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GENETICS AND PLANT BREEDING

SDS-Page based seed protein profiling and diversity assessment of indigenous genotypes of ridge gourd (*Luffa acutangula* (L.) Roxb.)

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Abstract

Genetic diversity through SDS-PAGE was assessed among the 28 monoecious and 14 hermaphrodite lines of ridge gourd, collected from different parts of the country. A total of 14 protein bands could be resolved which distributed in 3 zones namely zone A (6 bands) with Rf value from 0.13 to 0.31, Zone B (5 bands) with Rf value from 0.54 to 0.78, and zone C (3 bands) with Rf value from 0.82 to 0.92. Most of the protein bands were found in all the genotypes but a specific protein band C_2 with 0.85 Rf value was absent in genotype PCPGR-7267 (monoecious) and another specific protein band B_5 with 0.78 Rf value was absent in genotype PCPGR-7247 (hermaphrodite). Unweighted pair group method with arithmetic mean (UPGMA) analysis generated by SDS-PAGE based on genetic distance of genotypes displayed dendogram grouped the genotypes initially into one major cluster I and one independent genotype PCPGR-7267 with 65% similarity and further major cluster divided into sub-cluster to super small sub-sub clusters. The genotypes are grouped irrespective of their morphological distinctiveness indicating similarity between monoecious and hermaphrodite lines of ridge gourd at genotypic level in contrary that one monoecious genotype namely PCPGR-7267 and one hermaphrodite genotype namely PCPGR-7247 grouped into different cluster. Thus, genotypes which were found diverse may be included in ridge gourd future breeding programme to develop high yielding cultivars.

Highlights

- Easy quick and most economical method to study the genetic diversity
- Results are independent of environment fluctuations
- One monoecious genotype i.e. PCPGR-7267 and one hermaphrodite genotype PCPGR-7247 found most diverse.

Keywords: Ridge Gourd, *Luffa acutangula*, SDS-PAGE, Zymogram, UPGMA Analysis

Ridge gourd [*Luffa acutangula* (L.) Roxb.] having 2n=2x=26, is one of the important cucurbitaceous vegetable crop with old world origin in subtropical Asian region including particularly India. Sanskrit name 'Koshataki' of ridge gourd indicates its early cultivation in India (Kalloo, 1993). It also called Chinese okra is predominantly monoecious in sex expression(Reddy *et al.* 2013) but hermaphrodite, andromonoecious, trimonoecious, gynoecious flowering form has also been reported (Swarup 2006). Ridge gourd, is grown throughout India in tropical and subtropical climate, both as spring-summer and

rainy season crop known as ribbed gourd or angled gourd or silky gourd or angled loofah or vegetable gourd. Fruits of *Luffa* spp are very nutritious and good source of vitamin A,calcium, phosphorus, ascorbic acid and iron (Aykroyd 1963).Ridge gourd is low in saturated fat and cholesterol which makes it ideal diet for those who are looking for weight loss.It has excellent cooling properties. It contains a gelatinous compound called luffein which possess lot of medicinal uses. The juice prepared from ridge gourd is a natural remedy for jaundice, helpful in the purification, restoration and nourishment of the liver and is also helpful in the liver detoxification resulting from alcohol intoxication (Dubey *et al.* 2013). Ridge gourd has certain peptides which are exactly like insulin, alkaloids and charantin chemicals which help in reducing the blood sugar and urine sugar levels(Pullaiah, 2006). Genetic diversity in the germplasm is the foundation on which improvements are built. It is a useful and essential tool for parent's choice in hybridization to develop high yield potential cultivars and to meet the diversified goals of plant breeding (Gaur *et al.* 1978; Haydar *et al.* 2007 and Shekhawat 2001). If the genotypes do not have information on characterization, evaluation and biochemical analyses, their utilization is limited. Genotypes without utilization for crop improvement mean the wastage of resources (Gafoor and Arshad, 2008). Analysis of seed storage proteins provide aid for identification and characterization of diversity in genotypes, crop varieties, cultivars, their wild relatives, and phylogenetic relationship of the accessions (Nisar 2007). Polymorphism at protein level can help to measure genetic distance or genomic similarities between pairs of parental lines and hybrids. Seed proteins have the advantage of being scorable from inviable organs or tissue and the electrophoretic protocol for bulk protein assay is generally simpler than isozymes (Cooke 1984).Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is most economical simple, and extensively used biochemical marker (seed protein) based technique for analysis of genetic diversity of genotypes against the morphological markers as they are not influenced by environment. As seed storage proteins are largely independent of environmental fluctuation, their profiling using SDS-PAGE technology is particularly considered as a consistent tool for economic characterization of genotypes (Javid *et al.* 2004; Iqbal *et al.* 2005).Though, similar work i.e. SDS-PAGE based assessment of genetic diversity in different genotypes using seed storage protein, have been widely applied and used by several workers in different cucurbits namely Mariquita *et al.* 1997 in Luffa sps.; Yadav and Ram. (2003) in muskmelon; Upadhyay and Ram (2006) in bot le gourd; Pandey and Singh (2007) in sponge gourd; Chauhan *et al.* (2012) in sponge gourd but still very less information about the biochemical characterization is available in this crop. Thus, keeping above considerations in view, the present study was conducted to deal with assessment of genetic diversity of forty two (28 monoecious and 14 hermaphrodite) genotypes of ridge gourd including two checks (Pant Torai-1 and Satputia) through SDS-PAGE of seed protein.

Materials and Methods

A total of 42 genotypes including two checks (Pant Torai-1 and Satputia) employed in the study (Table 1) were procured from Pantnagar Centre for Plant Genetic Resources (PCPGR) of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Ut arakhand, India. The composite seed collected from each genotype was used for electrophoresis. Sodium Dodecyl Sulphate (SDS) extracts of seed proteins were used for SDS-PAGE as method described by Laemmli, 1970. Two to three seeds without seed coat were ground in 1 ml extraction buffer (0.0625 N Tris HCl, 2% SDS, 10% Glycerol, 1 mM PMSF, 2% Mercaptoethanol). The mixture was heated at 60^{o}C for 30 minutes. It was centrifuged at 10,000 rpm for 30 minutes. Supernatant was collected and stored at 4^{o}C for further use.Equal volume (25 µl) ofsupernatant i.e. protein sample and sample buffer(100 µl 1 M Tris (pH 6.8), 20 ml Glycerol, 2 g SDS, 2 ml 2 % Mercaptoethanol, 5 mg Bromophenol blue in final volume of 100 ml with distilled water) were mixed.

Then, it was heated at 100°C for 5 minutes. After that samples (50 µl)were loaded to each well along with marker protein (20 µl) in one well with help of micro syringe. The SDS solubilized protein samples were then subjected to vertical slab SDS-PAGE with 12% separating and 5% stacking gels using Tris-glycine electrode buffer (Tris-glycine and SDS, pH-8.6). The samples were electrophoresed at 80V initially and increased upto 100V with current 500mA, when the tracking dye passed from the stacking gel. The run was stopped when the dye was approximately 0.5 cm away from the bottom of the gel, which took around 5 to 6 hours. The gel was removed with the help of spatula and dipped for 12 hours in staining solution (0.25g Coomassie Brilliant Blue R-250, 60 g TCA, 180 ml methanol; and 60 ml glacial acetic acid). The staining solution was then replaced the next day with destaining solution (3% NaCl). The gel was intermittently and carefully shaken and destaining solution was changed till the blue colour of the background of the bands disappears. The position and intensity of the bands were visualized in the gels on a Syngen Gel Documentation system for documentation and photography. Thus, seed protein profiles were obtained used for the preparation of Zymogram. Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SAHN clustering function of NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System Programme) version 2.0 (Rohlf 1987). Relative front (Rf) of each band was calculated as follows:

Results and Discussion

To conserve the genetic diversity, elucidation of genetic diversity is extremely necessary for the effective maintenance, evaluation and utilization of germplasm because germplasm is the only source to be exploited for the development of new varieties during breeding programs (Baranger 2004). Proteins have been used as markers for the assessment of genetic diversity in many crops species (Nagy *et al.* 2009). Total seed storage proteins reflect high level of polymorphism, their function is the product of gene and their electrophoretic banding pattern is very minutely affected by environment (Gepts *et al.* 1986). Seed storage protein banding pattern is utilized for the identification of varieties, characterization of plant genotypes, studying phylogeny of different species and analysis in biosystematics (Sammour 1991). The SDS-PAGE technique is cheap and easy method to asses genotypes diversity. In the present study an attempt has been made to give a blue print of the genetic diversity of 42 indigenous genotypes of ridge gourd including two checks (Pant Torai-1 and Satputia) through SDS-PAGE technique. The seed protein fragments exhibited (Figure 1-4) appreciable polymorphism amongst genotypes used for the study and the diagrammatic representation has been depicted in Zymogram (Figure 5-6). A total of 14 protein bands were obtained which were further categorized under three distinct zones A, B and C depending on their decreasing molecular weights and increasing Rf (relative front) values. A standard medium range protein molecular weight marker of known molecular weight (14-95 kDa) was used along with samples.

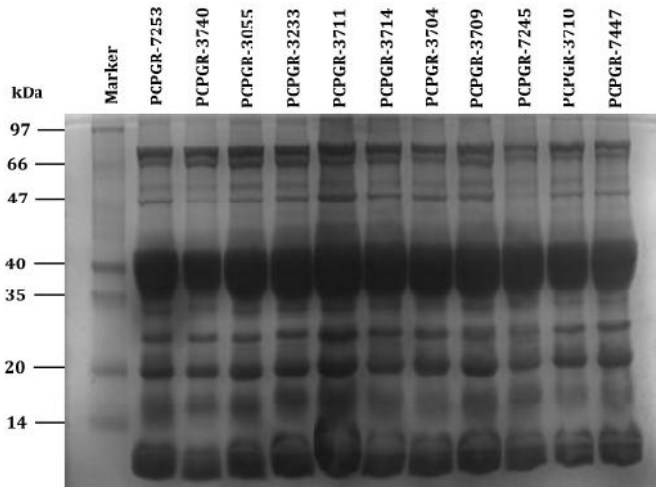
The zone A comprised of six bands and represented heaviest molecular weight proteins ranging from 95-47 kDa. The major differences in the protein banding pattern were mainly confined to this zone. The band A_1 (Rf- 0.13) was resolved in seventeen genotypes and absent in twenty-five genotypes along with two checks. The band A_2 (Rf- 0.20) was present in all genotypes along with two checks. The band A_3 (Rf- 0.22) was present in thirty-nine genotypes along with two checks and absent in three genotypes PCPGR-7267, PCPGR-7448, PCPGR-3708. The band A_4 (Rf- 0.25) was present in eleven genotypes and absent in thirty-one genotypes along with two checks. The band A_5 (Rf- 0.29) was resolved in twenty-eight genotypes including one check Satputia and absent in fourteen genotypes along with one check Pant Torai-1. The band A_6 (Rf- 0.31) was present in thirty-eight genotypes along with

two checks and absent in four genotypes GP 2014-1, PCPGR-3708, PCPGR-3111, PCPGR-3753. The zone B comprised of five bands and represents protein bands ranging from 40-20 kDa. The protein band B_1 (Rf- 0.54) exhibited highest intensity and thickest band width among all the fourteen protein bands of the profile and was discernibly present in all genotypes including two checks. The protein band B_2 (Rf- 0.62) was present in thirty-eight genotypes with variable band intensity along with two checks except genotypes PCPGR-3740, PCPGR-7447, PCPGR-7446, PCPGR-7267. The protein band B_3 (Rf- 0.69) was resolved in thirty-seven genotypes along with two checks and absent in five genotypes namely PCPGR-7252, PCPGR-7333, PCPGR-3234, PCPGR-3702 and PCPGR-3715. The protein band B_4 (Rf- 0.76) was present in twelve genotypes along with one check satputia and absent in thirty genotypes along with one check Pant Torai-1. The protein band B_5 (Rf- 0.78) was present in all genotypes including checks except genotype PCPGR-7247. Zone C comprised of three protein bands namely C_1, C_2 and C_3 with their corresponding molecular weights ranging from approximately 16 to below 14 kDa and Rf values from 0.82-0.92, respectively. The protein band C_1 (Rf- 0.82) was present in twenty genotypes including two checks and absent in twenty-two genotypes. The protein band C_2 (Rf- 0.85) was present in thirty-nine genotypes along with two checks and absent in three genotypes PCPGR-7267, PCPGR-3705, PCPGR-7252. The protein band C_3 (Rf- 0.92) was present in all genotypes along with two checks. The results obtained are in accordance with the findings of Pandey and Singh 2011; Barman *et al.* 2012; Sumathi and Balamurugan 2013.

The cluster analysis distinguishes genotypes on the basis of their diversity and could be used as basis of selection of genotypes for crop improvement (Bharose *et al.* 2014). Hence, On the basis of presence (+) and absence (0) of protein bands (Table 1); a dendogram (Figure 7) was constructed to group the genotypes on the basis of similarity in their protein banding pat ern. Forty two genotypes of ridge gourd including two checks was classified into one major cluster I and one independent genotype of monoecious ridge gourd PCPGR-7267 with 65% similarity. The major cluster was further divided into two sub clusters IA and IB with approximately 78 and 79% similarity, respectively. The sub cluster IA was further forked into sub- sub cluster IA1 with approximately82% similarity and one independent genotype of hermaphrodite ridge gourd i.e. PCPGR-7247 which showed only 78% similarity with sub-sub cluster IA1.

The sub sub-cluster IA1 was again forked into two sub-sub cluster 'Ia' and 'IIa' with approximately 84 and 86.4% similarity. The sub-sub cluster Ia further divided into two small sub-sub clusters 'Ia1' and 'Ia2' with approximately 85.3 and 92.8% similarity, respectively. The small sub-sub cluster Ia2 comprised one independent genotypes of monoecious ridge gourd *i.e*. PCPGR-7245 and two genotypes of hermaphrodite ridge gourd namely PCPGR-3716 and PCPGR-3700, showed closer affinities despite being morphologically different. While, small sub-sub cluster Ia1 was further branched into two super small sub-sub clusters 'Ia1.1' and 'Ia1.2' showed 89.3 and 88% similarity, respectively. The super small sub-sub cluster Ia1.1 comprised of a total of six monoecious ridge gourd genotypes, one genotype was independent namely PCPGR-7447 while other five were namely PCPGR-7253, PCPGR-3711 and PCPGR-3710 in one group and PCPGR-3055 and PCPGR-3233 in another group showed maximum similarity (100 per cent). The super small sub-sub cluster Ia1.2 comprised of five monoecious ridge gourd genotypes. One genotype as an independent namely PCPGR-7446 showed 88% similarity with genotypes of same super small sub-sub cluster (Ia1.2) and also had a group of three genotypes namely PCPGR-3714, PCPGR-3704 and PCPGR-3709 with 100% genetic similarity among them and one independent genotype namely PCPGR-3740. The sub-sub cluster IIa further divided into one independent monoecious ridge gourd genotype namely PCPGR-3112 and in one small sub-sub cluster IIa1 with approximately 86.4 and 90% similarity, respectively. The small sub-sub cluster IIa1 further

branched into one independent monoecious ridge gourd genotype namely PCPGR-5563 and in one super small sub-sub cluster IIa1.1 with 90 and 90.7% similarity, respectively. The super small sub sub-cluster IIa1.1comprised of a total of six genotypes of hermaphrodite ridge gourd. One genotype as independent namely PCPGR-3753 showed 90.7% similarity with other genotypes of same super small sub-sub cluster (IIa1.1) namely PCPGR-5991, PCPGR-3235 and PCPGR-3239 in one group and genotypes PCPGR-7261 and PCPGR-2264 in another one group which expressed genetic similarity of 100% within group. Hermaphrodite genotype PCPGR-7247 was found to be most diverse from the other genotypes of sub cluster IA. The sub cluster IB was further forked into two sub-sub cluster 'IB1' and 'IB2' denoted 84.8 and 81.4% similarity, respectively. The sub-sub cluster IB1 was again divided into two sub-sub cluster 'Ib1' and 'IIb1' with 91.2 and 92.8% similarity, respectively. The sub-sub cluster Ib1 further forked, showed one independent monoecious ridge gourd line namely Pant Torai-1 with 91.2% close affinity and one small sub-sub cluster namely Ib1with 92.8% similarity. The small sub-sub cluster (Ib1.1) comprised of one independent genotype of monoecious ridge gourd (PCPGR-7448), While, other four monoecious ridge gourd genotypes namely PCPGR-3713, PCPGR-7369, PCPGR-3774 and PCPGR-7255 in single cluster with 100% genetic similarity despite the fact being morphologically distinct.



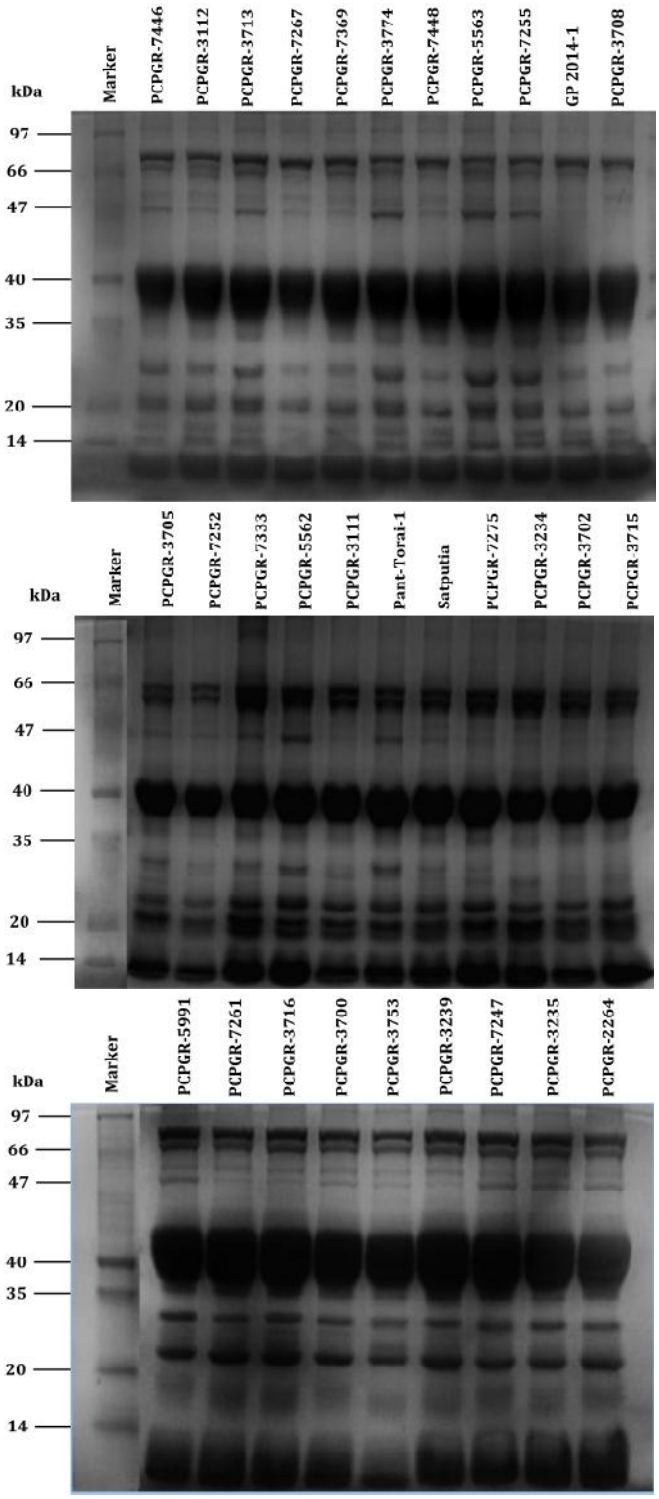


Fig. 1-4

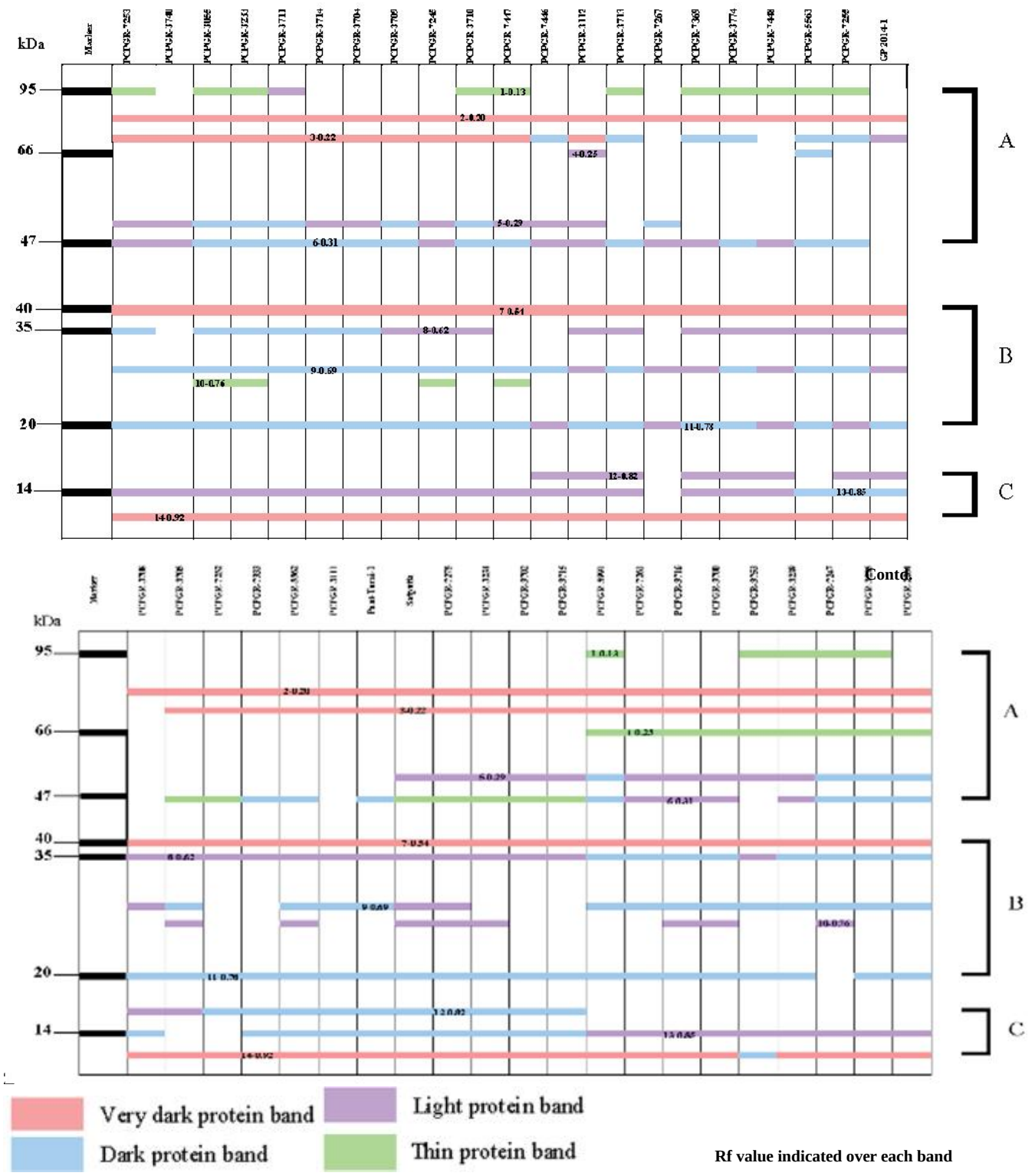
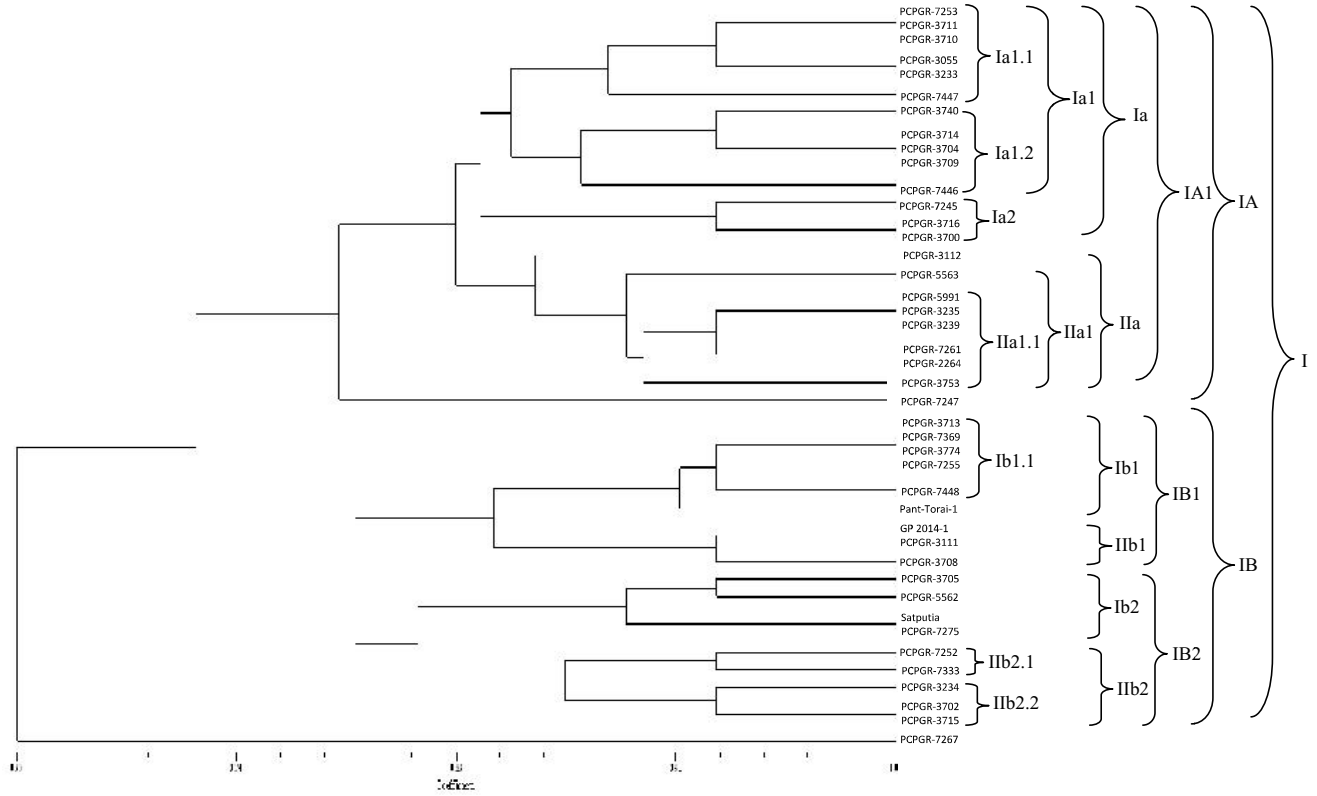


Fig. 6. Zymogram for the seed protein profile of ridge gourd genotypes

Table 1: Seed protein banding pattern in ridge gourd genotypes

SI. No.	Entry	Name of bands													
		A1	A2	A3	A4	A5	A6	B1	B2	B3	B4	B5	C1	C2	C3
1	PCPGR-7253	+	+	+	0	+	+	+	+	+	0	+	0	+	+
2	PCPGR-3740	0	+	+	0	+	+	+	0	+	0	+	0	+	+
3	PCPGR-3055	+	+	+	0	+	+	+	+	+	+	+	0	+	+
4	PCPGR-3233	+	+	+	0	+	+	+	+	+	+	+	0	+	+
5	PCPGR-3711	+	+	+	0	+	+	+	+	+	0	+	0	+	+
6	PCPGR-3714	0	+	+	0	+	+	+	+	+	0	+	0	+	+
7	PCPGR-3704	0	+	+	0	+	+	+	+	+	0	+	0	+	+
8	PCPGR-3709	0	+	+	0	+	+	+	+	+	0	+	0	+	+
9	PCPGR-7245	0	+	+	0	+	+	+	+	+	+	+	0	+	+
10	PCPGR-3710	+	+	+	0	+	+	+	+	+	0	+	0	+	+
11	PCPGR-7447	+	+	+	0	+	+	+	0	+	+	+	0	+	+
12	PCPGR-7446	0	+	+	0	+	+	+	0	+	0	+	+	+	+
13	PCPGR-3112	0	+	+	+	+	+	+	+	+	0	+	+	+	+
14	PCPGR-3713	+	+	+	0	0	+	+	+	+	0	+	+	+	+
15	PCPGR-7267	0	+	0	0	+	+	+	0	+	0	+	0	0	+
16	PCPGR-7369	+	+	+	0	0	+	+	+	+	0	+	+	+	+
17	PCPGR-3774	+	+	+	0	0	+	+	+	+	0	+	+	+	+
18	PCPGR-7448	+	+	0	0	0	+	+	+	+	0	+	+	+	+
19	PCPGR-5563	+	+	+	+	0	+	+	+	+	0	+	0	+	+
20	PCPGR-7255	+	+	+	0	0	+	+	+	+	0	+	+	+	+
21	GP 2014-1	0	+	+	0	0	0	+	+	+	0	+	+	+	+
22	PCPGR-3708	0	+	0	0	0	0	+	+	+	0	+	+	+	+
23	PCPGR-3705	0	+	+	0	0	+	+	+	+	+	+	+	0	+
24	PCPGR-7252	0	+	+	0	0	+	+	+	0	0	+	+	0	+
25	PCPGR-7333	0	+	+	0	0	+	+	+	0	0	+	+	+	+
26	PCPGR-5562	0	+	+	0	0	+	+	+	+	+	+	+	+	+
27	PCPGR-3111	0	+	+	0	0	0	+	+	+	0	+	+	+	+
28	Pant Torai-1(check)	0	+	+	0	0	+	+	+	+	0	+	+	+	+
29	Satputia (check)	0	+	+	0	+	+	+	+	+	+	+	+	+	+
30	PCPGR-7275	0	+	+	0	+	+	+	+	+	+	+	+	+	+
31	PCPGR-3234	0	+	+	0	+	+	+	+	0	+	+	+	+	+
32	PCPGR-3702	0	+	+	0	+	+	+	+	0	0	+	+	+	+
33	PCPGR-3715	0	+	+	0	+	+	+	+	0	0	+	+	+	+
34	PCPGR-5991	+	+	+	+	+	+	+	+	+	0	+	0	+	+
35	PCPGR-7261	0	+	+	+	+	+	+	+	+	0	+	0	+	+
36	PCPGR-3716	0	+	+	+	+	+	+	+	+	+	+	0	+	+
37	PCPGR-3700	0	+	+	+	+	+	+	+	+	+	+	0	+	+
38	PCPGR-3753	+	+	+	+	+	0	+	+	+	0	+	0	+	+

39	PCPGR-3239	+	+	+	+	+	+	+	+	+	0	+	0	+	+
40	PCPGR-7247	+	+	+	+	+	+	+	+	+	+	0	0	+	+
41	PCPGR-3235	+	+	+	+	+	+	+	+	+	0	+	0	+	+
42	PCPGR-2264	0	+	+	+	+	+	+	+	+	0	+	0	+	+



Thus, the small sub-sub cluster (Ib1.1) had a total of five genotypes of monoecious ridge. The sub-sub cluster (IIb1) had one independent genotype of monoecious ridge gourd *i.e*. PCPGR-3708 and other two monoecious ridge gourd genotype in single group namely GP 2014-1 and PCPGR-3111 with 100 similarities, revealed of having a total of three genotypes. The sub-sub cluster Ib2 further divided into small sub-sub cluster Ib2.1 with 92.8% similarity and had two independent monoecious ridge gourd genotypes *viz*., PCPGR-3705, PCPGR-5562. While, sub-sub cluster Ib2 also had two genotypes of hermaphrodite ridge gourd in single group *i.e*. Satputia and PCPGR-7275 showed 100% similarity between them. The sub-sub cluster IIb2 was further dividedinto two small sub-sub cluster IIb2.1 and IIb2.2 with 92.8% similarity each. The small sub-sub cluster IIb2.1 had two independent genotypes of monoecious ridge gourd namely PCPGR-7252, PCPGR-7333, while one independent hermaphrodite ridge gourd genotype *i.e*. PCPGR-3235 and two other genotypes of hermaphrodite ridge gourd *viz*., PCPGR-3702 and PCPGR-3715 were lied in small sub-sub cluster IIb2.2. Broadly, these clustering of genotypes to assess genetic diversity using seed protein profile as a time efficient and powerful tool (as seed protein not influenced by the environment) have been employed by several workers. The results obtained in the study are concordant with findings of Maurya *et al.* 2005; Singh *et al.* 2010; Chauhan *et al.* 2012; Sarkar 2013.

Conclusion

Study revealed that in all the genotypes despite of being morphologically different i.e monoecious v/s hermaphrodite ridge gourd, most of them grouped into similar sub or sub-sub clusters except one hermaphrodite ridge gourd genotype namely PCPGR-7247 and one monoecious ridge gourd genotype namely PCPGR-7267 were found most diverse among all genotypes and showed low similarity 78 and 65% with the other genotypes of clusters. The clustering pat ern seen indicates though, the monoecious and hermaphrodite genotypes are morphologically different but at the genotypic level they are close to each other. With the study, it is also concluded that the most diverse genotypes i.e. PCPGR- 7247 and PCPGR-7267 found most diverse among all genotypes and may be further utilized as potent genotypes in ridge gourd breeding programme for the development of open pollinated as well as hybrid varieties.

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