International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 8(3): 521-530 September 2015
DOI Number: 10.5958/2230-732X.2015.00059.5

GENETICS AND PLANT BREEDING

Molecular characterization of ridge gourd (*Luffa acutangula* L.) and sponge gourd (*Luffa cylindrica* l.) genotypes through PCR based molecular markers

Ravi R. Rathod[1], D. R. Mehta[2], H. P. Gajera[1]* and N. A. Delvadiya[1]

[1]Department of Biotechnology, Junagadh Agricultural University, Junagadh-362 001, Gujarat, India.
[2]Department of Genet cs and Plant Breeding, Junagadh Agricultural University, Junagadh-362 001, Gujarat, India.

*Corresponding author: harsukhgajera@yahoo.com

Paper No. 343 Received: 15 July 2014 Accepted: 22 August 2015

Abstract

The present study was carried out for the comparison of RAPD and ISSR markers for polymorphism pat ern and molecular diversity analysis among 17 ridge gourd (*Luffa acutangula* L.) and sponge gourd (*Luffa cylindrica* L.) genotypes using 20 RAPD and 12 ISSR markers. Twenty RAPD primers generated total of 94 bands of which 81 bands were polymorphic showing 86.17% polymorphism. The average bands per primer were found 4.05. The polymorphic information content (PIC) was recorded from 0.4828 to 0.8842 for RAPD. Jaccard's similarity coefficient ranged from 30.8% to 78.6% for RAPD. However, out of 30 ISSR primers screened, twelve ISSR primers produced 79 bands of which 66 bands were polymorphic and 83.54% polymorphism with an average of 7.16 bands per primer. The PIC ranged between 0.6548 and 0.8939 for ISSR. Jaccard's similarity coefficient ranged from 22.7 % to 81.2% for ISSR. This study showed that RAPD and ISSR markers produced specific DNA fragments for identification of ridge gourd and sponge gourd genotypes.

Highlights

RAPD and ISSR evident genetic diversity in the range of 30.8 to 78.6% and 22.7 to 81.2%, respectively among ridge gourd and sponge gourd genotypes.

Keywords: Ridge gourd, sponge gourd, genetic diversity, RAPD, ISSR

Ridge gourd (*Luffa acutangula* L.) and sponge gourd (*Luffa cylindrica* L.) are the important cucurbitaceae family vegetable crops with diploid chromosome number 2n = 26. Ridge gourd and sponge gourd are tropical plants believed to have originated in India and both are popular vegetables as spring, summer and rainy season crops (Kalloo 1993). They are annual, monoecious cross pollinating, running vine plants, with smooth, simple, sharply angled 5-lobed leaves. Fruits vary in size and may be oblong or club-shaped with dark green or green colour.

Molecular investigations are essential for collection, conservation and its utilization in future breeding programmes. The knowledge of genetic diversity in a crop species is fundamental to its improvement. The use of various molecular marker methods which are independent of environmental conditions such as RAPD, ISSR, and SSR 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 the evaluation of genetic diversity is receiving much at ention than morphological characterization.

Random amplified polymorphic DNA (RAPD) technique was developed by Williams *et al.* (1990). In the RAPD technique, DNA polymorphisms are produced using a single arbitrary primer that binds to the opposite strands of the genomic DNA template (Tingy *et al*. 1992). Compared to RFLPs, RAPDs are faster and easier to generate because of the fewer numbers of steps involved namely, extraction, amplification, and separation.

Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability and, they are, therefore, favored in population studies (Smith and Devey, 1994) and for the identification of closely related cultivars (Vosman *et al*. 1992). The ISSRs are DNA fragments of about 100–3000 bp located between adjacent, oppositely oriented microsatellite regions. The ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16–18 bp). These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objectively. The knowledge acquired through this investigation may play a pivotal role in the application of molecular markers in ridge gourd and sponge gourd improvement programmes.

Therefore, in the present experiment, the genetic diversity and molecular characterization among ridge gourd and sponge gourd genotypes were studied by using PCR based molecular markers for purity discrimination.

Materials and Methods

Plant material

The experimental material comprised of 17 ridge gourd and sponge gourd genotypes were collected from Vegetable Research Station, Junagadh Agricultural University (JAU), Junagadh, Gujarat (Table 1).

Table 1: List of ridge gourd and sponge gourd genotypes used in present study

Sr. No.	Name of Ridge gourd genotypes	Sr. No.	Name of Sponge gourd genotypes
1.	ARGS-07-40	9.	ASGS-04-23
2.	ARGS-07-50	10.	ASGS-06-30
3.	ARGS-07-52	11.	ASGS-08-40
4.	ARGS-09-56	12.	ASGS-08-41
5.	JRG-05-04	13.	ASGS-09-44
6.	JRG-05-06	14.	JSG-05-04
7.	PusaNasdar	15.	JSG-05-7
8.	GARG-1	16.	PusaChikni
		17.	GSG-1

DNA isolation

Total plant genomic DNA was extracted from young leaves of each genotype using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method as described in Doyle and Doyle (1987) with minor modifications as per Mohammed *et al.* (2012). The quantity and quality of the isolated DNA was determined by using Nanodrop Spectrophotometer (Thermo Scientific, U.S.A.). Dilutions of 50 ng/µl of each genotype were prepared and stored at 4°C for further use in PCR analysis.

RAPD analysis

Twenty oligonucleotide primers of 10-mer, each with at least 60% G+C content (Table 2), were obtained from Operon Technology Inc., USA and UBC primers, California. PCR reactions were performed as per Hoque and Rabbani (2009). The PCR master mix (25µl) contained 10x PCR buffer (10 mM Tris-HCl, pH 8.3), 100 mM each dNTPs, 25 pmoles primer, 50 ng of genomic DNA and 3 unit of Taq DNA polymerase. The samples were subjected to 40 repeats of the following cycle: 94°C 1 min, 36°C for 1 min, 72°C for 2 min with an initial denaturation of 5 minutes and a final extension of 10 minutes.

Table 2. List of RAPD primers used, number of amplified products, number of polymorphic bands, and percentage of polymorphism and PIC value obtained by analyzing 17 ridge gourd and sponge gourd genotypes

No.	RAPD Primers	Sequence (5‘→3‘)	Allele/Band size (bp)	Number of bands (A)	Polymorphic bands (B)	Polymorphic % (B/A)	PIC Value
1	OPA-04	AATCGGGCTG	260 – 1117	5	4	80	0.7246
2	OPA-07	GAAACGGGTG	233 – 1672	7	7	100	0.8220
3	OPC-02	GTGAGGCGTC	118 – 1442	4	3	75	0.7467
4	OPC-06	GAACGGACTC	261 – 841	3	2	66.66	0.6005
5	OPW-06	AGGCCCGATG	100 – 1710	6	6	100	0.7555
6	OPW-08	GACTGCCTCT	178 – 1754	4	4	100	0.7451
7	OPX-01	CTGGGCACGA	477 – 966	4	4	100	0.6592
8	OPW-01	CTCAGTGTCC	255 – 1319	4	2	50	0.7358
9	OPW-03	GTCCGGAGTG	228 – 1550	10	10	100	0.8842
10	OPF-08	GGGATATCGG	125 – 439	3	3	100	0.5267
11	OPF-10	GGAAGCTTGG	228 – 1545	2	2	100	0.4828
12	OPB-05	TGCGCCCTTC	198 – 1606	4	3	75	0.6519
13	UBC-106	CGTCTGCCCG	217 – 1932	5	3	60	0.7455
14	UBC-115	TTCCGCGGGC	167 – 337	4	4	100	0.7188
15	UBC-152	CGCACCGCAC	245 – 2450	5	4	80	0.7924
16	UBC-155	CTGGCGGCTG	180 – 1900	5	4	80	0.7474
17	UBC-157	CGTGGGCAGG	171 – 378	4	3	75	0.7102
18	UBC-199	GCTCCCCCAC	148 – 1617	3	3	100	0.5950
19	UBC-222	AAGCCTCCCC	120 – 1107	7	6	85.71	0.7821
20	UBC-228	GCTGGGCCGA	171 – 584	5	4	80	0.7485
Total				94	81	86.17	0.7088

Table 3. List of ISSR primers used, number of amplified products, number of polymorphic bands, and percentage of polymorphism and PIC value obtained by analyzing 17 ridge gourd and sponge gourd genotypes

No.	ISSR Primers	Sequence (5‘→3‘)	Allele/Band size (bp)	Number of bands (A)	Polymorphic bands (B)	Polymorphic % (B/A)	PIC Value
1	UBC-808	(AG)8C	96 – 631	7	5	71.42	0.8466
2	UBC-840	(GA)8CTT	167 – 356	3	1	33.33	0.6548
3	UBC-854	(TC)8AGG	165 – 411	6	4	66.66	0.7643
4	UBC-855	(AC)8CTT	199 – 423	6	4	66.66	0.8016
5	UBC-856	(AC)8CTA	154 – 554	6	5	83.33	0.8014
6	UBC-861	(ACC)6	153 – 379	5	4	80	0.7480
7	ISSR-834	(AGA)3 (GAG)2 GYT	143 – 1307	9	9	100	0.8872
8	ISSR-840	(GAG)3 (AGA)2 AYT	269 – 1208	11	11	100	0.8939
9	ISSR-856	(ACA)3 (CAC)2 CYA	185 – 676	9	9	100	0.8778
10	ISSR-865	(CCG)6	111 – 1283	8	8	100	0.8319
11	B3	(GA)8A	160 – 545	5	4	80	0.7866
12	B5	(GA)8T	151 – 436	4	2	50	0.6918
Total				79	66	83.54	0.7988

ISSR analysis

Total 15 ISSR were obtained from UBC primers, California (Table 3). PCR reactions were performed as per Behera *et al.* (2008b). The PCR master mix (25µl) contained 10x PCR buffer (10 mM Tris-HCl, pH 8.3), 100 mM each dNTPs, 25 pmoles primer, 50 ng of genomic DNA and 3 unit of Taq DNA polymerase. The samples were subjected to 40 repeats of the following cycle: 94°C 1 min, 55°C for 1 min, 72°C for 2 min with an initial denaturation of 5 minutes and a final extension of 10 minutes.

All the above PCR amplification (RAPD and ISSR) were performed in 0.2 ml thin-walled PCR tubes placed in a thermal cycler. The products of both RAPD and ISSR were analysed by electrophoresis in 1.5% agarose gel stained in ethidium bromide (10 mg/ml) and run in 1x TBE buffer at 100 V for 2 h. The separated bands were visualized under UV transilluminator and photographed using a gel documentation system (Alpha Innotech).

Statistical analysis

Clear and distinct bands amplified by RAPD and ISSR primers were scored for the presence (1) and absence (0) for the corresponding band among the genotypes. The binary data were subjected to UPGMA (Rohlf 2000) analysis using NTSYSpc version 2.02 (Anderson *et al.* 1993).

Results and Discussion

Total genomic DNA was extracted from leaf tissue of seventeen genotypes which were of good quality and quantity. The yield of DNA isolated ranged from 186.82 ng/µl in GSG-1 to 816.24 ng/µl in ARGS-07-50 with optical density near about 1.80 to 2.00 indicated that DNA extracted was pure in all the 17 genotypes.

RAPD analysis

Total 50 RAPD primers was used for amplification of ridge gourd and sponge gourd genomic DNA. Among them 20 polymorphic primers gave satisfactory results which were used for further analysis of all ridge gourd and sponge gourd genotypes. The banding pat ern of 20 RAPD primers using 17 ridge gourd and sponge gourd genotypes were shown in Table 2. Twenty RAPD primers generated total of 94 bands/alleles in which 81 bands were polymorphic showing 86.17% polymorphism (Table 2). The size varied from 100-2450 bp. Among them, there was maximum amplified allele size of 2450 bp (primer UBC-152) and minimum amplified allele size of 100 bp (primer OPW-06). Primer OPW-03 produced maximum 10 bands, while primer OPF-10 produced minimum 2 bands. A maximum 100% polymorphism was obtained with OPA-07, OPW-06, OPW-08, OPX-01, OPW-03, OPF-08, OPF-10, UBC-115 and UBC-199 primers. The average bands per primer were 4.05. The polymorphic information content (PIC) was recorded from 0.4828 to 0.8842 with an average of 0.7088. The highest PIC value of 0.8842 was recorded by OPW-03, while the lowest PIC value of 0.4828 was recorded by OPF-10. Based on PIC value, the primer OPW-03 was the best primer resulting in good amplification with maximum PIC value (0.8842). Similarly RAPD primer index (RPI) ranged from 0.9657 (OPF-10) to 8.8421 (OPW-03) with an average of 3.4701 per primer.

A dendrogram based on UPGMA analysis of 17 ridge gourd and sponge gourd genotypes with RAPD data is shown in (Figure 1). Jaccard's coefficient of similarity of 17 ridge gourd and sponge gourd genotypes ranged from 30.8% (between ARGS-07-40 and ASGS-04-23) to 78.6% (between ASGS-06-30 and JSG-05-07).

Dendrogram generated by RAPD molecular data 17 genotypes were grouped into two main clusters, cluster I and cluster II with an average similarity of 48% (Figure 2). The cluster I consisted of nine genotypes, while cluster II consisted of eight genotypes. The phylogenetic tree constructed by UPGMA method generated two main clusters which again sub-grouped in their respective sub-clusters. The cluster I was further sub-divided into subcluster IA and IB. The subcluster IA consisted of eight genotypes (seven of ridge gourd and one of sponge gourd) and these were again divided into two sub-

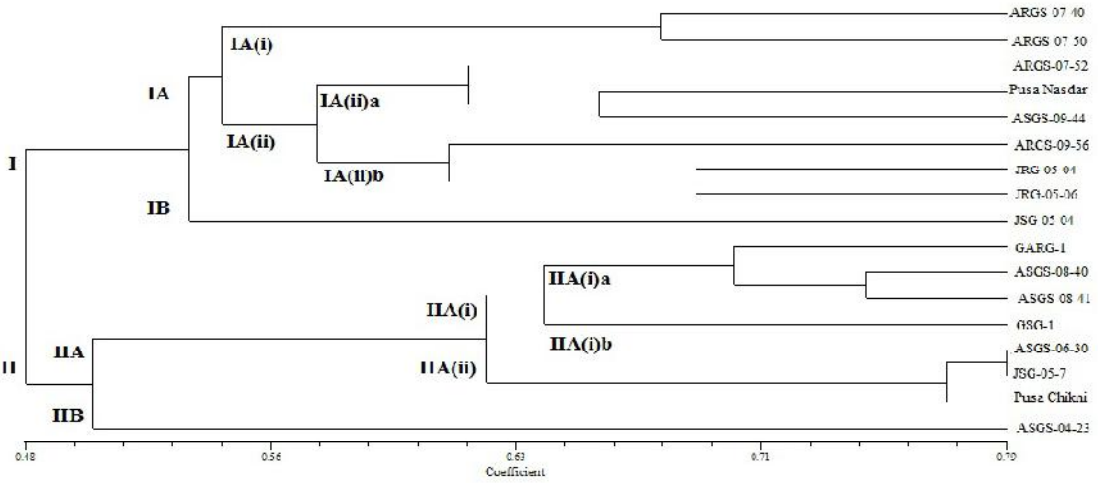

Fig. 1. Dendogram depicting the genetic relationship among 17 ridge gourd and sponge gourd genotypes based on RAPD markers

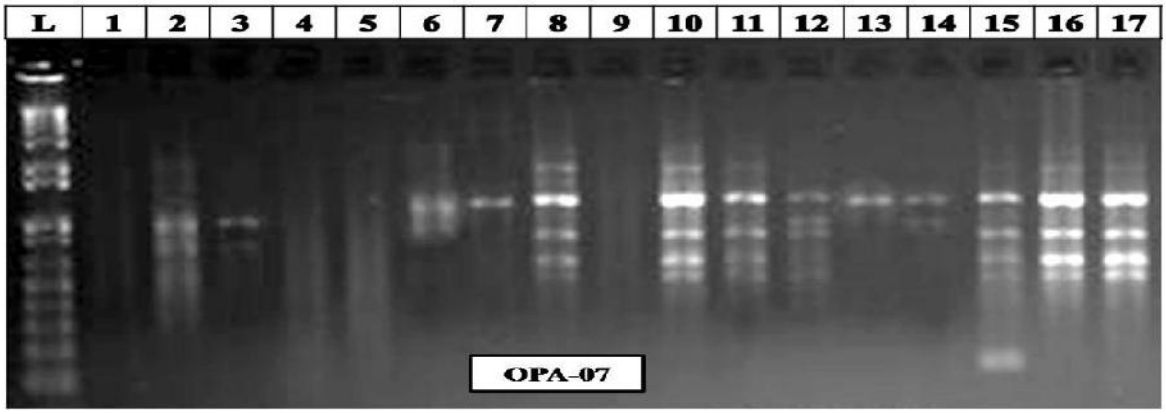

Fig. 2. RAPD marker profile amplified by OPA-07 in ridge gourd and sponge gourd genotypes

sub cluster IA(i) (with two ridge gourd genotypes i.e. ARGS-07-40 and ARGS-07-50) and IA(ii) (with six genotypes). The sub sub cluster IA(ii) was again divided into two cluster IA(ii)a and IA(ii)b each with three genotypes. The cluster IB consisted of solitary sponge gourd genotype (JSG-05-04). Likewise the cluster II was again split ed into two subclusters, cluster IIA (seven genotypes) and cluster IIB (one genotype). The sub cluster IIA was again divided into sub-sub cluster IIA(i) and IIA(ii). The cluster IIA(i) was again divided into cluster IIA(i)a with three genotypes (GARG-1, ASGS-08-40 and ASGS-08-41) and IIA(i)b with one genotype GSG-1. The sub cluster IIA(ii) consisted of three genotypes *viz*., ASGS-06-30 and JSG-05-07 with 78.6% similarity and pusa chikni, while sub cluster IIB consisted of one genotype of ASGS-04-23.

Similar findings were also recorded by Chang *et al.* (2003), Resmi and Sreelathakumary (2011) and Song *et al.* (2010). Chang *et al.* (2003) classified twenty pumpkin cultivars into three large categories and identified genetic distance of cluster ranging from 38% and 100%. Resmi and Sreelathakumary (2011) estimated Jaccard's similarity coefficients and constructed dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the twenty-five landraces of ashgourd. Pair-wise

genetic similarities among the landraces determined using Jaccard's coefficient ranged from 0.14 to 1.00. Song *et al.* (2010) recorded that genetic similarity between wax gourd and chieh-qua germplasm was in the range of 60% to 99%.

Latha (2012) studied RAPD analysis in cucumber and reported that OPA-19 primer recorded the highest polymorphism (86.6%). Out of 130 bands, 115 bands were polymorphic for a specific primer and can be used as differential markers for varietal differentiation. Likewise, At yaf (2014) studied RAPD profile in squash gourd and generated high level of polymorphism. OPA-03 and OPC-19 primers showed high value for number of main, amplified and unique bands and success in giving a distinct fingerprint for all genotypes. The highest value for primer efficiency and discriminatory value were produced by the primers OPD-13 and OPN 06, respectively. Thus, RAPD markers provides an excellent tool for future studies of squash genotypes fingerprinting using both OPA-03 and OPC-19 primers.

ISSR analysis

Screening of 30 ISSR primers was carried out using genomic DNA of ridge gourd (Pusa Nasdar) and sponge gourd (Pusa Chikni). Twelve primers gave satisfactory clear banding pat ern. Twelve ISSR primers produced 79 bands/alleles in which 66 bands were polymorphic and 83.54 % polymorphism with an average of 7.16 bands per primer (Table 3). The size varied from 96-1307 bp. Among them, there was maximum amplified allele size of 1307 bp (primer ISSR-834) and minimum amplified allele size of 96 bp (primer UBC-808). Primer ISSR-840 produced maximum 11 bands, while primer UBC-840 produced minimum 3 bands. A maximum 100% polymorphism was obtained with ISSR-834, ISSR-840, ISSR-856 and ISSR-865 primers. The polymorphic information content (PIC) ranged between 0.6548 (UBC-840) and 0.8939 (ISSR-840) with an average of 0.7988 per primer. Based on PIC value, the ISSR-840 was the best primer resulting in good amplification with maximum PIC value (0.8939). Likewise, ISSR primer index (IPI) ranged from 1.9644 to 9.8328 with an average of 5.4092 per primer. The maximum IPI value was obtained by UBC-840 and the minimum was obtained by ISSR-840.

Clustering pat ern using ISSR data of ridge gourd and sponge gourd genotypes is described in Figure 3. Jaccard's coefficient of similarity between 17 ridge gourd and sponge gourd genotypes ranged from 22.7% (between ARGS-07-40 and JSG-05-04) to 81.2% (between JSG-05-07 and Pusa Chikni). Dendrogram generated by ISSR molecular data seventeen ridge gourd and sponge gourd genotypes were grouped

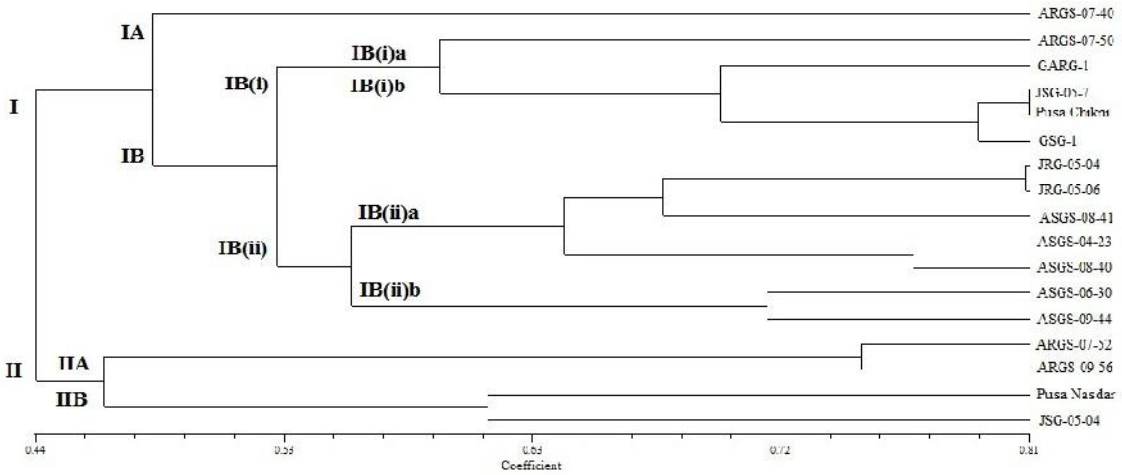

Fig. 3. Dendogram depicting the genetic relationship among 17 ridge gourd and sponge gourd genotypes based on ISSR markers

into two main clusters, cluster I and cluster II with an average similarity of 44% (Figure 3). The cluster analysis of ISSR revealed the two main clusters, which further divided into various sub-clusters. The cluster I consisted of thirteen genotypes and these were further divided into sub cluster IA and IB with one and twelve genotypes, respectively. The sub cluster IA was solitary cluster with genotype ARGS-07-40. On the other hand, the sub cluster IB was split ed into two sub cluster IB(i) and IB(ii) with five and seven genotypes, respectively. The sub cluster IB(i) was further divided into two cluster IB(i) a with only one genotype (ARGS-07-50) and IB(i)b with four genotypes. Similarly, sub cluster IB(ii) was again divided into two cluster IB(ii)a with five genotypes namely JRG-05-04, JRG-05-06, ASGS-08-11, ASGS-04-23 and ASGS-08-40, While sub cluster IB(ii)b with two sponge gourd genotypes (ASGS-06-30 and ASGS-09-44). The cluster II was again divided into two sub cluster IIA with two ridge gourd genotypes (ARGS-07-52 and ARGS-09-56) and IIB with two genotypes (Pusa Nasdar and JSG-05-04 of ridge gourd and sponge gourd, respectively).

Similar results have also been reported by Behera *et al.* (2008), Sikadar *et al.* (2010) and Haung *et al.* (2010). Behera *et al.* (2008) worked with fif een ISSR primers which produced 125 bands in the bit er gourd accessions with size ranged between 150 bp and 2700 bp. Sikadar *et al.* (2010) observed that out of the ten (ISSR) primers, eight produced informative data for phylogenetic analysis in *Cucurbita* species. These eight primers produced 139 ISSR fragments, an average of 17.37 bands per primer. Amplified product sizes ranged from 599 to 2399 bp. Haung *et al.* (2010) observed that size of amplicons ranged from 150 bp to 2700 bp with six ISSR primers in 38 diverse bit er gourd accessions.

Bhawna *et al.* (2014) studied 209 amplified bands from 20 ISSR primers in which 186 were polymorphic (89.00%) bands. Jaccard's similarity coefficient matrix was generated for pair-wise comparisons between individual ISSR profiles and UPGMA cluster analysis based on this matrix showed clustering into six groups. Jaccard's coefficient of similarity values ranged from 0.409 to 0.847, with a mean of 0.628 revealing a moderate level of genetic diversity in bot le gourd. Yildiz *et al.* (2014) also characterized 24 accessions covering different groups of *Cucumis melo* L. collected from Eastern and South-eastern Anatolian regions of Turkey by using 43 morphological traits and 207 markers obtained from 31 ISSR and 16 SSR primers. The findings indicated wide range of variations for investigated characteristics in Turkish gene pool that provides a good source of diversity to use in melon improvement program for bet er yield. De *et al.* (2015) studied phylogenetic pat erns and relatedness among selected species of cucurbits using ISSR markers. Total of 117 bands, of which 57

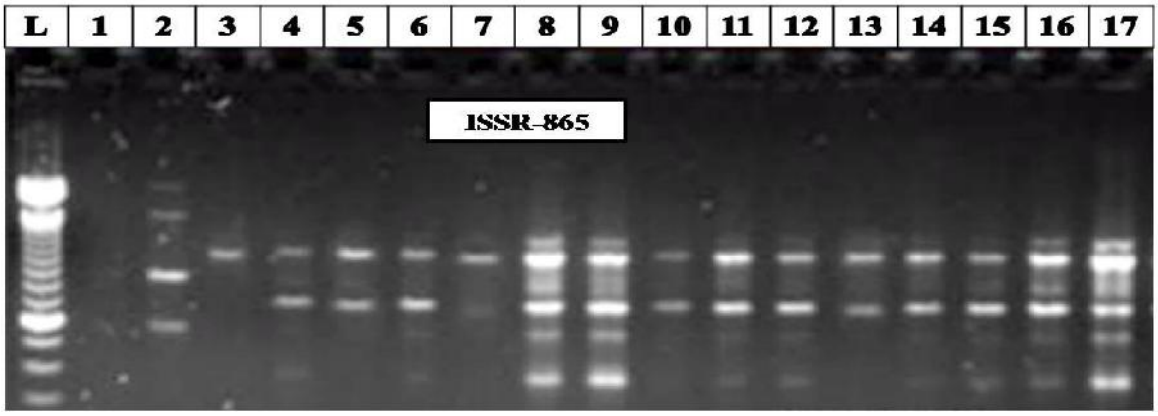

Fig. 4. ISSR marker profile and frequency of alleles amplified by ISSR-865 in ridge gourd and sponge genotypes

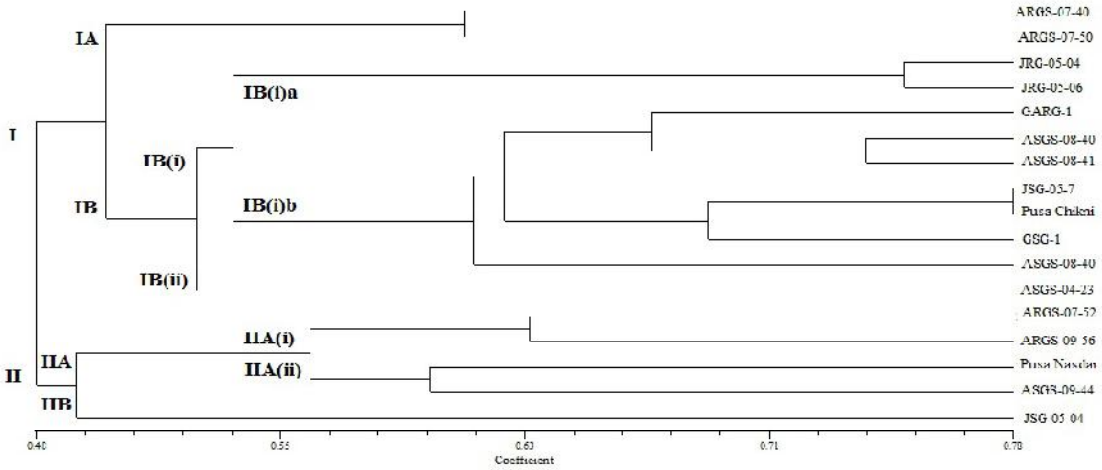

Fig. 5. Dendogram depicting the genetic relationship among 17 ridge gourd and sponge gourd genotypes based on pooled data of RAPD and ISSR markers

were polymorphic, were amplified by five primers. The phylogram generated on the basis of Jaccard's similarity coefficient revealed a close genetic relationship between *C. maderaspatanus* and *C. melo*, while *C. sativus*, a member of the same genus, was placed as a distant relative from both species, thereby demonstrating remarkable diversification among members of the same genus.

Clustering pat ern based on RAPD and ISSR combined data

The RAPD and ISSR data were combined for UPGMA cluster analysis. The UPGMA dendrogram obtained from the cluster analysis of RAPD and ISSR data is shown in Figure 5. The pooled study of molecular marker through RAPD and ISSR used to confirm the differences and similarity between 17 ridge gourd and sponge gourd genotypes. The Jaccard's similarity coefficient and UPGMA method showed the highest (78.1%) similarity between JSG-05-7 and Pusa Chikni and the lowest (36.5%) similarity between Pusa Nasdar and ASGS-04-23.

The dendrogram consisted of two main clusters I and II with an average similarity of 48% (Figure 5). The cluster I and cluster II consisted of twelve genotypes and five genotypes, respectively. The cluster I was further sub divided into two sub cluster, cluster IA with two genotypes namely ARGS-07-40 and ARGS-07-50 as well as cluster IB with ten genotypes. The sub cluster IB was again divided into two clusters, cluster IB(i) with nine genotypes and IB(ii) with solitary genotype ASGS-04-23. The sub sub cluster IB(i) was again divided into two cluster IB(i)a with two ridge gourd genotypes viz., JRG-05-04 and JRG-05-06. While sub cluster IB(i)b consisted six genotypes of sponge gourd (ASGS-08-40, ASGS-08-41, JSG-05-7, Pusa Chikni, GSG-1 and ASGS-06-30) and one ridge gourd genotype (GARG-1). Likewise, cluster II was again subdivided into two sub cluster IIA with four genotypes and IIB with solitary genotype of JSG-05-04. The sub cluster IIA was split ed into two sub sub cluster IIA(i) and IIA(ii) each with two genotypes.

Among the studied techniques, RAPD primers gave slightly higher polymorphism among the genotypes (86.17%) as compared to ISSR markers (83.54%). However, more number of polymorphic fragments, more PIC and higher percentage polymorphism per primer were amplified by ISSR as compared to RAPD markers. Both RAPD and ISSR markers gave distinct clustering pat erns. Based on molecular data of the present study, it can be concluded that the

molecular markers could be a bet er tool for studying the genetic diversity.

Similar findings were also recorded by Goswami and Tripathi (2010) and Manohar *et al.* (2012). Goswami and Tripathi (2010) recorded Jaccard's similarity coefficient and UPGMA clustering algorithm. The UPGMA dendogram obtained from the cluster analysis of RAPD and ISSR data gave similar clustering pat ern, with Jaccards similarity coefficient ranging from 0.23 to 0.93. This study showed that RAPD and ISSR markers could provide a practical and efficient tool in quality control of the *Trichosanthes dioica.*

Manohar *et al.* (2012) recorded the Jaccard's similarity coefficients ranged from 0.36 to 0.84 and the first two principal components explained 53.33 % of the total variance. The UPGMA phenogram and the Principle Component Analysis (PCA) indicated that the populations formed five major clusters. CSC 83 (774 g per fruit) and CSC 71 (yellow skin) are considered to be the most important collections to be stressed for further breeding purpose.

Unique markers for DNA fingerprinting of genotypes

In RAPD marker, one genotype specific band was observed by primer OPA-07 in genotype JSG-05-7 (Figure 2). Two genotype specific bands were observed by primer ISSR-865 in genotype ARGS-07-50 in case of ISSR (Figure 4). Similar findings were also reported by Anatala *et al.* (2014) in cowpea using ISSR and RAPD markers; by Dat a *et al.* (2014) in grass pea (*Lathyrus sativus*) and Prajapati *et al.* (2014) in pigeon pea using RAPD markers.

From the above study, it can be concluded that molecular markers RAPD and ISSR are effective for determining polymorphism and very useful to study the diversity analysis. Both the markers (RAPD and ISSR) produced unique positive and negative fragments as specific DNA finger print for identification of ridge gourd and sponge gourd genotypes.

Conclusion

Molecular markers represent new tools for a bet er understanding of genetic diversity. The formation of several subclusters within cluster I suggested the presence of moderate genetic diversity among the 17 ridge gourd and sponge gourd genotypes studied. Genetic diversity analysis through RAPD marker gave highest (86.17%) polymorphism percentage with primers *viz.*, OPA-07, OPW-06, OPW-08, OPX-01, OPW-03, OPF-08, OPF-10, UBC-115 and UBC-199. Therefore, these primers were most useful for genetic diversity analysis to generate DNA fingerprinting in ridge gourd and sponge gourd genotypes. Twelve ISSR primers selected in the present study gave 83.54% polymorphism. The highest polymorphism percentage (100%) was observed with primer ISSR-834, ISSR-840, ISSR-856 and ISSR-865. Therefore, this ISSR primer can be used further for diversity study in different ridge gourd and sponge gourd genotypes.

References

Anatala, T.J., Gajera, H.P., Savaliya, D.D., Domadiya, R.K., Patel, S.V. and Golakiya, B.A. 2014. Molecular diversity analysis of cowpea (*Vigna unguiculata* L.) genotypes determined by ISSR and RAPD markers. *International Journal of Agriculture, Environment and Biotechnology* **7**(2): 269-276.

Anderson, J.A., Churchill, G.A., Sutrique, J.E., Tanksley, S.D. and Sorrels, M.E. 1993. Optimizing parental selection for genetic linkage maps. *Genome* **36**: 181-186.

At yaf, J.T.A. 2014. Genetic Fingerprint of Some *Cucurbita pepo* (Summer squash) Genotypes Using Molecular and Biochemical Techniques. *Magazin of Al-Kufa University for Biology* **6**(1): 1-13.

Behera, T.K., Gaikward, A.B., Singh, A.K. and Staub, J.E. 2008. Relative efficiency of DNA markers (RAPD, ISSR and AFLP) in detecting genetic diversity of bit er gourd (*Momordica charantia* L.). *Journal of Science and Food Agricultre* **88**: 733-737.

Bhawna G., Abdin, M., Arya, L., Saha, D., Sureja, A., Pandey, C. and Verma, M. 2014. Population structure and genetic diversity in bot le gourd [*Lagenaria siceraria* (Mol.) Standl.] germplasm from India assessed by ISSR markers. *Plant Systematics and Evolution.*

Chang, H.B., Long, B., Park, Y., Jin Ma and Woojin, A. 2003. Assessment of genetic relationship among *cucurbitaceae* cultivars revealed by RAPD marker. *Journal of Life Science* **13**(5): 590-595.

Dat a, S.K., Mandal, R., Roy, P., Mandal, N. and Tarafdar, J. 2014. Detection of genetic diversity in *Lathyrus sativus* L. using RAPD marker system. *International Journal of Agriculture, Environment and Biotechnology* **7**(4): 729-733.

De, Payel, Parab, Mala and Singh, Sunita. 2015. Article; agriculture and environmental biotechnology Inter-genus variation analysis in few members of *Cucurbitaceae* based on ISSR markers. *Biotechnology and Biotechnological Equipment* **29**(5): 882-886.

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* **19**: 11-15.

Goswami, Sandhya and Tripathi, Vivek, 2010. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among *Trichosanthes dioica* Roxb. cultivars. *International Journal of Biodiversity and Conservation* **2**(12): 405-413.

Hoque, S. and Rabbani, M.G. 2009. Assessment of genetic relationship among landraces of Bangladeshi ridge gourd (*Luffa acutangula* Roxb.) using RAPD markers. *Journal of Scientific Research* **1**(3)**:** 615-623.

Huang, C.H., Wang, C.J. and Chyuan, J.H. 2010. Analysis of the genetic diversity and variety identification of bit er gourd (*Momordica charantia* L.) by ISSR marker. *Hualien District Agricultural Improvement Station, Bulletin* **28**: 21-33.

Kalloo, G. 1993. Egg plant *Solanum melongena*. In: Kalloo, G., Bergh, B.O. (Eds.), Genetic Improvement of Vegetable Crops. Pergamon Press Oxford pp. 587-604.

Latha, K. 2012. Genetic diversity in 6 local cucumber varieties (Cucumis *Satives*) in karnataka market by RAPD-PCR technique. *International Journal of Advanced Biological Research* **2**(1): 39-45.

Manohar, S.H., Murthy, H.N. and Ravishankar, K.V. 2012. Genetic diversity in a collection of *Cucumis sativus* L. assessed by RAPD and ISSR markers. *Journal of Plant Biochemistry and Biotechnology,* pp. 1-3.

Mohammed, Ismail, A., Gumaa, Abdel gabbar. N., Kamal, Nesreen, M., Alnor, Yasir, S. and Ali, Abdelbagi, M. 2012. Genetic diversity among some cucurbits species determined by Random Amplified Polymorphic DNA marker. *International Journal of Plant Research* **2**(4): 131-137.

Prajapati, V., Soni, N. and Sasidharan, N. 2014. Molecular study of pigeonpea [*Cajanus cajan* (L.) Mill sp.] genotypes for *Fusarium* wilt using RAPD markers. *International Journal of Agriculture, Environment and Biotechnology* 7: 459-465.

Resmi, J. and Sreelathakumary, I. 2011. RAPD markers for genetic variability studies in ashgourd [*Benincasa hispida* (Thunb.) Cogn.]. *Journal of Agricultural Technology* **7**(4): 1097-110.

Rohlf, F.J. 2000. *NTSYS-pc:* Numerical taxonomy and multivariate analysis system, version 2.02 manual. Exeter Sof ware, New York.

Sikadar, B., Bhat acharya, M., Mukherjee, A., Banerjee, A., Ghosh, E., Ghosh, B. and Roy, S.C. 2010. Genetic diversity in important members of *Cucurabitaceae* using isozyme, RAPD and ISSR markers. *Biologia Plantarum* **54**(1): 135-140.

Smith, N.D. and Devey, M.E. 1994. Occurrence and inheritance of microsatellites in *Pinus radiata*. *Genomics* **37**: 977-983.

Song, S.W., Li, Z., Liu, H.C., Sun, G.W. and Chen, R.Y. 2010. RAPD analysis of genetic diversity of wax gourd and chieh-qua germplasm. *China Vegetables* **22**: 47-53.

Tingey, S.V., Rafalski, J.A. and Williams, J.G.K. 1992. Genetic analysis with RAPD markers. Proceedings of the symposium: Applications of RAPD Technology to Plant Breeding. Minneapolis, MN, Joint Plant Breeding Symposium: Crop Science, Horticultural Science, American Genetic Association, pp. 3-8.

Vosman, M.I., Uzunova, M.I. and Ecke, W. 1992. Abundance, polymorphism and genetic mapping of microsatellites in *Allium. Plant Breed* **45 :**156-167.

Williams, J., Kubelik, A., Liviak, J.K., Rafalski, A.J. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* **18**: 6531-6535.

Yildiz, M., Akgul, N. and Sensoy, S. 2014. Morphological and molecular characterization of turkish landraces of *Cucumis melo* L. *Notulae Botanicae Horti Agrobotanici* **42**(1):51-58.