West Tripura, Tripura	Zone -III
VR SEM-8	Typicus	North Tripura, Tripura	Zone -III
VR SEM-6	Typicus	North Tripura, Tripura	Zone- III
VR SEM-3	Lignosus	North Tripura, Tripura	Zone -III

owing to their simplicity, speed and relatively low-cast (Rafalski and Tingey, 1993) compared to other types of molecular markers.

Another important association of divergence is with heterosis, since the extensiveness of this parameter also depends on the divergence among the cultivars. If these correlations are elevated, one may infer that the markers are good predictor of a population behavior. The genetic divergence of 30 Hyacinth bean cultivars from different races was evaluated using RAPD markers. The level of association of this divergence with some indirect variability estimates obtained in field experiment was also estimated to evaluate the usefulness of these markers as predictors of morphological agronomical variability among cultivars.

MATERIALS AND METHODS

Plant genetic material

The plant genetic material for this investigation comprised of thirty genotypes of Hyacinth bean including nineteen typicus var. and eleven lignosus var. germplasm collected from different parts of India (Table 1).

Field evaluation and data collection

The field evaluation of Hyacinth bean accessions was conducted at research farm of Indian Institute of Vegetable Research, Varanasi, India. Seeds were sown on the raised bed with spacing row to row 2 m and seed to seed 1 m. The experiment was led out in a complete randomized block design with three replications; each replication

had fifteen plants. Five plants were randomly chosen and data was recorded on eleven quantitative traits; days to first flower, days to first picking, pods per plant, per cent fruit set per cluster, pod length, pod width, seeds per pod, seed length, seed width, 100-seeds weight and pod yield per plant. Pods were harvested at maturity, for recording the data related to pods. The pod length, pod width, seed length, and seed width were measured with the help of meter scale and Vernier caliper. Two years pooled data for quantitative traits were analyzed for complete randomized block design (Gomez and Gomez, 1984). The effect of different scales of measurement for different quantitative trait were minimized by standardizing the data for each trait separately prior to cluster analysis; the STAND module of NTSYS–pc (Rholf, 1998) software was used to achieve the same. Pair wise distance matrix was used as an input for analysis of clusters. UPGMA-based clustering was done using SAHN module of NTSYS- pc.

Genomic DNA extraction

In the present investigation thirty germplasm belonging to two varieties that is, lignosus and typicus collected from different places of India (Table 1). Immature unaffiliated leaves were used for extracting DNA by CTAB (Cetyl trimethyl ammonium bromide) with some modifications in protocol (Doyle and Doyle, 1990). The quantity and quality of the isolated DNA were determined using Dyna Quant 200 Fluorimeter, 0.8% agarose gel stained with ethidium bromide (etbr).

PCR and agarose gel electrophoresis

The RAPD analysis was performed with 26 ten decamer primers supplied by Operon Technologies Inc. California USA (Table 2) following known protocol with minor modifications (William et al., 1990). The PCR amplification was carried out in 25 µl reaction mixture, containing 15 ng genomic DNA, 2.5 mm $MgCl_2$, 100 µm dNTPs, 10X assay buffer, 0.2 µm primer, and 0.6 U of Taq polymerase. Amplification was performed in dome shaped capillary tubes in Bio-Metra Thermal Cycler - programmed as one cycle of initial denaturation at 94°C for 1 min, 40 cycles each of denaturation at 94°C for 1 min annealing at 34°C for 1 min and elongation for 1 min at 72°C and final elongation at 72°C for 10 min. The amplified products were separated by electrophoresis in 1.4% (W/V) agarose gels, stained with ethidium bromide and photographed under ultraviolet light with Alfa InfoTech.

RESULTS

Performance of the genotypes based on quantitative traits

The mean of data on performance of the genotypes with respect to yield and yield-associated traits is shown (Table 2). The first flower, VR SEM-752 was very early in appearance (45 days after seed sowing), whereas VR SEM-946 was recorded to be late (123 days after seed sowing) flowering. The first picking is from genotype VR SEM-752 (121 days after seed sowing), while VR SEM-772 (151 days after seed sowing) recorded late picking. VRSEM-125 exhibited maximum number of pods/plant (724.00), while maximum percentage fruit set/cluster (66.33) was recorded in genotype VR SEM-772. The pod length (15.64) and pod width (3.51) was maximum in genotypes VR SEM-934 and VR SEM-501, respectively. The genotype VR SEM-930 showed maximum number of seeds/pod (5.67), whereas maximum 100-seeds weight (45 g) was recorded in genotype VR SEM-501. The longest seed length was in genotypes VR SEM-720 (1.85 cm), while the longest seed width is in VRSEM-3 (0.94). The maximum yield/plant was recorded in VR SEM-948 (3.55) followed by VR SEM-752 (3.06 kg).

Cluster analysis of the genotypes based on quantitative traits

The taxonomic distance matrix of 11 quantitative traits for the 30 genotypes was employed for cluster analysis and dendrogram was constructed (Figure 1). Distance between all pairs of genotypes was calculated using squared Euclidean distance method and genotypes were clustered based on Word's method. The 30 genotypes were mainly divided at the first node into 2 clusters with 26 and 4 genotypes in different groups. The cluster with 26 genotypes was again divided into 2 groups at the second node with 20 and 6 genotypes. Similarly, 20 genotypes were further divided into 2 groups at the third node with 11 and 9 genotypes. Cluster with 4 genotypes was again divided into 2 groups. It is inferred from the present investigation that hybridization involving the inter-cluster representatives of cluster would be more useful in Hyacinth bean breeding programme.

Performance of genotypes based on RAPD marker

In this study, RAPD markers were used to examine the degree of genetic variation within the thirty Hyacinth bean cultivars. RAPD banding patterns amplified by 26 random decamer primers (Table 3) generated a total of 148 amplification products, of which 70.27% (104) were found to be polymorphic. The size of amplified product from all the primers varied between 250 to 3000 bp. On an average, each primer amplified 5.69 bands per primer. Primers OPD 09, OPP 02, OPR 01 and OPT 05 generated only 4 amplification products, whereas a maximum of 9 products were amplified with primers OPT 15. The amplified DNA band was scored as 1 (present) or 0 (absent). The data matrix so generated through 26 RAPD primers from the 30 genotypes was used for calculation of similarity matrix based on Jaccords coefficients (Jaccard, 1908). A typical electrophoresis gel showing polymorphism detected by OPD 09 among all Hyacinth bean cultivars is illustrated in Figure 2.

RAPD marker diversity

According to the dendrogram of *L. purpureus*, all the genotypes except VRSEM-804 were grouped into a

Table 2. Mean of the 11 quantitative characters of 30 Hyacinth bean genotypes, average of two years (2006 and 2007).

Genotype	Days to First flower	Days to first picking	Pods /Plant (no.)	Fruit set /cluster (%)	Pod length (cm)	Pod width (cm)	Seeds /pod (no.)	Seed length (cm)	Seed width (cm)	100-Seed weight (gm)	Yield /plant (kg)
VR SEM-97	115.33	136.33	337.00	44.00	11.57	1.27	5.33	1.23	0.85	41.33	2.30
VR SEM-101	56.33	126.33	628.33	34.33	8.74	2.23	4.00	1.10	0.81	44.33	2.35
VR SEM-201	55.00	121.33	503.33	52.33	9.43	2.30	4.00	1.15	0.84	36.33	1.88
VR SEM-930	121.00	145.33	56.00	62.00	9.34	2.50	5.67	1.13	0.86	36.00	1.86
VR SEM-501	111.00	134.33	292.67	45.00	11.25	3.51	5.33	1.21	0.84	45.00	2.45
VR SEM-938	117.00	142.00	146.00	56.33	8.90	2.24	4.33	1.15	0.75	42.00	2.37
VR SEM-949	108.00	130.00	232.67	51.67	11.93	1.52	4.33	1.15	0.75	35.33	2.37
VR SEM-933	117.67	140.00	107.67	54.33	10.13	1.39	4.67	1.06	0.85	31.67	1.42
VR SEM-924	97.67	138.00	366.00	37.00	12.43	1.65	5.00	1.16	0.70	35.33	2.26
VR SEM-923	118.00	137.67	233.67	56.00	7.07	1.72	4.00	0.94	0.72	34.33	1.72
VR SEM-948	109.00	139.33	211.33	51.67	9.87	2.86	4.00	1.20	0.76	37.33	3.55
VR SEM-934	106.67	143.00	203.33	54.67	15.64	1.42	5.00	1.35	0.86	37.00	0.85
VR SEM-946	123.00	142.00	160.33	46.33	14.65	1.82	5.33	1.28	0.84	41.33	1.86
VR SEM-802	120.00	136.00	175.00	35.33	11.41	0.55	4.00	1.20	0.83	33.33	2.39
VR SEM-752	45.00	88.00	231.67	43.67	13.71	2.68	4.67	1.23	0.76	36.33	3.06
VR SEM-764	107.00	136.00	274.00	64.00	11.16	2.84	5.00	1.22	0.91	32.00	1.73
VR SEM-186	103.33	132.67	144.33	47.00	13.90	2.25	5.33	1.21	0.81	32.00	1.96
VR SEM-100	83.33	130.00	208.00	65.67	10.66	1.59	5.33	1.08	0.81	43.33	2.37
VR SEM-145	109.33	142.00	487.33	57.33	7.43	0.95	3.67	1.36	0.70	39.33	3.15
VR SEM-7	110.00	132.33	140.67	53.00	10.63	1.95	4.33	1.19	0.87	34.00	2.20
VR SEM-18	111.33	141.67	239.00	63.00	9.86	2.35	3.67	1.04	0.85	25.00	1.75
VR SEM-8	107.67	136.00	168.00	47.67	14.30	3.20	5.00	1.32	0.85	32.00	1.44
VR SEM-6	104.00	130.67	295.00	38.00	12.81	2.13	5.00	1.26	0.89	37.00	2.14
VR SEM-3	97.00	125.33	145.00	33.33	11.85	3.00	4.33	1.32	0.94	27.33	1.33
VR SEM-125	106.00	146.00	724.00	46.67	6.91	1.12	3.67	0.89	3.09	22.67	1.75
VR SEM-720	92.67	144.00	234.67	44.67	8.53	3.81	4.67	1.85	0.73	20.67	1.17
VR SEM-704	106.33	135.33	90.33	42.33	11.53	2.43	4.33	1.16	0.83	23.33	1.18
VR SEM-772	121.00	151.33	200.67	66.33	8.57	2.33	4.67	1.26	0.87	31.00	1.08
VR SEM-45	106.67	134.00	391.33	45.00	9.50	2.26	4.33	1.11	0.74	22.00	1.70
VR SEM-804	108.00	141.67	164.00	55.00	13.37	1.45	4.33	1.36	0.90	31.00	2.24
CD at 5%	2.759	3.142	9.213	3.993	1.568	0.157	0.941	0.079	1.206	3.777	0.116

single cluster at a similarity coefficient of 0.50 (Figure 3). In the bigger cluster, two genotypes VR SEM-186 and VR SEM-97 did not group into any cluster and both are separated alone at similarity coefficient of 0.50 and 0.57, respectively. All the remaining genotypes except 6, showed two sub clusters at the similarity coefficient of 0.75 (containing 21 genotypes) viz. VR SEM -101 and VR SEM-934, VR SEM-930 and VR SEM-948, VR SEM-501 and VR SEM-100 showed similarities with each other at similarity coefficient of 0.78, 0.81 and 0.89 respectively. As expected, the VR SEM-97 and VR SEM- 804 had the lowest genetic similarity when compared to the other genotypes. The VR SEM-938 and VR SEM-704 had the highest genetic similarity at the coefficient value of 0.96. The average genetic similarity value among all the cultivars was 0.67 with a range of 0.38 to 0.96 coefficient value.

DISCUSION

Germplasm characterization is an important component of breeding programme for an effective and efficient management/utilization of plant genetic resources. Morphological markers have been used for assessment of relationships among Hyacinth bean genotypes and for estimating genetic diversity among germplasm lines. The germplasm used in present studies showed a wide variation in morphological traits like number of pods/plant and pod length. The number of pods/plant was more in small pod accession. The 2 genotypes VR SEM-948 and VR SEM-752 showed maximum potential for pod yield/plant due to their pod weight. The genotypes VR SEM-948 and VR SEM -752 could be utilized in future breeding programs for development of high yielding cultivars. Yield per plant showed maximum deviation from

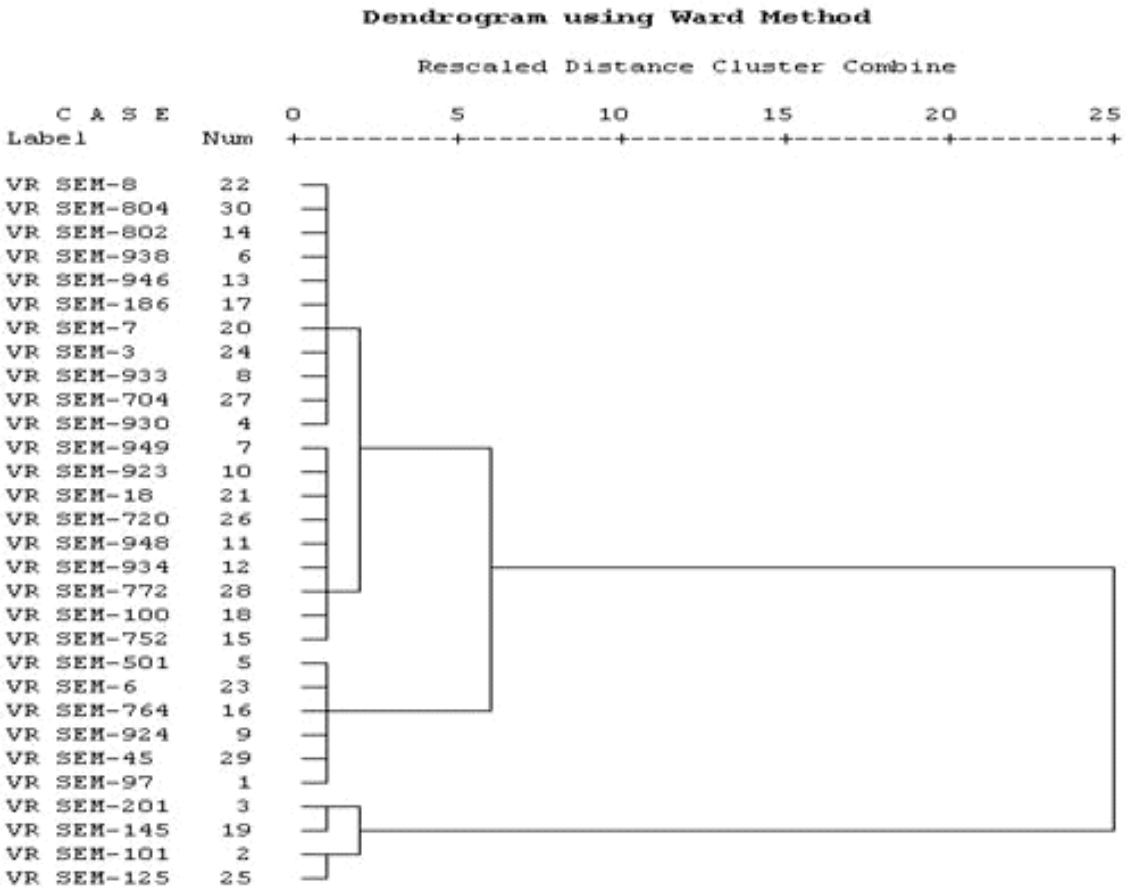

Figure 1. Genetic relationship among the 30 genotypes of Hyacinth bean based on 11 quantitative traits using Word's method of the distance matrix.

their mean and some of the traits like fruit weight, pod length, pod width, showed significant variation within the genotypes indicating the role of environment in expression of these traits. The level of genetic diversity between parents has been pro-posed as a mean of predicting hybrid performance and hetrosis of crosses.

DNA markers are preferable to morphological ones because they correlate variability directly at the genetic level and provide reliable and enormous data that permit a reproducible estimate of genetic diversity in the germplasm. The RAPD assay may generate a variety specific product in some of the genotypes. This may be used as a DNA fingerprint for variety identification. It would be of immense use for the establishment of proprietary rights and the determination of cultivar purity. Genetic erosion and habitat destruction by modern agriculture has increased the significance of collection of plant germplasm. Liu (1996) used RAPD marker for genetic relationship among 40 genotypes of *L. purpureus* and reported high level of genetic variation in various genotypes of common bean using RAPD molecular marker. Although morphological markers has been to study the relationship among common bean genotypes (Beebe et al., 1995) for assessment of genetic relationship among germplasm lines (Baswana et al., 1980; Baswana et al., 1980b; Biju et al., 2001), the information on the relationship among Hyacinth bean is limited.

In the cluster analysis, genotypes VR SEM-804, VR SEM-186, VR SEM-97 separate alone from the remaining 27 genotypes, indicating a high genetic diversity between these three and relationship among the other remaining genotypes. The remaining 27 genotypes separated into two sub cluster at similarity coefficient of 0.66. Sub cluster II contains genotypes VR SEM-930, VR SEM-948, VR-SEM 752, VR SEM-100, while sub cluster I contains the rest 23 genotypes. Further, sub cluster I separated into two sub clusters at similarity coefficient of 0.73, first one sub cluster containing only two genotypes VR SEM 101 and VR SEM-934. Second sub cluster is further divided into two sub clusters at similarity coefficient of 0.75, containing 11 and 10 genotypes. The genotypes from (Dewaria and Raipur), VR SEM-930 and VR SEM-145, (Dewaria and Varanasi) VR SEM-938 and VR SEM-704 grouped together in the same clustering based on quantitative trials as well as RAPD markers similarity. Genotypes VR SEM-186 and VR SEM-804 are grouped

Table 3. Random decamer primers used in present study, their sequence, number of polymorphic products, percent of polymorphic band and size of band produced by each primer.

RAPD primers	Primer sequence	Total number of bands amplified	Number of polymorphic bands	Percentage of polymorphic bands
OPA2	TGCCGAGCTG	5	3	60
OPA 4	AATCGGGCTG	6	4	66.66
OPB 1	GTTTCG CTC C	5	3	60
OPB 3	CATCCCCCTG	6	5	83.33
OPD 9	CTCTGGAGAC	6	5	83.33
OPD 10	GGTCTACACC	5	4	80
OPD 19	CTGGGGACTT	7	5	71.42
OPB 7	GGTGACGCAG	4	4	100
OPB 14	TCCGCTCTGG	5	3	60
OPB 15	GGAGGTGTT	7	5	71.42
OPB17	AGGGAACGAG	5	3	60
OPJ 9	TGAGCCTCAC	5	3	60
OPP 2	TCGGCACGCA	4	3	75
OPQ 4	AGTGCGCTGA	5	2	40
OPQ 5	CCGCGTCTTG	7	5	71.42
OPR 1	TGCGGGTCCT	4	3	75
OPR2	CACAGCTGCC	5	4	80
OPR6	GTCTACGGCA	8	6	75
OPR 17	CCGTACGTAG	7	4	57.14
OPR 18	GGCTTTGCCA	5	5	100
OPS1	CTACTGCGCT	6	4	66.66
OPS12	CTGGGTGAGT	7	5	71.42
OPT1	GGGCCACTCA	5	3	60
OPT5	GGGTTTGGCA	4	2	50
OPT14	AATGCCGCAG	6	4	66.66
OPT 15	GGATGCCACT	9	7	77.77
Total		148	104	

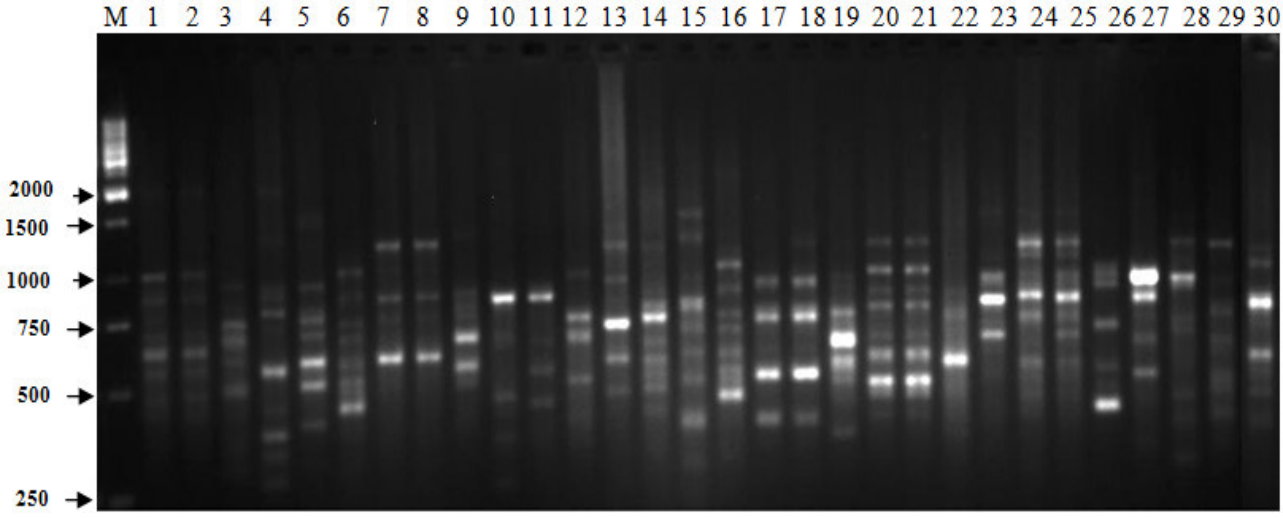

Figure 2. RAPD banding pattern of 30 genotypes of Hyacinth bean generated by random primer OPT 15. Molecular weight markers (in base pairs) are indicated on the right side (1 kb Ladder, Fermentas).

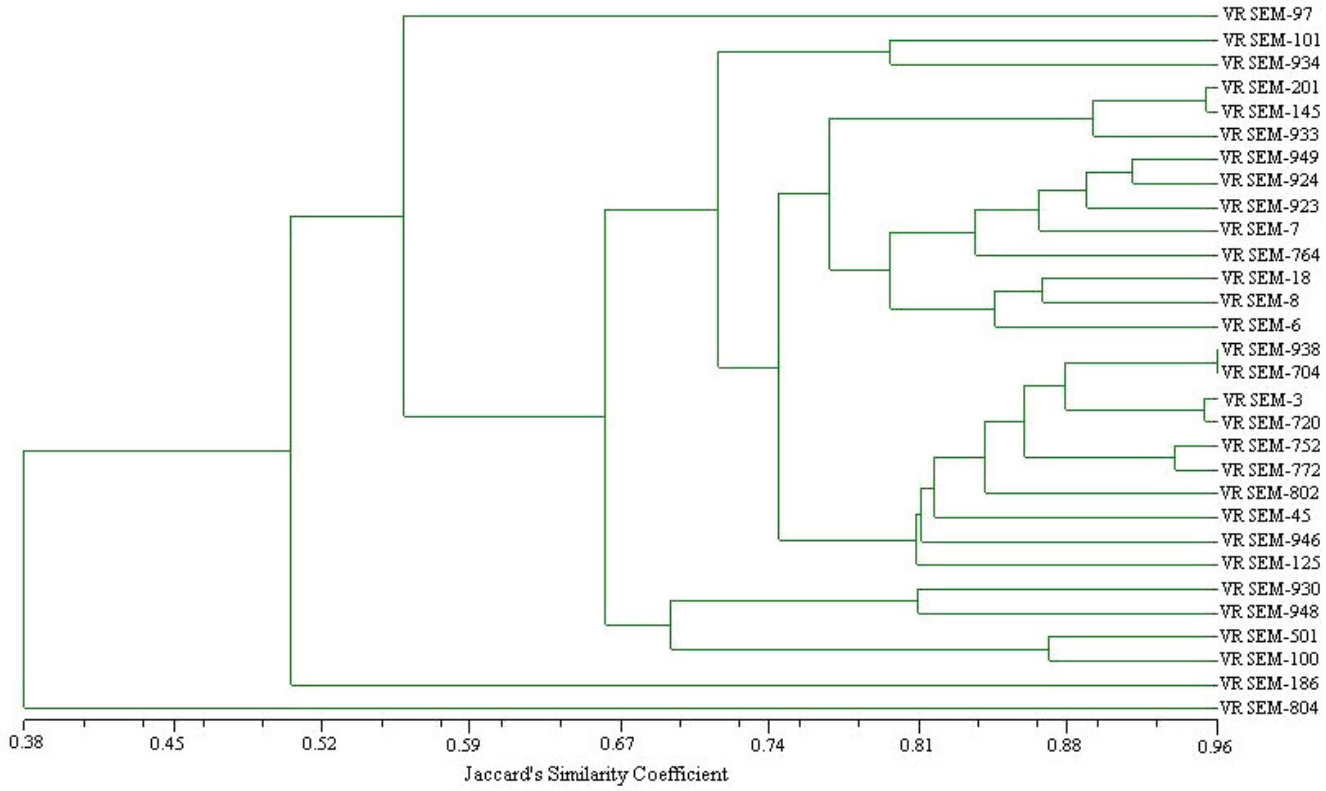

Figure 3. UPGMA clusters analysis showing the genetic relationship among 30 genotypes of Hyacinth bean based on 26 random decamer primers.

together in the same cluster based on quantitative traits but they are separated based on RAPD markers. The reason behind this is that the morphological traits are controlled by a subset of the genomic regions, while most molecular markers sample random genomic regions (Williams et al., 1990; Joyee et al., 1999; Dahlberg, 2000) most of which are likely to be related to the morphological traits. As a result, markers like RAPDs may accurately assay the degree of genetic change distinguishing two genomes, but they may not necessarily reflect the divergence in terms of changes in traits of agronomic importance, which are subjected to selective modifications.

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