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Genetic diversity analysis of spine gourd (*Momordica dioica* L.) genotypes using RAPD markers

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Abstract
Genetic diversity among ten genotypes of spine gourd (10 females and 10 males) was analyzed using Randomly Amplified Polymorphic DNA (RAPD) markers. A total of 50 RAPD primers were used, of which 28 primers gave amplification and generated 182 fragments. The numbers of polymorphic bands were 165 (90.66% polymorphism) with an average of 5.89 bands per primer. The Polymorphism Information Content (PIC) for RAPD marker ranged from 0.3598 (OPA-05) to 0.9011 (OPZ-19) with an average value of 0.7555 per primer and RAPD primer index (IPI) differed from 0.7196 (OPA-05) to 9.9121 (OPZ-19) with an average value of 5.1771 per primer. The Jaccard similarity based on RAPD profiles were subjected to UPGMA cluster analysis. The similarity coefficient of cluster analysis ranged from 29.1% to 72.1%. The dendogram generated by RAPD markers revealed two major groups, Cluster-I and Cluster-II. The formation of several sub-clusters with in cluster II suggested the presence of moderate genetic diversity among the 10 spine gourd genotypes of male and female plants. The highest (100%) polymorphi sm percentage was obtained with 23 primers *viz.*, OPA 05, OPA 03, OPC 05, OPD 18, OPD 20, OPL 01, OPL 03, OPZ 11, OPZ 17, OPZ 19, CMN A01, CMN A02, CMN A11, CMN A12, CMN A19, CMN A21, CMN A23, CMN A28, CMN A44, CMN A51, CMN A53, CMN A55 and CMN A57. Therefore, these primers may be most useful for genetic diversity analysis to generate DNA fingerprinting in spine gourd genotypes. However, RAPD primers were unable to distinguish male and female sex determinating plants, which may require another suitable molecular technique.

Keywords: spine gourd, genetic diversity, markers, PCR, RAPD, polymorphism etc

Introduction
Spine gourd (*Momordica dioica* Roxb. 2n=28) is one of the important members of the family *Cucurbitaceae*. The genus contains about 80 species (Raj *et al*., 1993) [8]. *Momordica dioica* commonly known as "spine gourd" or "teasle gourd" is a rhizomatous, distinctly dioeciously, annual or perennial climber in nature found in the forests of Southern India and West Bengal. Although considered an under-utilized and minor cucurbitaceous vegetable, it is widely cultivated in Orissa, Maharashtra, Bihar and West Bengal and is slowly gaining popularity as a commercial vegetable crop because of its rich taste and high nutritional value. It is rich in protein and vitamin C. Many species of the genus *Momordica* exist in the wild state in India and the surrounding geographical region in South and Central Asia (Hooker, 1879) [5] indicating that the center of origin of *M. dioica* might be in this region.

Nutritively, spine gourd fruit consists of moisture 84.1%, protein 3.1%, carbohydrate 7.7g, fibre 3g, and ash 1.1%. It also contains iron 4-6 mg, calcium 33 mg, phosphorus 42 mg, vitamin A 2,700 IU, thiamine 0.05mg, riboflavin 0.18 mg, and niacin 0.06 mg/100g. The fruit also contains 275.1 mg of ascorbic acid/100g. It has commercial importance and is exported market and also used locally. The fruits are cooked with spices, fried and eaten with meat and fish (Ram *et al*., 2004) [9].

Research efforts and outcomes have perhaps not been adequate in these important groups of vegetables which have largely remained under utilized and marginalized both by researchers and farmers. Due to its vegetative propagation very few variants are available for cultivation. Presently propagation entirely depends on underground tuberous roots. Propagation through seed was a major problem that the plants developed from seed segregate into 50:50 male: female ratio. For an economic crop production 10% of males are enough for effecting fertilization and fruit set. To maintain such ratio early determination of sex plays major role. Male and female plants can only be recognized when plants starts flowering. There are no any morphological markers to distinguish male and female plants before flowering. One pair of autosomes carry genes for sexual dimorphism in this crop was reported by Jha (1990) [6].

The development of molecular markers could complement these morphological markers. This enabled the plant breeders to overcome the limitations faced in the use of morphological markers. The knowledge of genetic and molecular diversity in a crops species is fundamental to its improvement. The use of various molecular marker such as RAPD, ISSR and SSR which are independent of environmental conditions such offers significant advantages for species identification in that they are rapid, relatively cheap, eliminate the need to grow plants up to maturity. The use of molecular marker for evaluation of molecular diversity is receiving much attention than morphological characterization. Hence, this particular study *i.e.*, use of molecular markers in determination of sex in spine gourd using RAPD's has been taken up.

Materials and Methods

Plant Material

The experimental material comprised of ten spine gourd genotypes collected from local farmers of Gujarat were used in present study (Table 1). All the molecular work was carried out at Department of Genetics and Plant Breeding, J.A.U., Junagadh.

Table 1: List of spine gourd genotypes used in present study

Sr. No.	Name of Genotypes	Origin of collection	Sr. No.	Name of Genotypes	Origin of collection
1	GP 1	Mangrol, Dist. Junagadh	6	GP 6	Manjola, Dist. Vadodara
2	GP 2	Keshod, Dist. Junagadh	7	GP 7	Manjola, Dist. Vadodara
3	GP 3	Una. Dist. Gir Somnath	8	GP 8	Dhavat, Dist. Vadodara
4	GP 4	Kodinar, Dist. Gir Somnath	9	GP 9	Dhavat, Dist. Vadodara
5	GP 5	Khapat, Dist. Gir Somnath	10	GP 10	Anastu, Dist. Vadodara

Experimental Details

The spine gourd plants were grown in pots in the green house at Department of Genetics and Plant Breeding, Junagadh Agricultural University, Junagadh under favorable conditions of germination. The leaf material compiled from five leaves per genotype after 15 days of germination were used for DNA extraction and molecular analysis.

DNA Extraction

Total genomic DNA extraction was carried out by CTAB method as described by Doyle and Doyle (1990) [4] with minor modifications as per Patil *et al*., (2012) [7]. For DNA extraction, 0.5 to 1.0 g of leaf tissues were weighed and grounded in pre-warmed (1.5 ml, 65 ^{0}C) DNA extraction buffer (2X CTAB extraction buffer) with the help of mortar and pestle. Homogenized material was transferred in capped polypropylene tubes. Tubes were incubated for 1 hr at 65^{0}C in water bath with gentle swirling. After incubation tubes were removed and spun at 12000 rpm for 10 minutes at room temperature (25^{0}C). One ml aqueous phase was transferred to another fresh tube and equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inversion for 15 minutes to ensure emulsification of the phase. Tubes were then spun at 4000 rpm for 30 minutes at room temperature (25^{0}C). Aqueous phase was transferred to another fresh tube. This steps was repeated once again. Equal volume of Ice-cold Iso-propanol was added to aqueous phase to precipitate DNA. DNA-CTAB complex was precipitated as a fibrous network. It was lifted by auto-pipette and was transformed to polypropylene tubes and centrifuged at 5000 rpm for 5 minutes at 4^{0}C. After removing supernatant 500 µl of 70% alcohol was added in pellet and was kept for 10 minutes with gentle agitation. It was centrifuged at 10,000 rpm for 5 minutes at 20^{0}C. The tubes were inverted and drained on a paper towel. The pellet was air dried for 30 minutes. Each pellet was re-dissolved in 300 µl of TE buffer by keeping 30 minutes at room temperature without agitation.

Purification of DNA

To get RNA free DNA sample, the purification was carried out. One µl of RNase was added to 100 µl of crude DNA preparation. It was mixed thoroughly and incubated at 37 ^{0}C for 30 minutes. Then ten µl of 3M sodium acetate was added and mixed thoroughly for 10 minutes till an emulsion was formed. Tubes were spunned at 12,000 rpm for 10 minutes at 4 ^{0}C. Supernatant was taken, avoiding the whitish layer at interface. The DNA was re-precipitated by adding double the quantity of absolute ethanol (isopropenol). To pellet the DNA, the tubes were centrifuged at 5000 rpm for 5 minutes at 4^{0}C. The pellet was washed with 500µl 70% ethanol and air dried for 30 minutes. Then, the DNA was re-dissolved in 300 µl of TE buffer for further use.

Estimation of quality and quantity of DNA

In order to perform PCR based analysis, the DNA concentration was determined by Nanodrop Spectrophotometer (Thermo Scientific, U.S.A.). Five micro-litres of DNA were loaded into the sample spot and measurement of quality at A_{260}/A_{280} ratio done. The A_{260}/A_{280} ratio was automatically calculated by the software and the quality was directly displayed as ng.µl^{-1}. The concentration of DNA was adjusted to 50 ng.µl^{-1}for further research work. To measure the integrity of DNA, agarose gel electrophoresis was carried out. Agarose gel of 0.8% was prepared (disolved 0.8 g agarose in 100 ml 1X TBE and 5.0 µl EtBr from 10 mg/ml stock). About 5 µl of DNA + 1 µl of 1 X gel loading dye were loaded in each well. The gel was run at 70 V for 1 hours and DNA bands were visualized under UV transilluminator (254nm) and photographed using Alpha Innotech Gel Documentation system using UV light. Presence of single compact band on agarose gel indicated integrity of isolated DNA.

DNA amplification using RAPD marker

The genomic DNA was amplified using RAPD primers as listed in Table 2. All the primers for RAPD were diluted by adding equal amount of deionized sterile distilled water equal to its concentration. For example, if the concentration of RAPD primer OPA-4 is 71 nMoles then adding 71 µl of deionized water to make concentration of 1 nMole.µl^{-1} = 1000 pMoles.µl^{-1}. This was kept as a stock solution of primer. By taking 1 µl of stock (1000 pMoles.µl^{-1}) and 99 µl of deionized sterile distilled water gave a final concentration of 10 pMoles.µl^{-1}. This working solution was used in PCR amplification.

Table 2: List of RAPD primers used in present study

Sr. No.	Primer	Sequence 5' – 3'	GC (%)	Tm (^{0}C)
1.	OPA-03	AGTCAGCCAC	2	60
2.	OPA-05	AGGGGTCTTG	2	60
3.	OPC-05	GATGACCGCC	7	70
4.	OPD-18	GAGAGCCAAC	32	60
5.	OPD-20	ACCCGGTCAC	32	60
6.	OPL-01	GGCATGACCT	34	60
7.	OPL-03	CCAGCAGCTT	32	70
8.	OPL-04	GACTGCACAC	35	70
9.	OPZ-11	CTCAGTCGCA	27	60
10.	OPZ-17	CCTTCCCACT	34	60
11.	OPZ-19	GTGCGAGCAA	40	70
12.	CMN A01	AGCAGCGCCTCA	40	66.7
13.	CMN A02	GCCAGCTGTACG	40	66.7
14.	CMN A11	ACTGACCTAGTT	34	41.7
15.	CMN A12	CTCCTGCTGTTG	38	58.3
16.	CMN A19	AAGGCGCGAACG	40	66.7
17.	CMN A21	GTGACCGATCCA	40	66.7
18.	CMN A23	AAGTGGTGGTAT	34	41.7
19.	CMN A24	GACGGTTCAAGC	38	58.3
20.	CMN A26	GGTGAGGATTCA	38	58.3
21.	CMN A28	TACCCTCAAGCT	36	50
22.	CMN A37	AGCGCGGCAAAA	38	58.3
23.	CMN A40	GCGGAGGAACCA	40	66.7
24.	CMN A44	AAGGACACAACA	34	41.7
25.	CMN A51	AAGTCGTTTGGG	36	50
26.	CMN A53	GACGCCCATTAT	36	50
27.	CMN A55	TACGCCGGAATA	36	50
28.	CMN A57	ATCATTGGCGAA	34	41.7

The PCR process for RAPD was performed according to method given by Williams *et al*. (1990) [11] with some modifications. The RAPD assays were performed using random 10-mer and 12-mer oligonucleotide primers from Operon Technology Inc., USA and UBC primers, california. The reactions for PCR were carried out in a final reaction volume of 15 µl (Table 3). To avoid pipetting error in measuring small volumes, a cocktail was prepared where constituents common to all the reactions were combines in one tube multiplying volume for one reaction with total number of samples.

Table 3: Preparation of reaction mixture for RAPD primers

Sr. No.	Reagent	Quantity
1.	Taq buffer A (10X) (with $MgCl_2$)	1.5 µl
2.	Taq polymerase (3 U/µl)	0.15 µl
3.	dNTPs mix (2.5 mM each)	1.2 µl
4.	Primer (25 pmoles/µl)	1.2 µl
5.	Template DNA (50 ng/µl)	1.2 µl
6.	Millipore sterile distilled water	9.75 µl
Total		15.0 µl

The amplification reaction was carried out in 15µl volume containing Millipore sterilized water, followed by Taq Buffer A (10X Tris with $MgCl_2$), dNTPs, TaqDNA polymerase, and template DNA in sequence as above quantity and finally the primer. The reagents were mixed thoroughly by a short spin using microfuge. The tubes were then placed on the Thermal Cycler for cyclic amplification (Table 4).

Table 4: PCR conditions for RAPD

Sr. No.	Steps	Temperature (ºC)	Duration	Cycle
1.	Initial denaturation	94	5.0 min	1
2.	Cycle Denaturation	94	45 sec	35
3.	Cycle Annealing	37	45 sec	
4.	Cycle Extension	72	1.0 min	
5.	Final extension	72	8.0 min	1
6.	Hold	4	∞	∞

Electrophoresis of amplified product

The amplified products of RAPD were analyzed using 1.5% agarose gel in TBE buffer. The gel was buffered with 1X TBE (1.5 g agarose in 100 ml 1X TBE and 4.0 µl Ethidium bromide from 10 mg/ml stock). PCR amplified products (15 µl and 4 µl 6X gel loading dye) were loaded into the wells. Low range standard DNA ladder (3 kb) was also run along with the samples. The electrophoresis was conducted at 100 V current (constant) for 1.5 hour to separate the amplified bands. The separated bands were visualized under UV trans-illuminator and photographed using Gel Documentation System.

Molecular Data Analysis

In order to score and preserve banding pattern, photograph of the gel was taken in a Gel Documentation System. The presence of each band was scored as '1' and its absence as '0'. The data were entered in to MS-Excel data sheet and subsequently analyzed using NTSYS pc version 2.02 (Rohlf, 2000) [10]. Similarity matrices generated according to the Jaccard similarity coeeficient and were used to perform cluster analysis using the unweighted pair group method with arithmetic average (UPGMA). Dendrogram was constructed with the TREE programme of NTSYSpc. Polymorphic information content (PIC) for RAPD was calculated on the basis of allele frequency (Anderson *et al*., 1993) [1]. PIC values were used to calculate RAPD primer indexes (RPI) which were generated by multiplying the PIC values of all the markers amplified by the same primer.

Results and Discussion

DNA was extracted from leaf tissue of 10 genotypes (males and females plants separately). The yield of DNA isolated ranged from 60.58 ng/µl (GP-1 M) to 352.05 ng/µl (GP-9 M) in male plants and from 107.04 ng/µl (GP-9 F) to 325.13 ng/µl (GP-7 F) in female plants. The optical density near about 1.73 to 1.98 indicated that DNA extracted was pure in all the samples (Table 5).

Table 5: Purity and concentration of DNA samples in spine gourd

Sr. No	Genotypes	Absorbance A_{260}/A_{280} ratio	Concentration ng.µl^{-1}	Genotypes	Absorbance A_{260}/A_{280} ratio	Concentration ng.µl^{-1}
1	GP 1 M	1.83	60.58	GP 1 F	1.78	142.49
2	GP 2 M	1.89	87.88	GP 2 F	1.82	136.09
3	GP 3 M	1.92	103.19	GP 3 F	1.79	214.73
4	GP 4 M	1.83	237.13	GP 4 F	1.84	156.65
5	GP 5 M	1.98	171.78	GP 5 F	1.83	214.80
6	GP 6 M	1.73	192.97	GP 6 F	1.82	223.04
7	GP 7 M	1.76	96.94	GP 7 F	1.78	325.93
8	GP 8 M	1.82	97.98	GP 8 F	1.84	213.31

9	GP 9 M	1.85	352.05	GP 9 F	1.89	107.04
10	GP 10 M	1.88	142.68	GP 10 F	1.88	224.83

Ten spine gourd genotypes were subjected to RAPD analysis using 50 primers such as OPA, OPC, OPD, OPL, OPZ and CMN A series to investigate the level of polymorphism among these genotypes. Out of 50 RAPD primers screened, 28 primers amplified a total of 182 bands. Two RAPD markers (OPZ-19 and CMN-A01) produced maximum number of 11 bands, while OPA-05 produced minimum number of 2 bands. Out of 182 bands, 165 bands/alleles were pollymorphic in nature with an average of 5.89 bands per primer, while 17 bands/alleles were monomorphic in nature. Out of 165 polymorphic bands, 160 were shaded polymorphic and five were unique bands (Table 6). These five unique polymorphic bands were observed in genotype GP-8 F by OPL-03; GP-9 F by OPZ-17; GP-5 M by CMN-A01; GP-9 F by CMN-A53 and GP-7 F by CMN-A57 with allelic size of 442 bp, 181 bp, 826 bp, 1027 bp and 450 bp, respectively (Plate 1 and 2). From the data in Table 6, it was observed that 90.66% polymorphic based and remaining 9.34% monomorphic bands were observed (Fig. 1).

In present study, the amplified fragments of RAPD were ranged from 110 bp (CMN-A01) to 2082bp (CMN-A44) (Table 6). Behera *et al.* (2008a) [3] reported that 208 amplicons by examining 38 *Momordica charantia* accessions with 29 RAPD primers. Amplicon numbers per primer ranged from 3 (OPE-19, OPW-09) to 15 (OPW-05) and varied in size between 200 bp and 3000 bp. of the 208 amplified bands, 76 were polymorphic, with an average of 2.6 polymorphic fragments per primer. Behera *et al.* (2008b) [2] observed amplicon numbers per primer ranged from 3 (OPE-19 and OPW-09) to 15 (OPW-05) and varied in size between 200 bp and 3000 bp in Indian bitter gourd.

The polymorphic information content (PIC) was calculated for each primer presented in Table 6. The polymorphic information content was recorded from 0.3598 to 0.9011. The highest PIC value of 0.3598 was recorded by OPA-05, while lowest PIC value of 0.9011 was recorded by OPZ-19. Similarly RAPD primer index (RPI) ranged from 0.7196 to 9.9121 with an average of 5.1771 per primer. The highest RPI value was obtained by OPZ-19 and the lowest was obtained by OPA-05 (Table 6).

Genetic similarity

Similarity index and cluster analysis was done by Jaccard's coefficient and UPGMA, respectively using NTSYSpc-2.02i software. The dendrogram was generated by using Jaccard's coefficient values (Fig. 2) to estimate the genetic similarity among ten spine gourd genotypes. Jaccard's coefficient of similarity of 10 genotypes of spine gourd ranged from 29.1% (GP 1 male and GP 8 female) to 72.1% (GP 2 female and GP 4 female).

Table 6: Size, number of amplified bands, percent polymorphism and PIC obtained by RAPD primers

Sr. No.	RAPD primer	Allele/ Band Size (bp)	Total no. of Bands (A)	No. of Polymorphic Bands (B)			No. of Monomorphic Bands	% Polymorphism	PIC Value	RPI (PIC×A)
				S	U	T				
1	OPA-03	207-1462	8	8	0	8	0	100	0.8551	6.8408
2	OPA-05	266-430	2	2	0	2	0	100	0.3598	0.7196
3	OPC-05	328-1405	4	4	0	4	0	100	0.6903	2.7612
4	OPD-18	283-798	4	4	0	4	0	100	0.7117	2.8468
5	OPD-20	465-783	3	3	0	3	0	100	0.5102	1.5306
6	OPL-01	242-1258	6	6	0	6	0	100	0.7684	4.6104
7	OPL-03	235-1560	7	6	1	7	0	100	0.7897	5.5279
8	OPL-04	146-808	4	2	0	2	2	50	0.6481	2.5924
9	OPZ-11	164-1500	4	4	0	4	0	100	0.6805	2.722
10	OPZ-17	134-591	5	4	1	5	0	100	0.7551	3.7755
11	OPZ-19	143-1495	11	11	0	11	0	100	0.9011	9.9121
12	CMN-A01	110-1597	11	10	1	11	0	100	0.8868	9.7548
13	CMN-A02	172-1906	7	7	0	7	0	100	0.8288	5.8016
14	CMN-A11	148-1336	7	7	0	7	0	100	0.811	5.677
15	CMN-A12	245-950	4	4	0	4	0	100	0.6666	2.6664
16	CMN-A19	564-886	3	3	0	3	0	100	0.5726	1.7178
17	CMN-A21	278-1106	7	7	0	7	0	100	0.8269	5.7883
18	CMN-A23	181-1332	8	8	0	8	0	100	0.8405	6.724
19	CMN-A24	449-1490	6	1	0	1	5	16.67	0.8310	4.986
20	CMN-A26	239-1441	10	5	0	5	5	50	0.8948	8.948
21	CMN-A28	304-1454	10	10	0	10	0	100	0.8887	8.887
22	CMN-A37	214-1529	8	4	0	4	4	50	0.8593	6.8744
23	CMN-A40	142-699	4	3	0	3	1	75	0.6746	2.6984
24	CMN-A44	148-2082	10	10	0	10	0	100	0.8425	8.425
25	CMN-A51	115-1415	7	7	0	7	0	100	0.8073	5.6511
26	CMN-A53	193-1423	8	7	1	8	0	100	0.7576	6.0608
27	CMN-A55	118-1427	7	7	0	7	0	100	0.7804	5.4628
28	CMN-A57	127-1053	7	6	1	7	0	100	0.7140	4.998
Total			182	160	5	165	17	2541.6	21.153	144.960
Average			6.5	-	-	5.89	-	90.66	0.7555	5.1771

S = Shared; **U** = Unique; **T** = Total Polymorphic bands; **PIC** = Polymorphism Information Content; **RPI** = RAPD Primer Index = Number of Bands × PIC

Cluster analysis of RAPD

The dendrogram constructed using UPGMA based on Jaccard's similarity coefficient for RAPD data of 10 spine gourd genotypes (males and females) are depicted in Fig 2. Ten males and females genotypes were grouped into two main clusters, cluster I and cluster II with an average similarity of 41% (Fig 2). The cluster I consisted of only one genotype (GP-1 male), while cluster II consisted of nine males and ten females genotypes. The cluster II was further sub-divided into subcluster IIA and IIB. The subcluster IIA consisted of only one genotype (GP-2 male) and IIB was divided into two sub-sub cluster IIB (i) and IIB (ii) with 17 and one genotypes, respectively. IIB (i) was also divided into further sub-sub-sub cluster IIB (i) a and IIB (i) b. IIB(ii) sub-sub cluster consisted only one genotype (GP-6 Male). The sub cluster IIB (i) a consisted of sixteen genotypes and sub cluster IIB (ii) b consisted of one genotype of (GP-10M). Likewise, IIB (i) a was further splitted in two sub dividing cluster, IIB (i) a_1 and IIB (i) a_2 with seven and nine genotypes, respectively. It is interesting to note that all the four solitary clusters grouped male genotype separately. However, there eas no specific pattern of separating sex related plants of male and femle plants. In sub cluster IIB (i)a_2, genotypes GP-2 F and GP-4 F shared 72% genetic similarity. Overall, classing pattern was quite complex and do not differentiate male and female plants with RAPD markers.

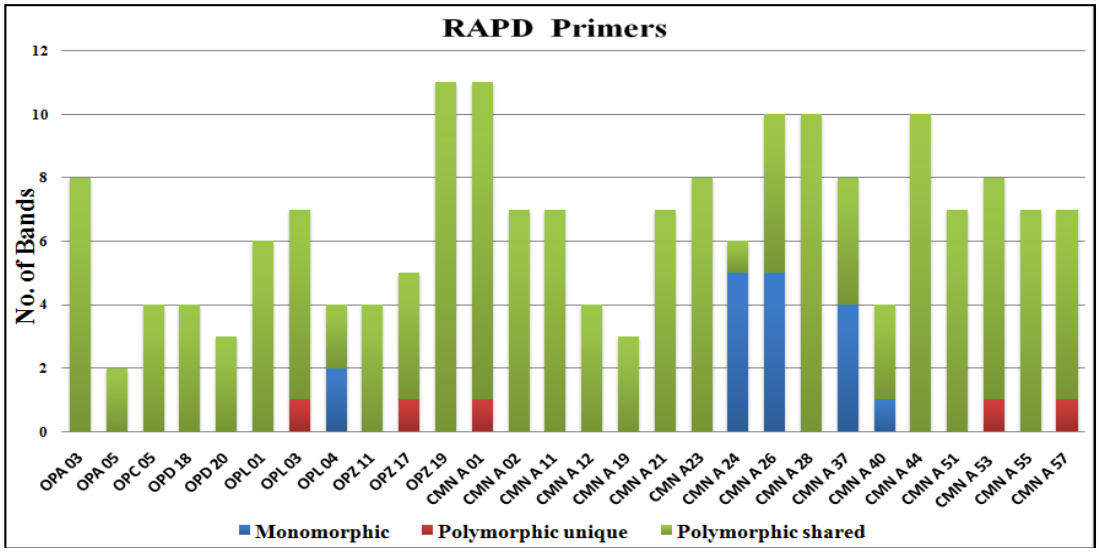


Fig 1: Properties of polymorphic and monomorphic bands amplified by RAPD primer

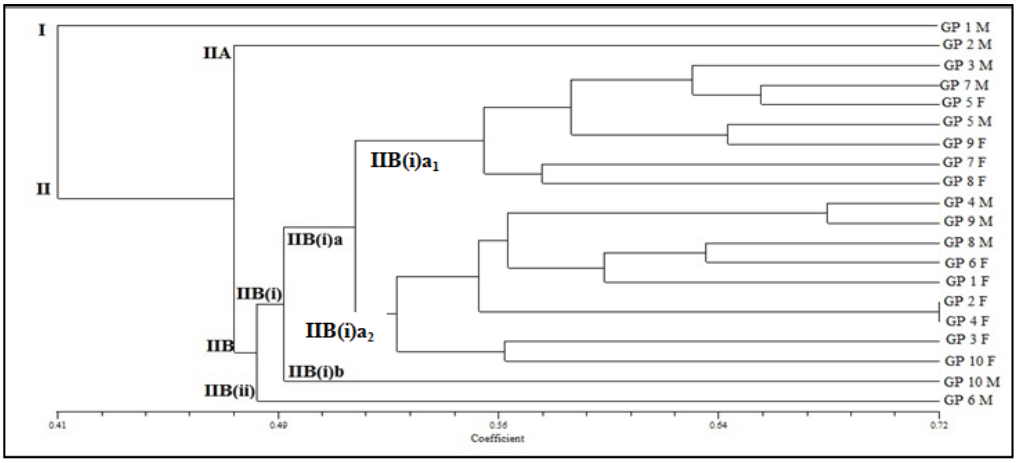
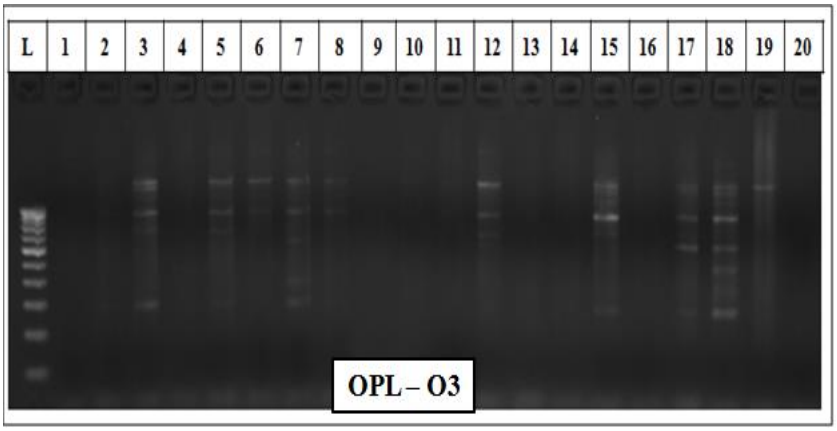


Fig 2: Dendogram depicting the genetic relationship among 20 spine gourd genotypes based on RAPD markers



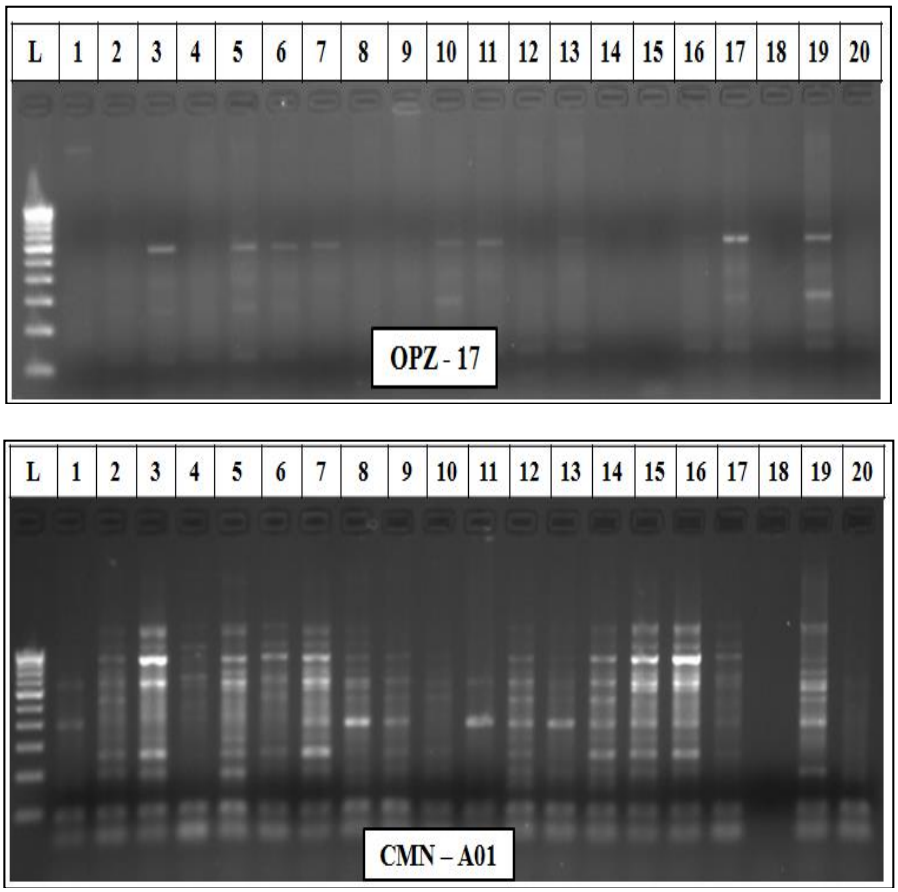
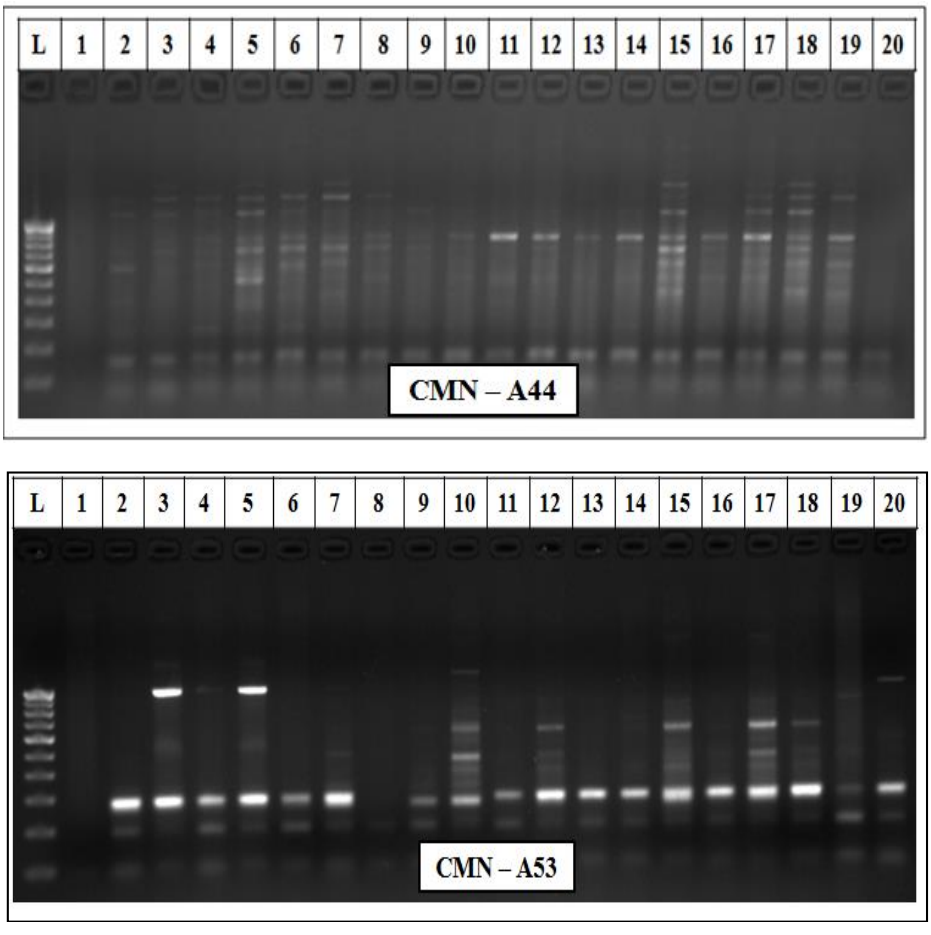


Plate 1: Agarose gel electrophoresis of amplified products obtained with RAPD Primers OPL-03, OPZ-17 and CMN-A01



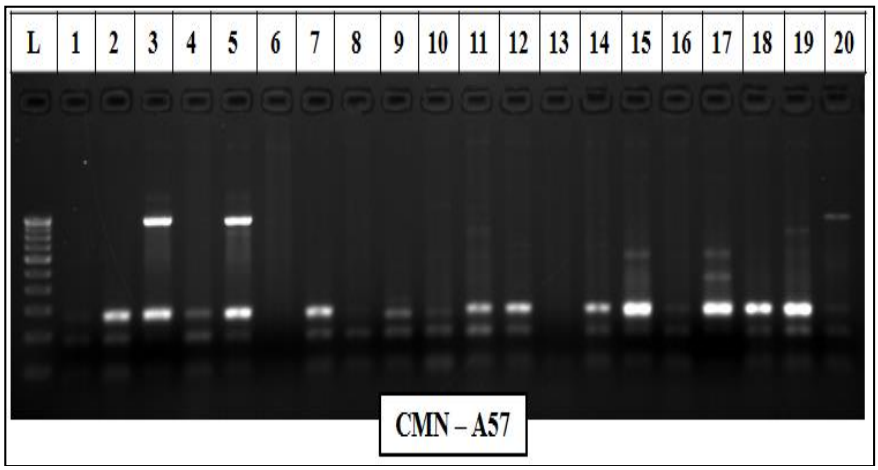


Plate 2: Agarose gel electrophoresis of amplified products obtained with RAPD Primers CMN-A44, CMN-A53 and CMN-A57.

Conclusion

Twenty-eight RAPD primers generated total of 182 bands/alleles in which 165 bands were polymorphic showing 90.66% polymorphism. The average bands per primer were 6.5 and 5.89 polymorphic bands per primer. The polymorphic information content (PIC) was recorded from 0.3598 (OPA-05) to 0.9011 (OPZ-19). Similarly, RAPD primer index (RPI) ranged from 0.7196 (OPA-05) to 9.9121 (OPZ-19) with an average of 5.1771 per primer. The phylogenetic tree constructed by UPGMA method generated two main clusters I and II with an average similarity of 41%. The jaccard's similarity coefficient and UPGMA method showed the highest (72.1%) similarity between GP-2 female and GP-4 female and the lowest (29.1%) similarity between GP-1 male and GP-8 female.

Genetic diversity analysis through RAPD marker gave highest (100%) polymorphism percentage with 23 primers *viz.*, OPA 05, OPA 03, OPC 05, OPD 18, OPD 20, OPL 01, OPL 03, OPZ 11, OPZ 17, OPZ 19, CMN A01, CMN A02, CMN A11, CMN A12, CMN A19, CMN A21, CMN A23, CMN A28, CMN A44, CMN A51, CMN A53, CMN A55 and CMN A57. Therefore, these primers were most useful for genetic diversity analysis to generate DNA fingerprinting in spine gourd genotypes but these primers were unable to distinguish male and female sex determinating plants.

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