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Electrophoretic protein profile and ISSR marker based genetic relationship study in ridge gourd [*Luffa acatangula* (Roxb.)L.]

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Abstract

An experiment was conducted to study the biochemical as well as molecular traits among eight ridge gourd inbred lines by using SDS-PAGE analysis and ISSR marker data. The entire seed protein profile comprised of 24 protein bands, distributed into four major zones A, B, C and D in the increasing order of electrophoretic mobility as analysed by SDS-PAGE. Maximum sixteen bands were found in PRG 120 and minimum thirteen bands were observed in PCPGR 7256 and PRG 142. Similarity index value ranged from 25% to 100%. Hundred percent similarities were observed for genotype PCPGR 7256 with PRG 117. Genotype PRG 131 showed least similarity (25%) with PRG 132 as PRG 137 with PRG 7. The UPGMA clustering divided all the genotypes into two major clusters (I and II) with 43% similarity among them. Cluster I comprised of 5 genotypes and cluster II comprised of 3 genotypes. Among 20 ISSR markers, only 4 were selected due to their polymorphic bands. A total of 27 bands were produced and the number of loci ranged from 6 to 7. The per cent polymorphism varied from 66.67 to 85.71 with an average of 74.07. The similarity matrix value ranged from 43% to 90%. The maximum similarity value was recorded for PCPGR 7256 (0.90) with PRG 142. The lowest similarity was recorded for PCPGR 7256 (0.43) with PRG 137. In ISSR profile based dendogram eight genotypes were classified into two main clusters. Cluster I comprised of 5 genotypes with 62% similarity among themselves whereas cluster II comprised of 3 genotypes with 67% similarity. The information generated in the present study could be further utilized for ridge gourd improvement programme in future.

Key words: Ridge gourd, genetic diversity, SDS-PAGE, ISSR

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Introduction

Ridge gourd [*Luffa acutangula* (Roxb.) L.], 2n=2x=26, is one of the important cucurbitaceous vegetable crops. India being a centre of diversity is endowed with great variability in terms of morphological characters of this vegetable, especially growth habit, shape, skin colour, size and flesh thickness of fruits. Ridge gourd fruits contain fair amount of antioxidants and minerals (Karmakar et al. 2013). The predominant sex form in ridge gourd is monoecious but hermaphrodite sex form is reported in Satputia. Hermaphrodite lines have huge potential to improve plant architecture, earliness and yield traits (Karmakar et al. 2014); antioxidant content in monoecious ridge gourd (Karmakar et al. 2013) and also provide heterotic cross combination for yield parameter in ridge gourd (Karmakar et al. 2014, Sarkar and Singh 2017). In spite of substantial variation it is difficult to distinguish genotypes on their external morphology alone. Assessment of genetic diversity based on phenotype has limitations, since most of the morphological characters are influenced by environment and developmental stage of the plant. Biochemical methods using storage proteins, isozymes have been widely used for assessing the genetic diversity (Smith and Smith 1992). These methods used in routine testing of parents, maintaining genetic purity and also serve as additional descriptors in DUS testing. Identification of cultivars by the use of protein profiles is more suitable as this is expression of genetic makeup of plants and protein profiles are species specific. Polymorphism at protein level can help to measure genetic distance or genomic similarities between pair of parental lines and hybrids. Thus, inbreeds can be assigned into respective heterotic groups in hybrid breeding programme. The major disadvantages of biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental 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 ridge gourd using the modern molecular approaches. This would allow a more efficient utilization of plant characters in developing suitable stable varieties with higher yield. Besides these, molecular marker based characterization is an unambiguous, reliable, fast and cost-effective assessment of genetic diversity and 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). Inter-simple sequence repeats (ISSR) is a dominant and semi-arbitrary DNA marker amplified by PCR in the presence of one primer complementary to a target microsatellite region of a genome. It is easy and quick to handle and like RAPD and it does not require prior knowledge of the genome, cloning or specific primer design. It has higher reproducibility than RAPD due to high annealing temperature and the cost of analysis is lower than the cost of AFLP (Reddy et al. 2002). The potential supply of ISSR markers depend on the variety and frequency of microsatellites, which changes with species and the repeated motifs that are targeted (Depeiges et al. 1995). ISSR has been used in genetic diversity analysis of different cucurbits (Dje et al. 2006) and phylogenetic studies in *Citrullus* spp., *Cucumis* spp. and *Praecitrullus fistulosus* (Levi et al. 2005) and among different subspecies of *Cucurbita pepo* (Paris et al. 2003). In this paper we studied the extent of genetic diversity of ridge gourd using electrophoretic protein profiling (SDS-PAGE) and ISSR markers.

Materials and Methods

Plant materials: A total of eight ridge gourd inbred lines were analyzed by SDS-PAGE for biochemical characterization as well as by molecular marker (ISSR) to assess diversity. In this experiment seven genotypes were monoecious and one was hermaphrodite in sex form (Table 1).

SDS-PAGE: SDS-PAGE of total soluble seed proteins was carried out by using 12.5% gels as per Laemmli (1970). Two to three seeds without seed coat were taken and ground to fine powder with the help of pestle and mortar, 0.1g of this powder was added to Tris HCl extraction buffer (25mM, *p*H 8.8) and vortexed briefly for 1-2 minutes. The tubes were kept in hot water at 100^0C for 5 minutes and cooled to room temperature. The contents were centrifuged (Systonic) for 30 minutes at 10,000 rpm in a refrigerated centrifuge at 4°C temperature. The supernatant was collected in separate tubes and kept at 4°C for the electrophoresis use. This protein extract was dissolved in an equal volume of working buffer (0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.025% bromophenol blue) and incubated at 60-70ºC for 10 minutes, cooled immediately for 5 minutes and centrifuged at 10,000 rpm for 5 minutes. The supernatant was used for loading on to a vertical SDS-polyacrilamide gel. A medium range (14.3 to 97.4 kD) marker provided by Bangalore Genei Pvt. Ltd., Bangalore was used. The gel was run at a voltage of 80 V until the tracking dye crossed the stacking gel and then increased up to 120 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. The gel was stained using Coomassie brilliant blue solution overnight and destained using a mixture of 227 ml of methanol, 46 ml of acetic acid and 227 ml of distilled water until the bands were clearly visible. Protein bands of different intensities and positions were obtained after destaining and counted by keeping the gel on a light box. On the basis of intensity of bands they were classified into dark, medium dark and light bands. The electrophoregrams of protein profile were prepared and gel was photographed. Obtained electrophoreograms were analysed to calculate the Jaccard's coefficient of genetic similarity matrix using the software, SPSS version 11.0. Cluster analysis was performed to produce a dendrogram using unweighted pair group method using arithmetic averages (UPGMA).

Table 1: Details of experimental materials

S. No.	Inbred Line	Nature
1.	PCPGR 7256	Hermaphrodite
2.	PRG 117	Monoecious
3.	PRG 142	Monoecious
4.	PRG 131	Monoecious
5.	PRG 137	Monoecious
6.	PRG 132	Monoecious
7.	PRG 120	Monoecious
8.	PRG 7 (Pant Torai 1)	Monoecious

ISSR: The genomic DNA was isolated from a bulk of 3 week old seedling leaf tissues taken from 5 plants from each inbred line. 3-4 g leaf tissue was grounded in liquid nitrogen until it formed a very fine powder. DNA was extracted by using the CTAB method (Doyle and Doyle 1987). The extracted DNA solution was purified by RNase and the concentration of DNA was estimated by optical density (@ 260 and 280 nm; Systronics India Ltd.). The selection of ISSR markers was based on their previous performance successfully in other cucurbits. Among 20 markers used in other cucurbits only 4 were selected due to their polymorphism shown in ridge gourd (Table 2). PCR amplifications were carried out in 25il (final reaction) volume containing 20 ng template DNA, 10 mM dNTPs, 0.50 il TaqDNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) and 0.8 mM of each primer (1.5 il). Amplification reactions were carried out in 0.2 ml tubes using an

Table 2: Details of ISSR Primers

S. N.	Genei Code no.	Sequence 5'- 3'	GC content (%)	Tm (°C)
1	Primer 809	AGAGAGAGAGAGAGAGYG	52.7	50.5
2	Primer 843	CTCTCTCTCTCTCTCTRA	47.2	47.1
3	UBC 854	TCTCTCTCTCTCTCTCAGG	52.6	51.5
4	UBC 855	ACACACACACACACACCTT	47.3	54.6
5	UBC 856	ACACACACACACACACCTA	47.3	53.8
6	UBC 861	ACCACCACCACCACCACC	66.6	60.6
7	UBC 890	ACGACTACGGTGTGTGTTTGTGT	47.8	58.8
8	UBC 840	GAGAGAGAGAGAGAGACTT	47.3	49.2
9	UBC 808	AGAGAGAGAGAGAGAGC	52.9	48.8
10	UBC 825	ACACACACACACACACT	47	51.4
11	ISSR CR-2	CACACACACACACACAAG	50	51.6
12	UBC 866	CTCCTCCTCCTCCTCCTC	66.6	55.7
13	UBC 846	CACACACACACACACART	47.2	51.8
14	Sola 1	BDBACAACAACAACAACA	37	47.8
15	Sola 5	DBDACACACACACACAC	49	50.1
16	Sola 11	GAGCAACAACAACAACAA	38.8	48.7
17	B 5	GAGAGAGAGAGAGAGAT	47	45.4
18	B 10	AGAGAGAGAGAGAGAGG	52.9	48.2
19	Sola 2	DDCGACGACGACGACGA	62.7	57.4
20	Sola 4	VHVGTGTGTGTGTGTGTG	53.7	53.7

eppendorf Thermal cycler (Bio-Rad). The amplification consisted of a 5 minutes initial denaturation step at 94°C followed by denaturation at 94°C for 1 minute. Annealing of the primer was done at 51°C for 1.30 minutes followed by an extension period for 2 minutes at 72°C. The reactions were subsequently subjected to 35 additional cycles after reaching the final annealing temperature. This was followed by a final extension at 72°C for 7 min. PCR products were separated in 1.5% agarose gels in 1X TBE buffer by electrophoresis at 80 V for 3-4 hour using a horizontal gel electrophoresis system (Sigma-Aldrich). Gels were stained with ethidium bromide. The amplified fragments were visualized and photographed under UV light. Reproducible DNA bands were scored manually. Weak bands with negligible intensity were excluded from the final data analysis. Band profiles for each parent were scored in a binary mode (1 indicating its presence; 0 indicating its absence). The binary data were used to compute pair wise similarity coefficient (Jaccard 1908). A dendrogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA) and computation for multivariate analysis was done using the computer programme NTSYS-pc Version 2.0 (Rohlf 1998).

Results and Discussion

The protein profile (Fig. 1) and zymogram (Fig. 2) of banding pattern comprised of twenty four protein bands, distributed into four major zones A, B, C and D in the increasing order of electrophoretic mobility. The proteins separated on 12.5 per cent acrylamide gel were distinguished and grouped based on the standard marker (14.3 to 97.4 kD). Each major zone was further subdivided into a number of bands. Zone A representing the heaviest molecular weight protein was subdivided into 5 intense to light and sharp band of subzones A1, A2, A3, A4 and A5. Zone B was representing thin and light bands of subzones B1, B2, B3, B4, B5, B6 and B7. Zone C was representing thick and sharp bands of subzones C1, C2, C3, C4, C5, C6 and C7. Zone D was representing thick bands of subzones D1, D2, D3, D4 and D5. Subzone A1 was only present in PRG 7. Subzone A2 was absent only in PRG 7. Subzone A3 and A5 were present in all 8 genotypes. Subzone A4 was absent in PCPGR 7256 and PRG 117 and present

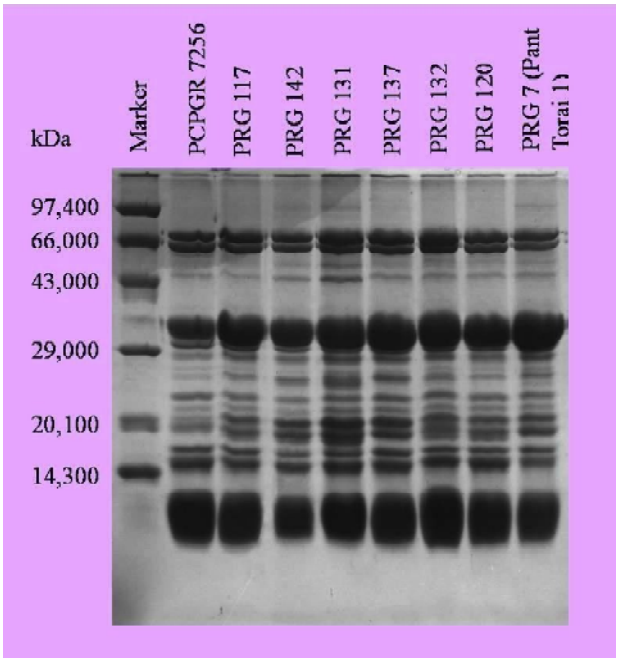

Figure 1: seed protein profile of ridge gourd inbred lines

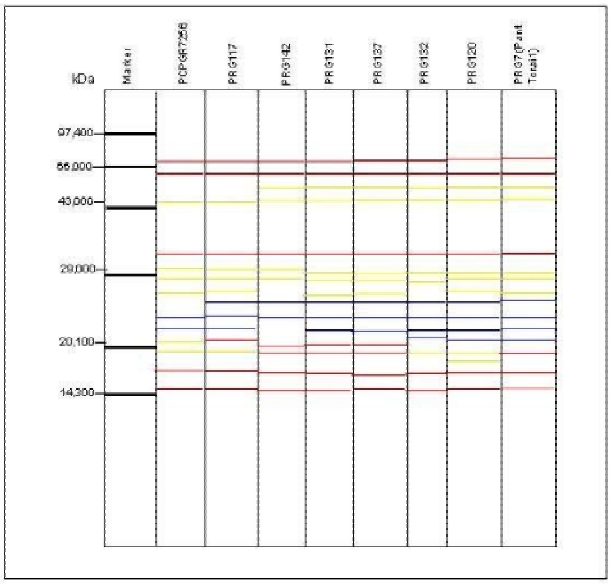

Figure 2: Electrophoretic banding pattern (zymogram) of seed protein profile in ridge gourd inbred lines

in all the rest genotypes. Subzone B1 was present in all the parents. Subzone B2 was present in PCPGR 7256, PRG 117 and PRG 142. Subzone B3 was present in PRG 131, PRG 137, PRG 132, PRG 120 and PRG 7. B4 was present in PRG 120 and PRG 7. Subzone B5 was present in PCPGR 7256, PRG 117, PRG 142, PRG 131, PRG 137 and PRG 132. Subzone B6 was present in PCPGR 7256, PRG 117, PRG 120 and PRG 7. Subzone B7 was present in PRG 131 and PRG 137. Subzone C1 was absent only in PCPGR 7256, subzone C4 and C5 was present only in PRG 137 and PRG 132 respectively. Subzone C2 was present in all genotypes. Subzone C3 was present in PCPGR 7256, PRG 117, PRG 131, PRG 132, PRG 120 and PRG 7. Subzone C6 was present in PCPGR 7256, PRG 117, PRG 120 and PRG 7. Subzone C7 was present in PRG 142, PRG 131 and PRG 137. Subzone D1 and D5 were present in all the genotypes. Subzone D2 and D4 were only present in PRG 120 and PRG 137. Subzone D3 was present in all genotypes except PRG 137. Maximum 16 bands were found in PRG 120 and minimum 13 bands were present in PCPGR 7256 and PRG 142. These results were in conformity with previous reports of Singh (1996) in bottle gourd, Yadav et al. (1998) and Chaudhary and Ram (2000) in muskmelon and Singh and Ram (2001) in cucumber. However, differences among genotypes for darkness and thickness of protein bands were also evident. Ladizinsky and Hymowitz (1979) reported such variation as the commonly reported ones, suggesting that the formation of many of the bands in the seed protein profile are under control of quantitative gene system and such variation may be due to lack of separation of several proteins having similar migration rates on the gels. A perusal of SI value in Table 3 revealed that similarity index value ranged from 25% to 100% among all the genotypes. Hundred percent similarity was observed for PCPGR 7256 with PRG 117. PRG 131 and PRG 137 showed least similarity (25%) with PRG 132 and PRG 7 respectively. The dendrogram (Fig 3) clustered all genotypes into two major clusters (I and II) with 43% similarity among them. Cluster I comprised 5 genotypes and cluster II comprised 3 genotypes. Cluster I was further subdivided into 2 sub-clusters IA and IB with 58% similarity. Sub-cluster IA comprised 2 genotypes with 100% similarity. Sub-cluster IB further forked into two small clusters IB1 and IB2. IB1 again divided into IB1-1 comprising PRG 132 and IB1-2 comprising PRG 120. IB2 comprised PRG 7. Main cluster II further subdivided into two sub-clusters IIA and IIB with 86% similarity among them. IIA further forked into two small clusters IIA1 comprising PRG 142 and IIA2 comprising PRG 131. IIB comprised PRG 137. In previous studies, Singh and Ram (2001) classified nineteen cucumber germplasm in eight different groups.

In this present study, very few ISSR primers were taken for analysis, hence, it is essential to draw strong conclusion related to diversity among the inbreds. The similarity value among eight inbred lines ranged from 43% to 90% (Table 4). The maximum similarity value recorded for the PCPGR 7256 (0.90) with PRG 142. The lowest similarity recorded for PCPGR 7256 (0.43) with PRG 137. The UPGMA analysis was done and dendrogram (Fig 4) was constructed using Jaccard s similarity matrix of ISSR markers. Eight genotypes were classified into two main clusters. Cluster I comprised 5 genotypes with 62% similarity among themselves.

Table 3: Similarity index of protein profile in ridge gourd

	PCPGR 7256	PRG 117	PRG 142	PRG 131	PRG 137	PRG 132	PRG 120	PRG 7
PCPGR 7256	1.00							
PRG 117	1.00	1.00						
PRG 142	0.50	0.50	1.00					
PRG 131	0.45	0.45	0.87	1.00				
PRG 137	0.58	0.58	0.83	0.87	1.00			
PRG 132	0.54	0.54	0.37	0.25	0.37	1.00		
PRG 120	0.70	0.70	0.54	0.41	0.54	0.83	1.00	
PRG 7	0.50	0.50	0.33	0.29	0.25	0.79	0.62	1.00

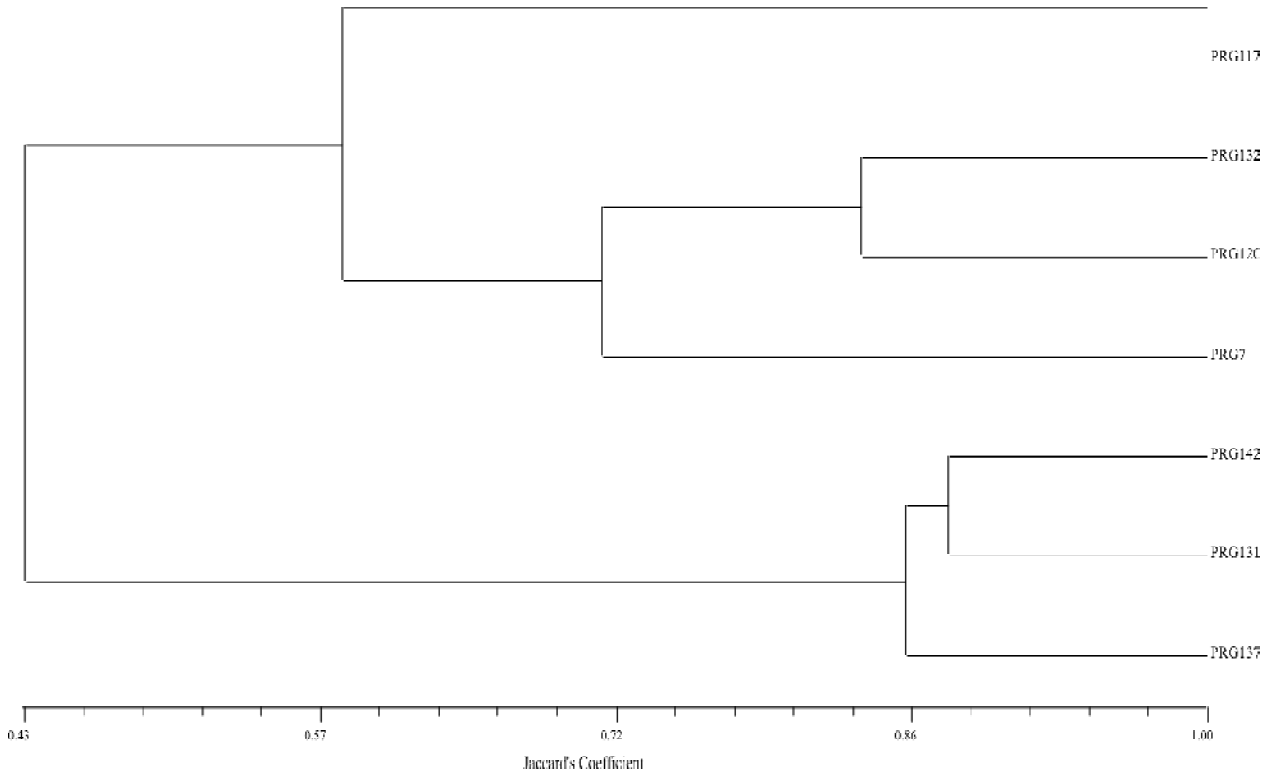

Fig. 3: Dendogram of protein profile of ridge gourd genotypes

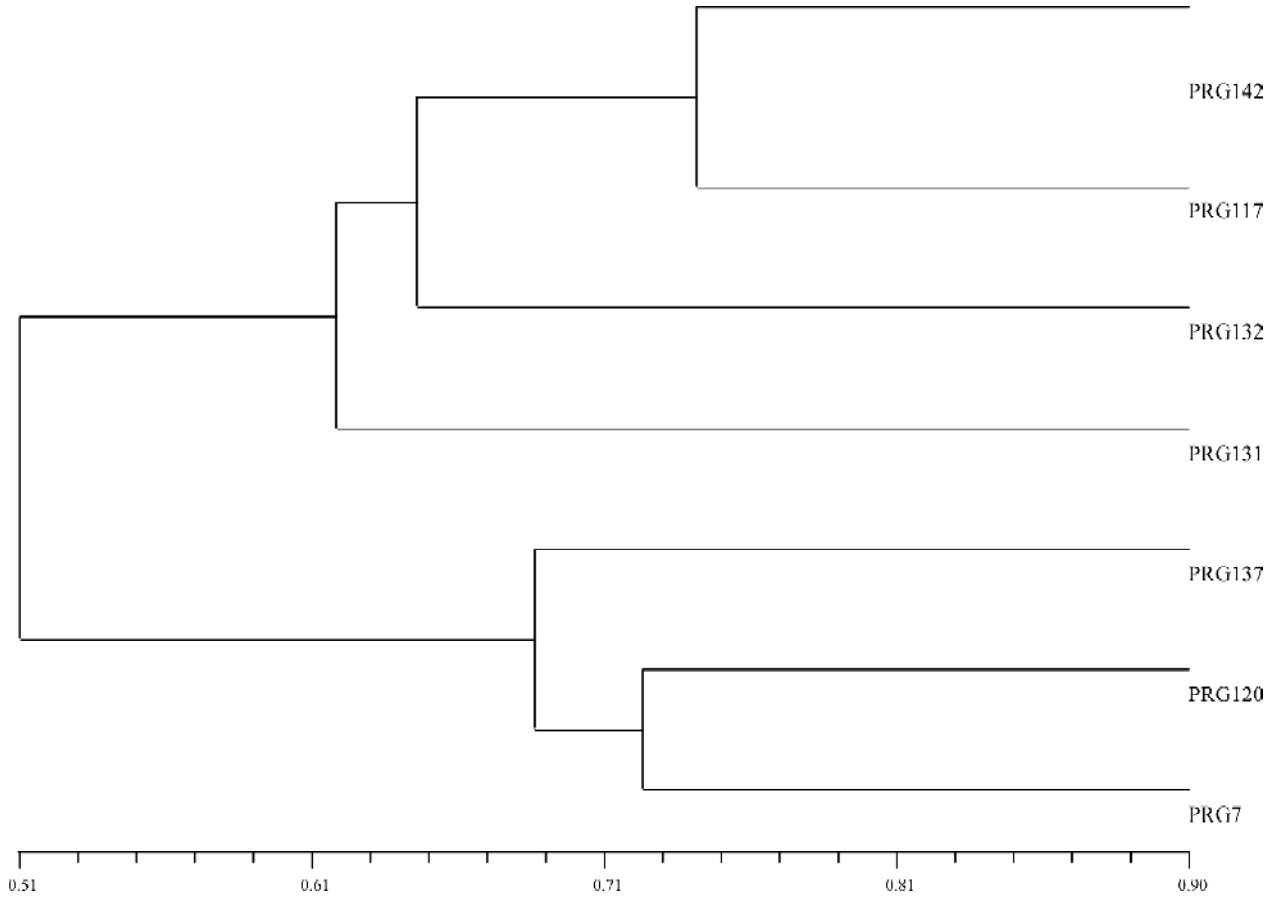

Fig. 4: UPGMA dendogram of ridge gourd genotypes based on Jaccard's Coefficient (ISSR)

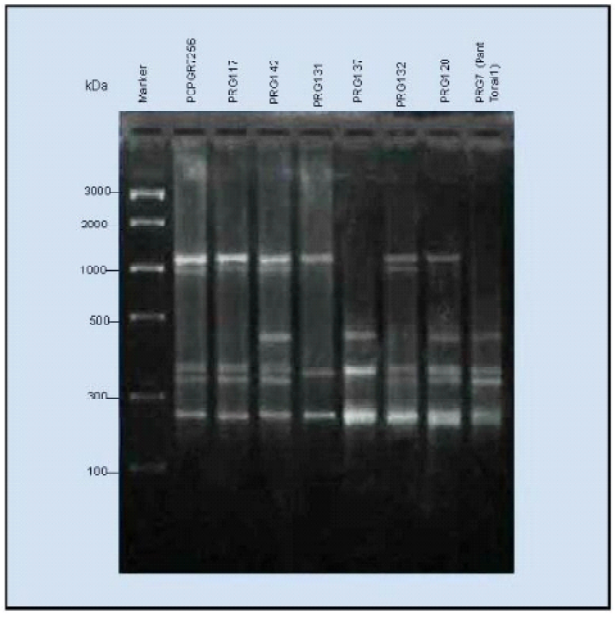

Fig. 5: ISSR profile of hermaphrodite and monoecious ridge gourd genotypes obtained with primer UBC-861

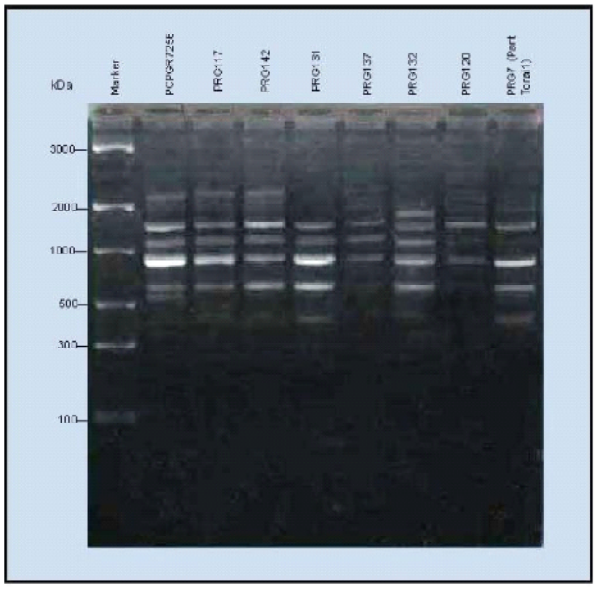

Fig. 6: ISSR profile of hermaphrodite and monoecious ridge gourd genotypes obtained with primer Sola-4

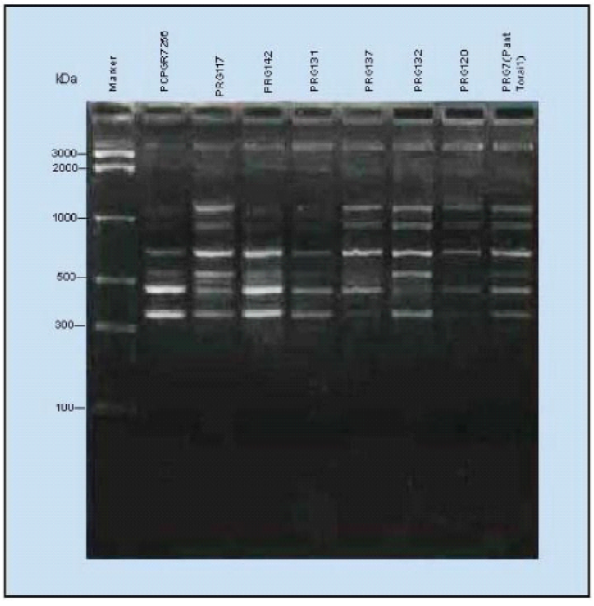

Fig. 7: ISSR profile of hermaphrodite and monoecious ridge gourd genotypes obtained with primer Sola-11

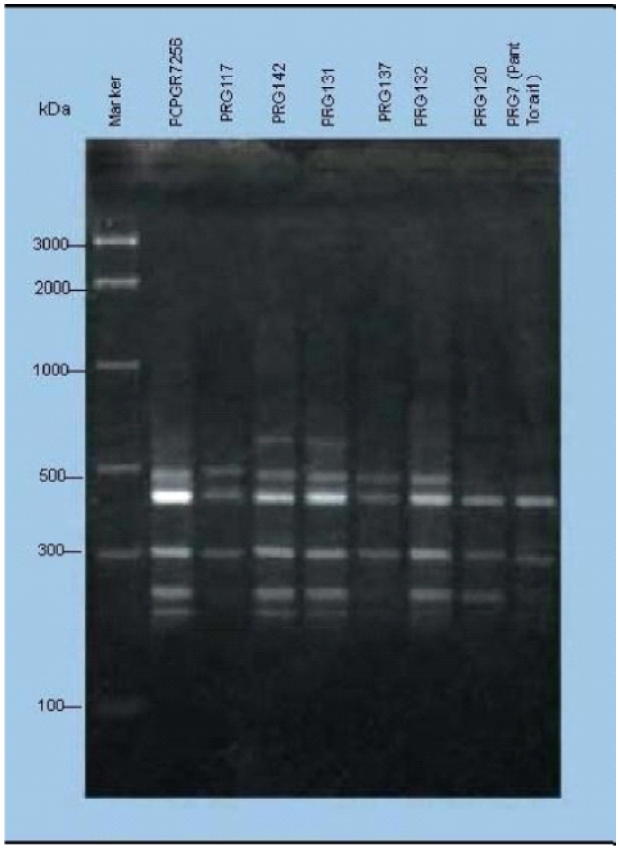

Fig. 8: ISSR profile of hermaphrodite and monoecious ridge gourd genotypes obtained with primer B-5

Cluster II comprised three genotypes with 67% similarity. Cluster I further subdivided into two sub-clusters IA and IB. Sub-cluster IA further forked in to two small clusters IA1 and IA2. IA1 again divided into two super small clusters IA1-1 and IA1-2. IA1-1 comprised two genotypes PCPGR 7256 and PRG 142 with 90% similarity. IA1-2 comprised PRG 117. IA2 comprised PRG 132. Sub-cluster IB comprised PRG 131. Main cluster II further divided in to two subcluster IIA and IIB. IIA comprised PRG 137. IIB again forked into two small cluster IIB-1 and IIB-2 with 72% similarity among them. IIB-1 comprised PRG 120 and IIB-2 comprised PRG 7. Yaboah et al. (2007) constructed genetic map based on ISSR and SRAP markers for cucumber and found 26% (ISSR) and 20% (SRAP)

Table 4: Pair wise similarity matrix for eight genotypes of ridge gourd (ISSR)

	PCPGR 7256	PRG 117	PRG 142	PRG 131	PRG 137	PRG 132	PRG 120	PRG 7
PCPGR 7256	1.00							
PRG 117	0.77	1.00						
PRG 142	0.90	0.70	1.00					
PRG 131	0.68	0.52	0.69	1.00				
PRG 137	0.43	0.54	0.45	0.52	1.00			
PRG 132	0.62	0.73	0.57	0.58	0.54	1.00		
PRG 120	0.47	0.52	0.50	0.50	0.70	0.59	1.00	
PRG 7	0.45	0.56	0.48	0.54	0.66	0.56	0.72	1.00

Table 5: Suitable ISSR markers and their polymorphism in ridge gourd

S. No.	Primer	Total number of ISSR loci	Polymorphic loci	
			Number	Percent
1	UBC-861	7	6	85.71
2	Sola-4	7	5	71.42
3	Sola-11	7	5	71.42
4	B-5	6	4	66.67
Total		27	20	74.07

polymorphism. The above results are in conformity with Levi et al. (2005) in *Citrullus* and Sikdar et al. (2010) in family Cucurbitceae. Total numbers of ISSR bands generated by four markers in eight genotypes were 27 (Table 5). The amplification product with primer UBC-861, Sola-4, Sola-11 and B-5 are depicted in Fig. 5 to Fig. 8. The total number of ISSR loci ranged from 6 (B-5) to 7 (UBC-861, Sola-4 and Sola-11). The highest number of polymorphic bands were observed in UBC-861 (6) followed by Sola-4 (5), Sola-11 (5) and B-5 (4). The polymorphism varied from 66.67 % (B-5) to 85.71 % (UBC-861) with an average of 74.07 %. Seed protein and ISSR profiling offered an effective means of assessing genetic variation and thus would be useful for differentiation of elite germplasm and varieties. In the present study cluster analysis revealed that the range of genetic distance among the inbred lines by seed protein profiling varies 0 to 75 % whereas the ISSR data suggests 10 to 57%. The present results are reliable with the results based on variations in nuclear DNA. Hence, this experiment confirms high degree of divergence among the inbred lines those are genetically diverse and can be exploited for ridge gourd improvement programme in future.

Lkkjk'k

धारीदार तोरई के आठ वंशक्रमों की जैव रसायन एवं अण्विक घटकों की जानकारी हेतु 'एस डी एस पेज' एनालाइसिस विधि व चिन्हित 'आई एस एस आर' आकड़ों के द्वारा प्रयास किया गया। पूर्ण बीज प्रोटीन में 24 प्रोटीन बैंड पाये गये हैं जिनको मुख्यतः चार भागों अ, ब, स एवं द को बढ़ते क्रम में इलेक्ट्रोफोरोसिस गतिशीलता व विश्लेषण 'एस डी एस पेज' विधि द्वारा किया गया है। अधिकतम सोलह बैंड एक ही जननद्रव्य पी आर जी–120 में एवं न्यूनतम 13 बैंड पी सी पी जी आर 7256 व पी आर जी 142 में पाए गए। समानता सूचकांक मूल्य परिसर 25 से 100 प्रतिशत पाया गया। शत–प्रतिशत समरूपता जननद्रव्य पी सी पी जी आर 7256 के साथ पी आर जी 117 में पायी गयी। जननद्रव्य पी आर जी 131 में सबसे न्यूनतम समरूपता (25 प्रतिशत) पी आर जी 132 के साथ मिली जैसे कि पी आर जी 7 व पी आर जी 137 में था। यू पी जी एम ए डेन्डोग्राम के द्वारा जननद्रव्यों को मुख्य रूप से दो गुच्छों/कलस्टर में 43 प्रतिशत समरूपता के साथ बाँटा गया। प्रथम गुच्छ/कलस्टर में पाँच जननद्रव्य एवं द्वितीय गुच्छ/कलस्टर में 3 जननद्रव्यों को रखा गया। कुल 20 आई एस एस आर में से केवल 4 बहुरूपी प्राइमर चयनित किय गये। कुल 27 आइ एस एस आर बैंड उत्पन्न हुए जो कुल आइ एस एस आर की संख्या "लोसाई" परिसर 6 एवं 7 थी। बहुरूपिता परिसर 66.67 से 85.71 प्रतिशत है जिसका औसत 74.07 प्रतिशत है। मैट्रिक मूल्य परिसर 43 से 90 प्रतिशत है। सबसे अधिकतम समरूपता जननद्रव्य सूचकांक पी सी पी जी आर 7256 (0.90) एवं पी आर जी 142 के साथ है। न्यूनतम समरूपता/समानता पी सी पी जी आर 7256 (0.43) वपी आर जी 137 में पायी गयी। डेन्डोग्राम के अनुसार आठ जननद्रव्यों को दो मुख्य गुच्छों में विभाजित किया गया है। प्रथम गुच्छ में 5 जननद्रव्यों के 62 प्रतिशत समानता के साथ सम्मिलित हैं। द्वितीय गुच्छ में 3 जननद्रव्यों में 67 प्रतिशत समरूपता वालो को सम्मिलित किया गया। यह सूचना भविष्य में प्रजाति/संकर प्रजाति के विकास में प्रयोग की जा सकती है।

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