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Analysis of seed storage protein diversity among twelve different cultivars of Pea (*Pissum sativum* L.) using SDS-PAGE.

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Abstract
The present investigation was carried out during 2012-13 with twelve Pea (*Pisum sativum* L.) cultivar for protein profiling through SDS-PAGE in The department of Genetics and Plant Breeding, CSA University of Agriculture and Technology, Kanpur (U.P). The Seed protein profiles of pea cultivars were studied by extracting the total proteins from five single seeds in each cultivar and performed SDS-Polyacrylamide gel electrophoresis. All the cultivars were clearly revealed remarkable polymorphism from their protein banding patterns. On the basis of banding patterns through SDS-PAGE, indicated that the number of bands found in cultivars ranged from 12 to 19 with Rm value 0.12 to 0.9. The results found that, the cultivar KPMR-400 had recorded highest number of bands (19) whereas, the minimum number of bands (12) observed in three cultivars *viz.*, KPMR-921, KPMR-902 and KPMR-913. The total seed protein variation were also analyzed using Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) and resultant cluster analysis based on the data of protein profiling, classified twelve cultivars into six major groups. The finally study concluded that, the protein variability analysis clearly showed that there was sufficient genetic divergence among these cultivars of pea with respect to seed storage protein. Among all the cultivars, the KPMR-906 in cluster IV having wider genetic diversity and suggested to utilize in future crop improvement program.

Key words: Genetic diversity, pea, protein profiling, seed storage protein, SDS-PAGE.

Introduction

Pea (*Pisum sativum* L.) is widely grown around the world and it is the second most consumed legume after chickpea and unquestionably the most popular garden crop. It is a short duration legume crop belongs to Leguminace or Fabaceae family. In India, it is a important legume crop after chickpea and pigeon pea as well as an important vegetable crop due to its high nutritive value, particularly proteins; 7.2g/100g (Singh *et* al., 2007). In addition, it is also important source of vegetable protein (21-32%) in major part of the world and also consumed as green vegetables (whole pods or immature Seed) in Asian countries as well as dry seed in Europe, Australia, America and Mediterranean regions (Ghafoor *et* al., 2008)

The seed storage proteins have been used as genetic markers in four major areas: (1) analysis of genetic diversity within and between accessions, (2) plant domestication in relation to genetic resource conservation and breeding, (3) establishing genome relationships, and (4) as a tool in crop improvement (Iqbal *et al.*, 2005; Hameed *et al.*, 2009_a; Hameed *et al.*, 2012_b). The seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary relationships among crops and their wild relatives (Das & Mukarjee, 1995).

The SDS-PAGE is the most commonly methods used for studying protein differences between species. However previously varieties were described on the basis of their morphological characters. But now a day's SDS is an anionic detergent which binds strongly and denatures proteins. Hence, the number of SDS molecule bound to a polypeptide chain is approximately half the number of amino acid residues in that chain. There after the protein SDS complex carries net negative charges. And move towards the anode and the finally separation is based on the size of the protein. Therefore, modification of PAGE called SDS-PAGE, an oligomer protein may be dissociated into its sub-units and the molecular weight of sub-units are determined.

Thus, the aim of the present investigation is the analysis of seed storage protein diversity among twelve different cultivars of Pea (*Pissum Sativum* L.) using SDS-PAGE.

Materials and Methods

The present investigation included 12 varieties of pea (Table 1**).** The genetically pure nucleus seed were obtained from Pea Breeder of the university. Analysis of total soluble proteins for characterization of genotypes and total soluble proteins were analysis by SDS-PAGE using the method recommended by UPOV, 1994.

A total of Five seeds from each cultivars were grinded in mortar and pestle after removing the seed coat and defatted by defeating solution 4 times. Take the defatted material in 1.5 ml centrifuge tubes and 1 ml Tris-glysine extraction buffer (pH 8.3) was added to 0.5g of defatted powder and left over night and 10 % solution of SDS (10µl), 2-mercapto ethanol (10µl) with bromophenol blue (10 µl) was added. It was mixed well and left over night in a refrigerator. The mixture was kept for 10 minutes in water bath at 100ºC after that mixture was centrifuged at 10,000 rpm for 15 minutes. The clean suspension supernatant was used for study. After pouring the stacking gel, set a comb without trapping any bubble. After the gel polymerized (it takes about 10-15 minute) remove the comb and wash the wells with tank buffer.

Electrophoresis was conducted using Auto Electrophoresis Unit having fourteen well for loading the sample. The gel cassette was fixed into the electrophoresis unit, as per the design of the equipment. 50 µl of clear supernatant to each well was loaded. The electrode tank buffer was filled in the electrophoresis unit and added 2-3 drop of bromophenol blue dye to the electrode buffer in the upper tank. The electrophoresis apparatus was connected to the power with the anode connected to the lower reservoir and cathode to the upper reservoir. The electrode was fitted into sockets with identical colour. Connect the power pack to the main and switch it on.Conduct electrophoresis at 42 mA (@ 1.5 mA per well) till the sample migrates into the running gel, and subsequently at 56 mA unit the tracking dye reaches the bottom of the gel. When tracking dye reaches the bottom of the gel, switch off the power supply and remove the cassette. Removed the cassette from the unit and take out the gel gently. Then after, it was placed in a staining tray and incubated overnight in 15% TCA (Trichloroacetic acid) solution and washed thoroughly the excess SDS which might precipitate on the surface sufficient staining solution to cover the gel uniformly incubation for 16 hr to stain and rinsed with water. Destaining in water and 5% Acetic acid for a day or two till clears the gel background, resulting in a better resolution. Place the gel over a trans-illuminator and draw the electrophoregram for calculating Rm values. Photograph of the gel was also taken.

Results and Discussion

In the present study, the SDS-PAGE of seed proteins of twelve pea cultivars was carried out to investigate the genetic diversity in protein level. The seed storage profiling showed distinct polymorphism in electrophoretic banding patterns and led to the detection of total of 75 polypeptide bands (Fig No. 1). Polymorphism was evident in all storage proteins fraction of the selected pea cultivars on the basis of their molecular weight. Pea cultivars were distinguished on the bases of presence and absence of protein bands at particular Rm value (Table 2). The numbers of bands for tris soluble SDS-PAGE presents in each cultivar ranged from 12-19 with Rm value 0.12 to 0.93 and the bands having Rm value 0.50, 0.71, 0.83 and 0.86 were found common in all pea genotypes (Table 3). The cultivar KPMR-400 had recorded highest number of bands (19) whereas, the minimum number of bands (12) observed in three cultivars *viz.*, KPMR-921, KPMR-902 and KPMR-913. Whereas, 13 bands were found in KPMR-820, 14 bands in KPMR-763 and KPMR-918, 15 bands in KPMR-906, KPMR-920, KPMR-525 and KPMR-870, and 17 bands were present only in KPMR-922 respectively (Table 4). The similar results were associated with Chang *et al.*, (2012).

The similarity distance dendrogram of 12 pea genotypes of tris soluble protein banding pattern using UPGMA clusters analysis revealed that, the twelve pea genotypes were grouped into 6 clusters (Fig. 2). Clusters I contains two genotype KPMR-763 and KPMR-921 are grouped that are close to each other. In clusters II, KPMR-922 and KPMR-400 are grouped that are close to each other. Cluster III contains three genotypes viz., KPMR-920, KPMR-870, KPMR-820 in which KPMR-920 and KPMR-870 are more close than KPMR-820. Cluster IV contains one genotypes i.e. KPMR-906 and has wider distances to other genotypes. Cluster V contains two genotypes i.e. KPMR-918 and KPMR-902 that are more close each other. Cluster VII contain two genotypes KPMR-913 and KPMR-525 that are close to each other. In present study no clear differentiation was recorded for origin of genotypes based on seed storage proteins. Similar results were found in Ghafoor *et al*., 2002.

Conclusion

The study concluded that, the protein variability analysis clearly showed that there was divergence among these cultivars of pea with respect to seed storage protein. Among all the cultivars, the KPMR-906 in cluster IV having wider genetic diversity and suggested to utilize in crop improvement program.

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Table 1: Varieties under study for protein profiling.

S.No.	Name of Variety	Source
1	KPMR-763	C.S.A.U.A&T., Kanpur
	KPMR-906	C.S.A.U.A&T., Kanpur
3	KPMR-918	C.S.A.U.A&T.,Kanpur
4	KPMR-921	C.S.A.U.A&T., Kanpur
5	KPMR-922	C.S.A.U.A&T., Kanpur
6	KPMR-920	C.S.A.U.A&T., Kanpur
7	KPMR-913	C.S.A.U.A&T., Kanpur
8	KPMR-525	C.S.A.U.A&T., Kanpur
9	KPMR-902	C.S.A.U.A&T., Kanpur
10	KPMR-870	C.S.A.U.A&T., Kanpur
11	KPMR-820	C.S.A.U.A&T., Kanpur
12	KPMR-400	C.S.A.U.A&T., Kanpur

Table. 2 Relative mobility Rm values and presence/absence of total soluble seed protein in Pea genotype (SDS-PAGE)

Band No.	Genotype RM value	KPMR-763 (1)	KPMR-906 (2)	KPMR-918 (3)	KPMR-921 (4)	KPMR-922 (5)	KPMR-920 (6)	KPMR-913 (7)	KPMR-525 (8)	KPMR-902 (9)	KPMR-870 (10)	KPMR-820 (11)	KPMR-400 (12)
1.	0.12	-	-	-	-	+	-	-	+	-	+	-	+
2.	0.20	+	+	+	+	+	+	+	-	-	-	-	+
3.	0.26	-	-	+	+	+	+	+	+	+	+	+	+
4.	0.29	+	+	+	+	+	-	-	+	-	-	+	+
5.	0.31	-	-	+	-	-	+	+	-	+	+	-	+
6.	0.34	-	+	-	-	+	+	-	+	-	+	-	-
7.	0.36	+	-	-	+	+	-	+	+	+	-	-	+
8.	0.40	-	+	+	-	-	+	-	-	+	+	+	+
9.	0.45	+	-	+	-	+	+	-	-	-	+	+	+
10.	0.50	+	+	+	+	+	+	+	+	+	+	+	+
11.	0.52	-	-	-	-	+	+	-	-	-	-	+	+
12.	0.54	+	-	+	-	-	-	-	-	+	+	-	-
13.	0.57	+	+	-	-	+	+	-	-	+	+	+	+
14.	0.63	+	+	+	+	+	+	+	+	-	+	+	+
15.	0.66	+	+	-	+	+	-	-	-	-	-	-	+
16.	0.68	+	+	-	+	+	+	+	+	-	+	+	+
17.	0.71	+	+	+	+	+	+	+	+	+	+	+	+
18.	0.73	-	-	-	-	-	-	+	+	-	-	-	-
19.	0.77	-	+	-	-	-	-	+	+	-	-	-	+
20.	0.81	+	+	+	+	+	+	-	+	+	+	+	+
21.	0.83	+	+	+	+	+	+	+	+	+	+	+	+
22.	0.86	+	+	+	+	+	+	+	+	+	+	+	+
23.	0.93	-	+	+	-	-	-	-	+	+	-	-	-
	Total	14	15	14	12	17	15	12	15	12	15	13	19

Fig. 1. Electrophorogram of 12% polyacrylamide gel banding pattern showing diversity in total seed storage proteins of *Pisum sativum*.

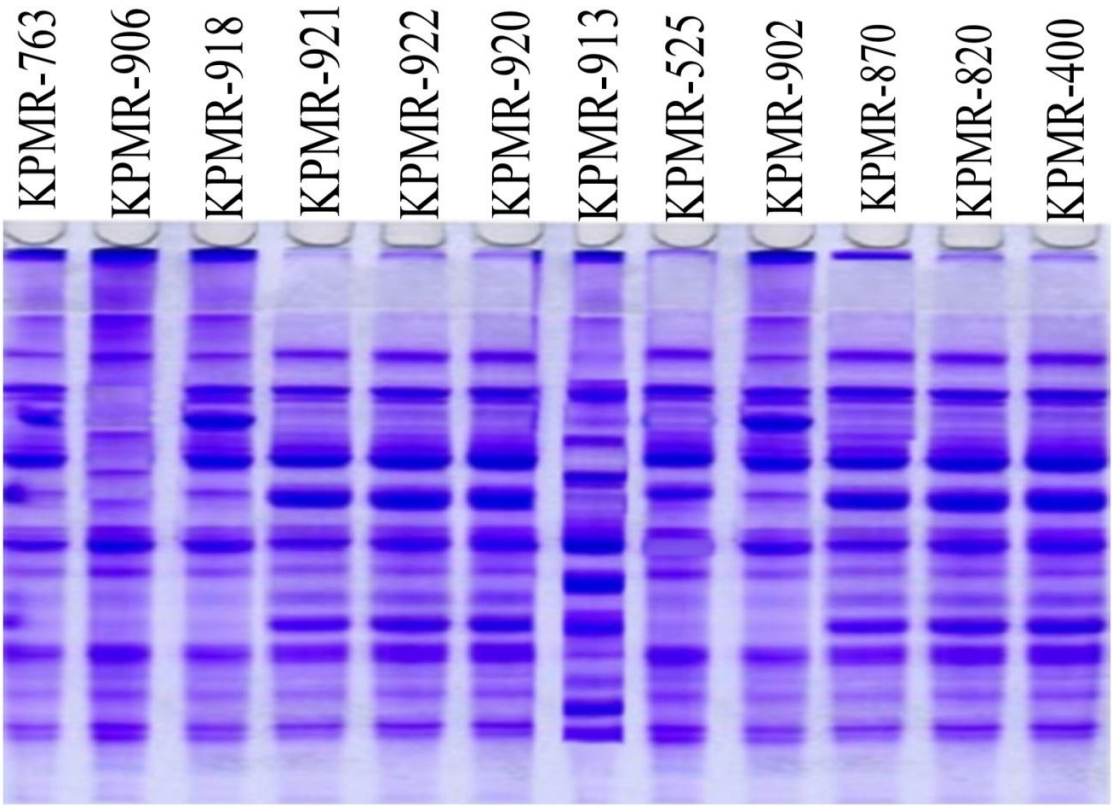

Fig. 2.Electrophoregram of pea verities showing protein banding pattern through SDS-PAGE

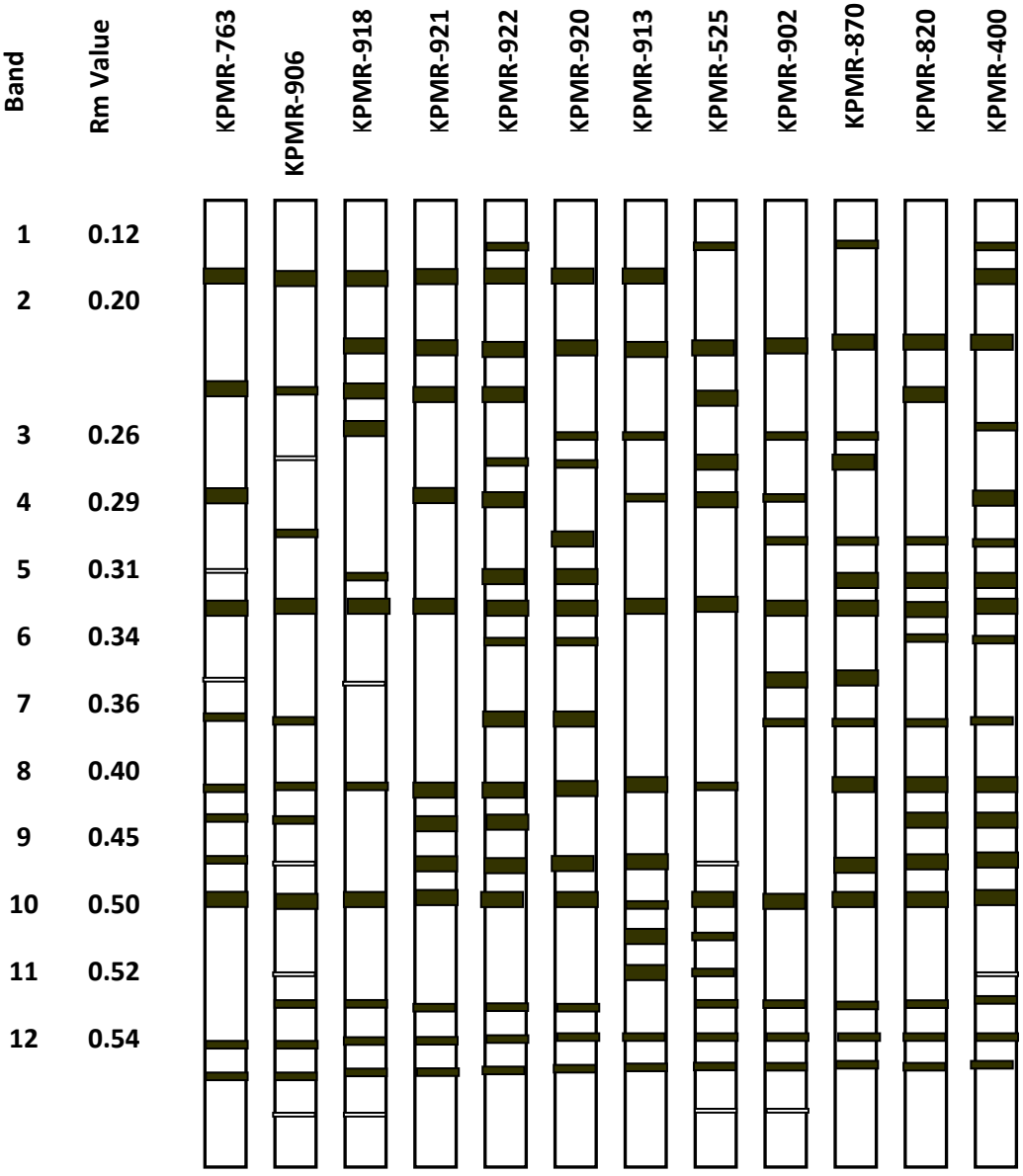

Fig. 3: Total seed protein Based, cluster analysis and grouping pattern of 12 pea cultivars