Pak. J. Bot., 40(6): 2315-2321, 2008.

SEED PROTEIN PROFILING OF *PISUM SATIVUM* L., GERMPLASM USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) FOR INVESTIGATION OF BIODIVERSITY

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Abstract

Sixty-seven pea genotypes originating from 5 countries were investigated for genetic divergence based on seed protein profile using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Based on seed proteins, 25 subunits were observed and among these 20 were polymorphic. The un-weighted paired group method with arithmetic averages (UPGMA) exhibited 9 clusters which were grouped into 5 distinct clusters when plotted for first two components that contributed 62 % of variability. The genotypes from Romania and India were separated clearly that might be due to their unique genetic make up. Other genotypes were more or less scattered indicating shared protein profile that might be due to common parentage or exchange of pea germplasm by different breeders. 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 evaluated for 2 years 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.

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 (Ghafoor *et al*., 2002). Seed storage proteins have been used as genetic markers obtained by electrophoresis to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky & Hymowitz 1979; Das & Mukarjee 1995). 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 was initiated to study genetic diversity on the basis of seed protein profile and its relationship with agronomic traits in peas.

Materials and Methods

Sixty-seven pea genotypes were selected to study the extent of genetic variation based on SDS-PAGE markers. Out of 67 genotypes, 49 were collected from Pakistan, 11 were obtained from Australia, three from ICARDA originating from India, two from Romania, one from Syria and one check variety (Green Feast). These genotypes were also evaluated under field conditions for physiological, vegetable and dry pod traits in augmented design for two consecutive years i.e., 2004-05 and 2005-06 (planted during last week of October and harvested in April for both the years) at National Agricultural Centre, Islamabad (Anon., 2006). Two rows of 4 meter length for each genotype were planted with 15 cm intra-row spacing, whereas inter-row distance of one meter was kept. The data on vegetable traits were recorded on 10 plants sampled at random and dry pod traits were recorded on different set of 10 randomly sampled plants within same genotype. This paper presents results on seed protein profiling and impact of grouping on quantitative traits.

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. Seed protein was analyzed through slab type SDS-PAGE using 11.25% polyacrylamide gel. The gel size was 12.0 x 13.8 cm^2. In order to check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions. The molecular weights of the dissociated polypeptides were determined by using molecular weight protein standards "MW-SDS-70 kit" from Sigma Chemical Company, USA. The SDS-PAGE of total seed protein was carried out in the discontinuous buffer system according to the method of Laemmli (1970).

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 types electrophoregrams. The similarity matrix thus generated was converted to a dissimilarity matrix and used to construct dendrogram by the UPGMA (Sneath & Sokal, 1973). Quantitative data were analysed for means along with standard deviation for various clusters on the basis of seed protein profile using the computer software MS Excel for Windows XP.

Results and Discussion

Figure 1 represents the banding pattern of protein peptides in *Pisum sativum*. In total, 25 protein subunits were observed and out of these 20 were polymorphic. Variability in intensity was observed in some bands that indicated the quantity of protein peptides cumulating at a particular molecular weight. The protein markers plotted for first 2 principal components that explained 40% of variability revealed 5 distinct groups (Fig. 2). Principal component analysis based on SDS-PAGE revealed clear grouping pattern when investigated for geographic origin. The genotypes from Romania and India were separated clearly, although the genotypes from Romania were in a bigger group in the left upper half. All the three genotypes, viz., DMR- 4, DMR- 7and DMR- 20 originated from India were grouped together in the right half of the graph (Figs. 1 & 2).

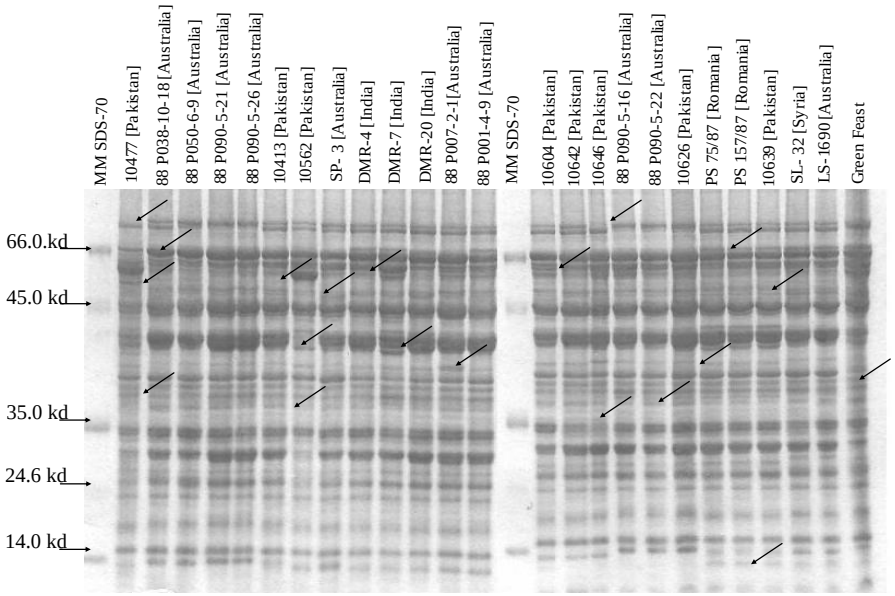

Fig. 1. Seed protein profile of *Pisum sativum* (L.). the arrows represent 20 polymorphic bands. Molecular marker used in this gel was SDS-70 KIT from SIGMA Chemical Company.

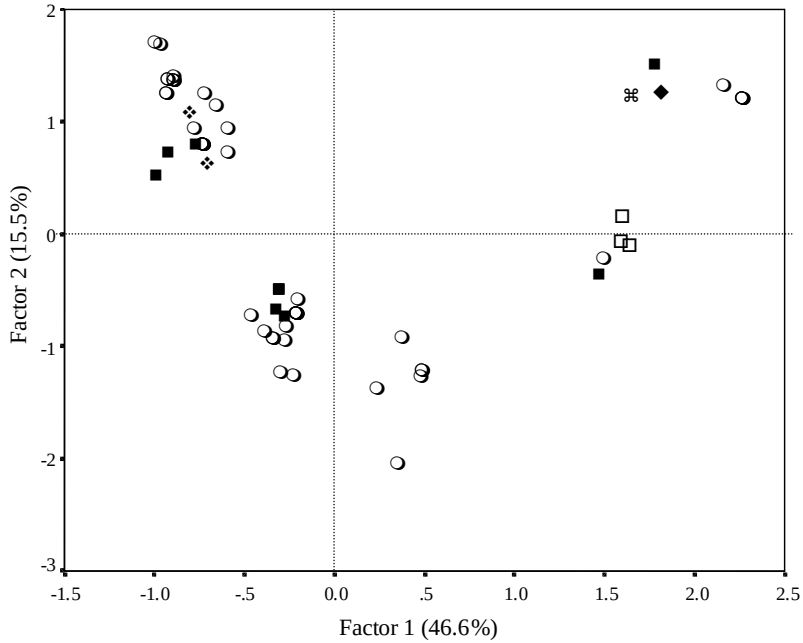

Fig. 2. Scatter diagram for 67 genotypes of *Pisum sativum* based on seed protein. The symbols represent as, ○-Pakisan, ■- Australia, □-India, ❖-Romania, ◆-Syria, ⌘-Variety.

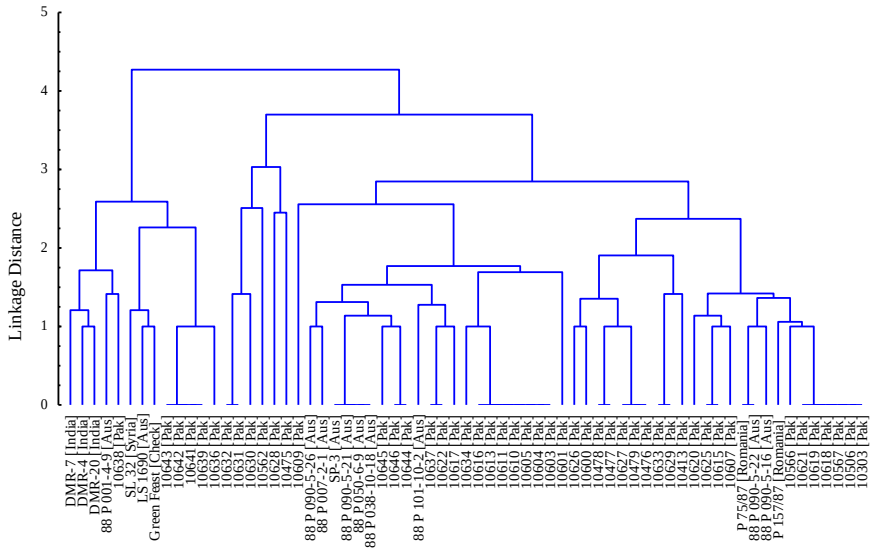

Fig. 3. Dendrogram based on protein in markers in *Pisum sativum*.

The genotypes from Pakistan and Australia were scattered in different groups, whereas some of these were mixed in a single cluster in the lower left half of the graph. On the basis of UPGMA, 9 clusters were observed for seed protein bands (Fig. 3). Cluster IV and VI consisted one genotype in each case, Cluster I consisted 5 genotypes, cluster II 8, cluster III 3, cluster V 2, cluster VII 22, cluster VIII 10 and cluster IX consisted 10 genotypes. These clusters were analysed for individual character for both the years and mean values alongwith standard deviation for each cluster revealed that genotypes (10628, 10475) in clusters V were high yielding and were followed by the genotypes (DMR 4, DMR- 7, DMR- 20, 88P 001-4-9 and 10638) in I (Table 1). The mean values for both the years were in linear relationship for most of the traits that enhanced the validity of results. On the basis of average performance for various traits, it was observed that the genotypes in cluster IV, V, VI and VIII were better for vegetable purposes and the best genotypes could be selected from these clusters.

It is important to note that a low level of intra-specific variation has been reported in various legumes, i.e., chickpea (Ghafoor *et al*., 2003*b*), lentil (Piergiovanni & Taranto, 2003; Sultana & Ghafoor. 2007), groundnut (Javaid *et al*., 2004), pigeon pea (Jha & Ohri, 1996) and black gram (Ghafoor *et al*., 2003*a*) but in the case of pea, a considerable amount of variation was observed based on SDS-PAGE that indicated the valid utilization of seed protein markers for germplasm classification in pea. Ladizinsky (1979) used morphological and seed protein comparisons but found no biological basis for separating closely related small and large seeded lentils. Similarly, although high level of polymorphism was observed in peas but no separation could be made on the basis of consumptive use i.e., dry pods or green vegetable types, although the genotypes originated from India and Romania were grouped based on SDS-PAGE. There was pattern in grouping of genotypes related to agronomic traits and selected genotypes from various clusters are suggested to use in pea improvement program. The technique is simple and can be used on half seed without embryo, the remaining part (with embryo) could be multiplied and evaluated.

Acknowledgements

The cooperation by the donors for providing seed material is highly appreciated. This work is a part of ALP funded project under Agricultural Linkage Program and financial support is highly acknowledged.

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(Received for publication 3, May 2008)