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Seed storage Protein profiling of Pea (*pisum sativum* l.) Genotypes using SDS-PAGE

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Abstract

Ten pea genotypes of different origin from were investigated for genetic divergence based on seed protein profile using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) in G.B. Pant University of Agriculture and Technology, Pantnagar during 2012-13. Based on seed proteins, 13 subunits were observed and among these 7 were polymorphic. The un-weighted paired group method with arithmetic averages (UPGMA) exhibited 2 clusters which were further grouped into distinct subgroups. Further, bifurcation at different levels leads to the different similarity index groups at the 90 per cent. In broader spectrum, the genotypes from various sources differed in grouping and it was difficult to establish relationship between origin and cluster pattern. The protein banding data were investigated in relation to agronomic traits that indicated influence of polymorphic bands on quantitative traits. Particular clusters were better for specific traits that are suggested to utilize in crop improvement program.

Keywords: Electropherogram, Genotypes, Pea, SDS-PAGE, Seed protein.

Introduction

Pea (*Pisum sativum* L.) is an important source of vegetable protein (21-32%) in major part of the world. It is consumed as green vegetables (whole pods or immature seed) in Asian countries and dry seed in Europe, Australia, America and Mediterranean regions. It ranks third in the world production amongst the food legumes. Diversity in the germplasm is the foundation on which improvements are built. If the germplasm do not have information on characterization, evaluation and biochemical analyses, their utilization is limited. The germplasm without utilization for crop improvement means the wastage of resources. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is used due to its validity and simplicity for describing genetic structure of crop germplasm, but its implication has been limited mainly to cereals due to less polymorphism in most of the legumes[1]. Seed storage proteins have been used as genetic markers obtained by electrophoresis to resolve the taxonomic and evolutionary problems of several crop plants[2,3]. Researchers can use genetic similarity information to make decisions regarding the choice for selecting superior genotypes for improvement or to be used as parents for the development of future cultivars through hybridization. The present study has been undertaken to get better knowledge on the architecture of genetic diversity within *Pisum sativum* L. In view of above considerations, the present study was initiated to study genetic diversity on the basis of seed protein profile and its relationship with agronomic traits in peas.

Materials and Methods

One-dimensional SDS-polyacrylamide vertical slab gel electrophoresis was carried out to determine the protein profiles per banding patterns of buffer extracted (Tris base, SDS and Mercaptoethanol) seed proteins excluding seed coat in 10 vegetable pea genotypes[4]. The extraction procedure of Matta and Gatehouse[5] was followed. For the extraction of proteins, single seed was ground to fine powder with mortar and pestle. Sample buffer (400 µl) was added to 0.01 g of seed flour as extraction liquid and mixed thoroughly in Eppendorf tube with a small glass rod. The extraction buffer contained the following final concentrations: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol Blue (BPB) was added to the sample buffer as tracking dye to watch the movement of protein in the gel. The procedure developed by Laemmli[6] was followed for gel preparation and running. After staining and destaining the gels, depending upon the presence or absence of polypeptide bands, similarity index was calculated for all possible pairs of protein types. To avoid taxonomic weighing, the intensity of bands was not taken into consideration, only the presence of the bands was taken as indicative. Presence and absence of the bands were entered in a binary data matrix. Based on results of electrophoretic band spectra, similarity index was calculated for all possible pairs of protein type's electrophoregrams. The similarity matrix thus generated was converted to a dissimilarity matrix and used to construct dendrogram by the UPGMA[7]. The different bands of HMW (97-43 kDa) were used for calculation of similarity indices. Presence of bands was scored as 1 and its absence as 0

for all the genotypes. These data matrix were then entered into NTSYS-PC.

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[8, 9]. Proteins have been used as markers for the assessment of genetic diversity in many crops species[10]. In the present study an attempt has been made to give a blue print of the genetic diversity of indigenous genotypes of garden pea through SDS-PAGE technique. The seed protein fragments exhibited (Figure-1) appreciable polymorphism amongst genotypes used for the study and the diagrammatic representation has been depicted in Zymogram (Figure-2). The cultivars which were indistinguishable on the basis of simple identifiable morphological traits like growth habit; flower colour etc. could be distinguished on the basis of their electrophoretic patterns, example, cultivars Arkel, Arka Ajit, PMR-62, PMR-53 got separated which was not possible by morphological markers. Variability in intensity was observed in some bands that indicated the quantity of protein peptides accumulating at a particular molecular weight. The banding patterns were characterized by 4 clear distinct zones viz., A, B, C, D. Zone A (66 kDa) was nearest and F (around 14kDa) was farthest from the origin *i.e.* the point of protein sample application. The protein migrated from cathode to anode passing through separating gel. Figure 1 represents the banding pattern of protein peptides in *Pisum sativum*. In total, 13 protein subunits were observed and out of these 7 were polymorphic. The protein bands were stacked according to their molecular weight *i.e.* high molecular weight protein were located in upper region and low molecular weight proteins in the middle to lower regions of the gel, respectively. Different protein electrophoretic patterns exhibited by the cultivars could be identified solely by the cultivar specific.

Variation in band intensity observed within electropherograms of different groups may be attributed to large variation in the amount of various polypeptides present in the protein extract. Similar trends were observed in the protein-banding pattern of other leguminous crops like cowpea[11], fieldpea [12] and also pea[13]. SDSPAGE of seed storage proteins is, however, considered as practical, cost effective and reliable method as it is largely independent of environmental fluctuations[14,15] as compared to soluble protein and isozyme analysis. Cluster analysis (Figure-3) after quantifying the protein bands, using UPGMA procedure, indicated that broadly the genotypes were grouped into two mega groups at about 68% similarity index. I mega group formed of six genotypes (Arkel, PSM-4, PSM-5, E-6, PMR-53, PM-69) While, II mega group consisted of four genotypes subgroup V (PM-69). However, II mega group consisted of three subgroups viz., sub group I (PMR-62), sub group II (Arka Ajit and PSM-3), subgroup II (AP-3). Within sub group II (Arka Ajit and PSM-3) both the cultivars were having normal leaves, wrinkled seeds (Arka Ajit, PSM-3, PMR-62, AP-3) but the color of the seeds of Arka Ajit are yellow. Further, bifurcation at different levels leads to the different similarity index groups at the 90 per cent. I megagroup consisted of 5 sub groups viz., subgroup I (Arkel and PSM-5), Sub group II (PSM-4), subgroup III (E-6), subgroup IV (PMR-53). The results obtained are in accordance with the other findings[16-18] as well.

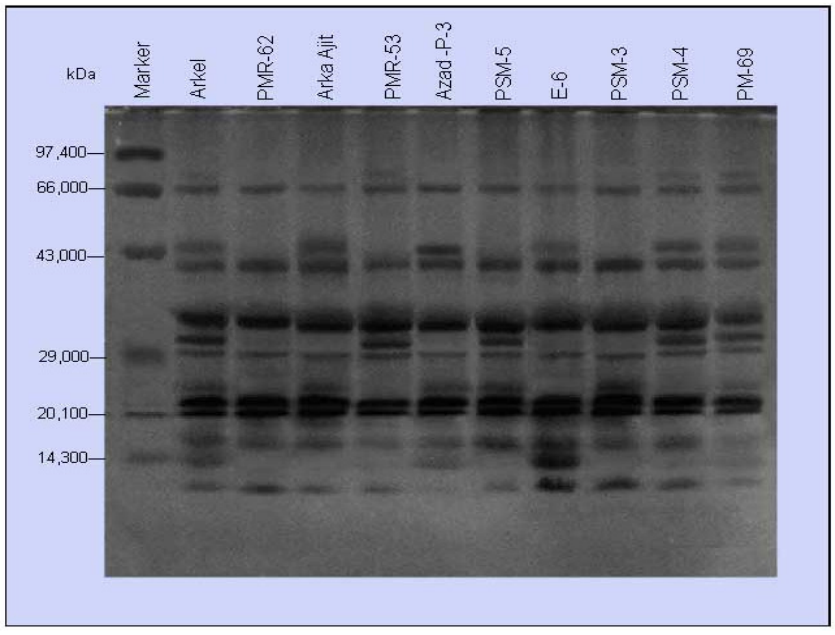

Figure-1
SDS-PAGE Electrophoretic profile product of seed storage proteins of studied *Pisum* genotypes
***Pisum* genotypes**

The phylogenetic evolutionary tree developed from the analysis indicated that most of the pea varieties did not form distinct clusters; rather, the relatively close and small clusters of varieties were distributed within one broad cluster as can be seen in the phenogram obtained in cluster 1 with 6 groups of 10 varieties of pea The relatedness of varieties in dendrogram were of mixed pattern as per their geographical distribution. This may be due to the fact that till now different selection pressure must have been applied for different yield and yield related characters in different genotypes which caused the diverse expression of genes for those characters in genotypes[19,20].

This need to the fact that formation of many bands in seed protein profiles are under control of quantitative gene systems and proteins are primary products of structural gene changes in coding base sequence will under many circumstances result in corresponding changes in the primary structure of protein. Even single amino acid substitution, deletion or addition can have marked effects on the migration of proteins under an electric field during electrophoresis. In pea improvement program, grouping of genotypes on the basis of clustering patterns can be used based on agronomic traits.

Thus, the seed storage protein profiling emerge as a potent technique to generate wide array of polymorphism and as such, it could serve valuable information for varietal identification and extent of genetic diversity. Further, the perspective of such a biochemical approach could be more successful in conjunction with morphoeconomic traits for genotype sorting and to support pea breeding programme.

Conclusion

Study revealed that in all the genotypes despite of being morphologically different, most of them grouped into similar sub or sub-sub clusters. It's concluded from the study that Arkel, PSM-5 and AP-3 are most diverse among all genotypes and may be further utilized as potent genotypes in pea breeding programme for the varietal development.

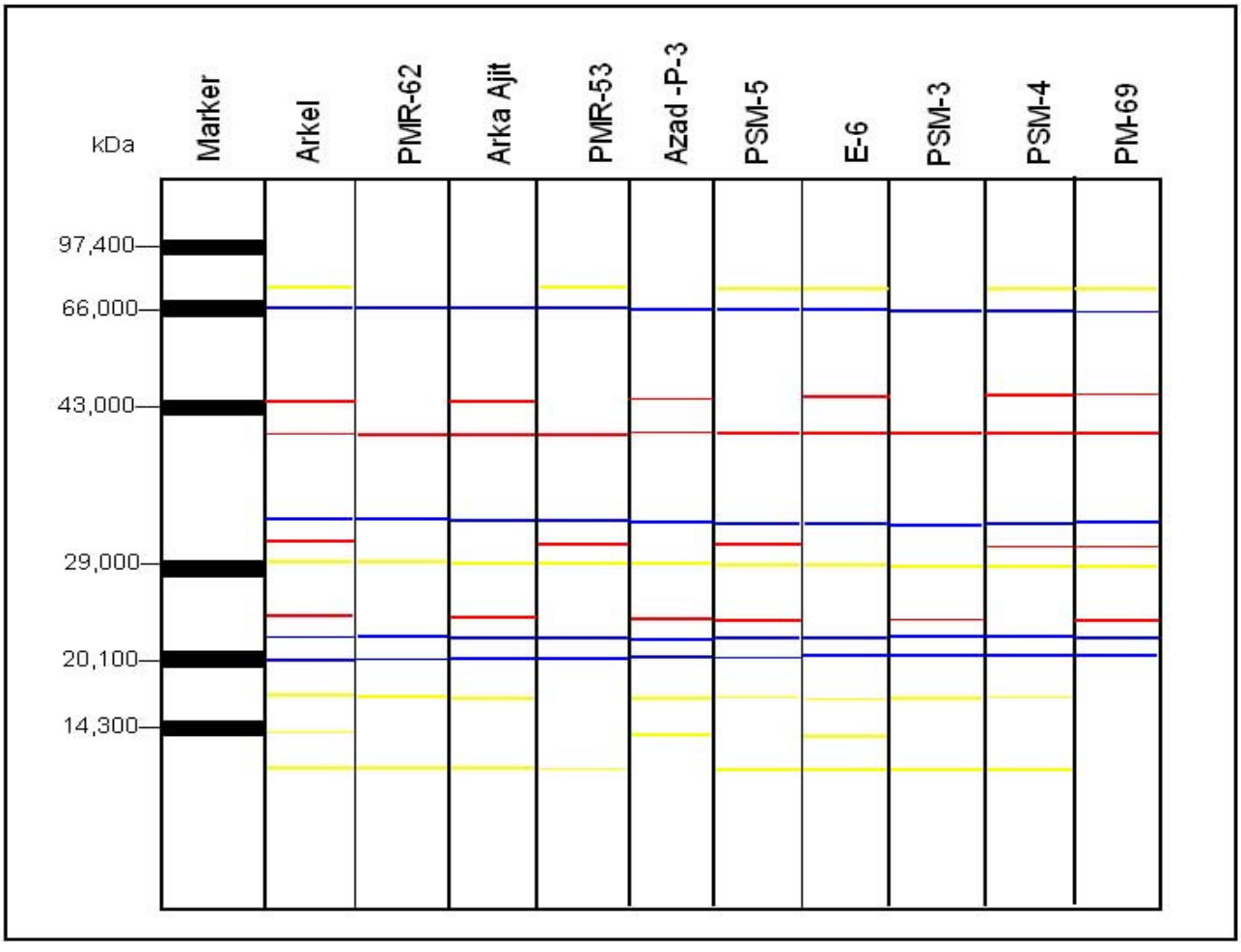

Figure-2
Zymogram (Seed protein profiles as resolved through SDS-PAGE)

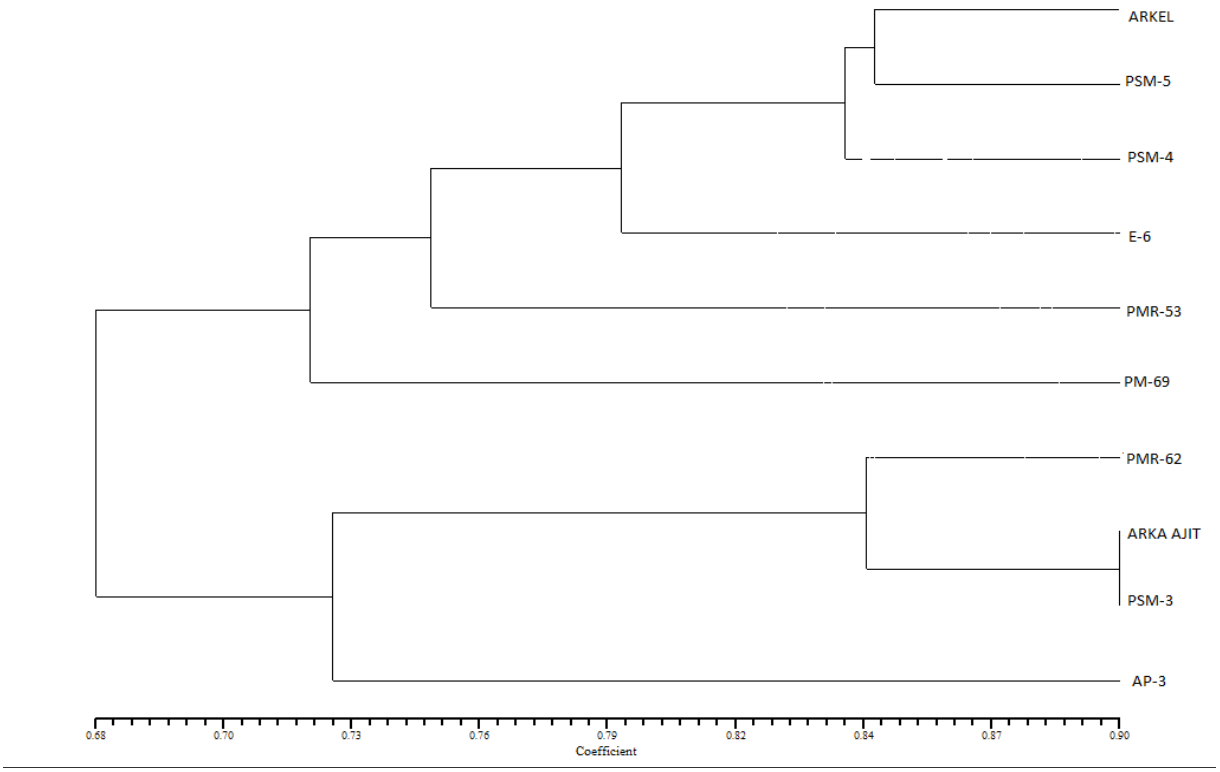

Figure-3
Dendrogram showing cluster groups of pea genotypes based on protein profiles through SDS-PAGE

Table-1
List of genotypes, their Origin and features

Genotype	Origin	Plant Height	Growth	Flower Color	Foliage color	Seed color	Seed shape
Arkel	Europe	Dwarf	Early	W	G	Green	Wrinkled
PMR-62	Pantnagar	Dwarf	Early	W	G	Green	Wrinkled
Arka Ajit (FC-1)	IIHR, Bangalore	Med- tall	Mid	W	G	Yellow	Wrinkled
PMR-53	Pantnagar	Med- tall	Mid	W	G	Green	Wrinkled
Azad Pea-3	CSU, Kanpur	Dwarf	Early	W	G	Green	Wrinkled
PSM-5	Pantnagar	Dwarf	Mid	W	DG	Green	Wrinkled
E-6	PAU, Ludhiana	Dwarf	Early	W	G	Green	Wrinkled
PSM-3	Pantnagar	Medium	Mid	W	G	Green	Wrinkled
PSM-4 (PMR-21)	Pantnagar	Med- tall	Early-Mid	W	G	Yellow	Smooth
PM-69	Pantnagar	Med	Mid	W	DG	Green	Wrinkled

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