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EVALUATION OF GENETIC DIVERSITY PRESENT IN PEA (*PISUM SATIVUM* L.) GERMPLASM BASED ON MORPHOLOGICAL TRAITS, RESISTANCE TO POWDERY MILDEW AND MOLECULAR CHARACTERISTICS

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Abstract

In order to investigate the genetic diversity present in the pea germplasm provided by IBGRI, NARC, Islamabad, 153 local and exotic genotypes were analyzed for 27 morphological traits, resistance to powdery mildew, and at molecular level using SDS-PAGE. The data was analyzed by descriptive statistics which reveal considerable extent of diversity and the association among different traits was estimated by Correlation analysis. Most of the morphological traits showed significant variance, and only 12 accessions were found resistant to powdery mildew. Correlation analysis showed high significant positive correlation among different important agro economic traits. SDS-PAGE results showed comparatively significant variation in major bands although significant variations in minor bands were there. Cluster analysis (Ward's method) carried out separately for morphological and SDS-PAGE data divide the whole germplasm into two major groups in respect of phylogenetic or pedigree relationship, sorting centers of origin, genetic diversity and similarity.

Introduction

Genetic diversity is a prerequisite, for increasing yields and for stabilizing production in the face of disease epidemic and fluctuation environmental condition. However there has been relatively little utilization of these genetic resources to enhance the performance of a germplasm (Moeljopawiro *et al*., 2000). Knowledge of genetic diversity is a useful tool in gene bank management and in planning experiments, as it facilitates efficient sampling and utilization of germplasm by identifying and/or eliminating duplicates in the gene stock, and helps in the establishment of core collections (Ghafoor *et al*., 2005). One practical application of knowledge of genetic diversity is in the design of populations for genome mapping experiments (Kaga *et al*., 1996). In order to maintain, evaluate and utilize germplasm efficiently and effectively, it is important to investigate the extent of genetic diversity it contains (Smith & Smith, 1989). Many of the landraces and wild species are maintained in the world as genetic resources for crop improvement, but their use for breeding is still limited and we are challenged as to how to use this biodiversity for practical crop improvement (Tsujimoto *et al*., 2000). Intra-specific genetic variation creates genetic diversity and is a fundamental material for plant breeding. Increasing in intra locus variation by the

enhancement of genetic diversity in the available genetic resources has been a drastic gain in the agronomic trait (Wolfe, 2000). Representative samples from the complete geographical range of the crop species can help to ensure conservation of co-adapted gene complexes (Frankel, 1984; Frankel & Soule, 1981; Frankel *et al*., 1995; Brown, 1978; Beuselinch & Steiner, 1992). Genetically heterogeneous populations produce more and stable yield than genetically homogeneous lines (Simmonds, 1979). The variation within the country appears largely attribute to different province rather than smaller unit of crop ecological zone and altitude intervals (Ghafoor *et al*., 2001). Therefore local plant genetic recourse is of greater choice for evaluation as they are in synchronization with the environmental conditions and free from introduction of pests and diseases.

Different methodologies are now adopted in germplasm evaluation for genetic diversity in desirable traits that may include morphological and agronomic evaluation (qualitative and quantitative), biochemical evaluation at protein level (SDS PAGE, Isozyme assay, isoelectric focusing) and at DNA level (Blotting and PCR based analysis).

Morphological characterization is the first step in the description and classification of the germplasm (Smith & Smith, 1989). An understanding of morphological characters facilitate the identification, selection of desirable traits, designing new populations, in transferring their desirable genes into widely grown food legumes through biotechnological means, resistance to biotic and a biotic stresses that are known to individual accessions increase the importance of the germplasm (Santall *et al*., 2001).The economic value of a population is related to plant morphology, agronomic performance, seed quality and nutritional qualities (Piergiovarrni *et al*., 2000). Only those characters that show consistent genetic versus environmental basis should be taken into account, because those markedly influenced by the environment are unreliable and uneven from one place to another. They are useful along with the traditional quantitative ones when the material is intended for a breeding program (Amurrio *et al*., 1993). The high yielding accessions selected from the local germplasm often proved their superiority in advance testing under various agro ecological conditions (Ghafoor *et al*., 1989).

Uses of genetic markers are more useful for screening germplasm with the minimum cost in time and labour (Nakajima, 1994). Bio- chemical markers received more attention in recent years from the crop geneticists for assessment of genetic variability (Akhtar, 2001; Rabbani *et al*., 2001). Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary relationships among crops and their wild relatives (Rao *et al*., 1992; Das & Mukarjee, 1995), in biosystematic study of several plant species (Badr, 1995; Sanches-Yelamo *et al*., 1995; Sheidai *et al*., 1999) and also in studying the origin of different species (Kole & Pianigrahi, 2001). These patterns can also be used for distinguishing cultivars of particular crop species (Jha & Ohri, 1996). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the total seed protein is a practical, reliable and effective method because seed storage proteins are largely independent of environmental fluctuations (Gepts, 1989; Javiad *et al*., 2004; Iqbal *et al*., 2005). Moreover, seed protein profile is hardly affected by experimental conditions (Gray *et al*., 1973; Ladizinsky, 1983).

In the present study the superior genotypes for agronomic characters, resistance to powdery mildew were selected on morphological bases and their protein patterns were obtained using SDS-PAGE. This information can be used for the development of cultivars.

Materials and Methods

This research project had two experiments, morphological evaluation of 153 accessions representing different countries for 27 traits according to the IABGR descriptor for 10 plants randomly sampled in field and SDS-PAGE of all the 153 accessions in laboratory. For SDS-PAGE analysis total seed storage proteins were extracted from 0.1g of the fine powder of a single seed using protein extraction 0.05M Tris-HCL pH 8, 0.2% SDS, 5M urea, 1% B-mercaptoethanol. Electrophoresis was carried out in the discontinuous Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) system of Leammli (1970) using 12.25%(w/v) separating gel and 4% (w/v) stacking gel (Walter *et al*., 2003). The molecular weight of the dissociated protein were estimated by using molecular weight standard proteins "MW-SDS-70 Kit" Gels were shaked gently until the background of the gel became clear and polypeptide bands were clearly visible (Masood *et al*., 2003).

The data recorded were analyzed for simple statistics: Range, Mean, Std. Deviation,Variance for agronomic traits. For protein profile data to avoid taxonomic weighing, the intensity of bands was not taken into consideration, only the presence of bands was taken as indicative. The scores were 1 for the presence and 0 for the absence of a band. The similarity matrix thus generated was converted to a dissimilarity matrix (Dissimilarity = 1-similarity) and used to construct dendrogram by the unweighed pair group method with arithmetic means (Sneath & Sokal 1973) using STATISTIA (Ward's method) for Windows 98.

Results

Agronomic characteristics: The results in Table 1 are based on the agronomic characteristics shows high genetic variability with the maximum in the stem length followed by biological yield. The cluster analysis of 153 accessions based on agronomic characteristics is found in Fig. 1. This dendrogram reveled two major groups A and B. Group A comprised of two clusters one containing 35 accessions and the other containing 41 accessions. Cluster B also composed of two clusters one containing 43 accessions and other containing 32 accessions. This shows that the accessions in one cluster are mostly identical and have less diversity.

Correlation analysis: Table 2 represents the correlation coefficients among all the quantitative traits, which revealed that the harvest index has a highly significant positive correlation with grain yield, 100-seed weight and a positive significant correlation with dry pod weight. Grain yield has a highly significant positive correlation with number of pod/plant, 100-seed weight, biological yield, dry pod weight and positive significant correlation with fresh pod thickness. Biological yield has a positive highly significant correlation with grain yield, number of pods/plant.100-seed weight has a positive highly significant correlation with fresh pod length, fresh pod thickness, fresh pod width, fresh pod weight, dry pod length, thickness, width, weight, grain yield and harvest index. Fresh pod weight has a positive highly significant correlation with fresh pod length, dry pod weight, dry pod length, thickness, width and 100-seed weight. Number of seeds /pod has a highly significant positive correlation with number of locules/ pod.

Table 1. Summary of basic statistics for quantitative traits of 153 accessions.

Variables	Range	Minimum	Maximum	Mean	Std. error	Std. deviation	Variance
Stem length	163.8	33.4	197.2	99.9	2.980	36.86	1358.913
Fresh pod length	5.2	3.5	8.7	5.6	0.076	0.94	0.880
Fresh pod width	10.5	5.5	15.98	11.0	0.147	1.81	3.289
Freshpod thickness	8.7	3.1	11.78	7.9	0.117	1.45	2.091
Fresh pod weight	4.9	0.3	5.26	1.9	0.067	0.83	0.695
Number of seeds/pod	5.4	2.2	8.0	4.6	0.093	1.15	1.334
Number of locules/pod	6.2	3.6	9.8	6.3	0.071	0.88	0.776
Dry pod length	4.5	3.5	7.98	5.5	0.061	0.75	0.569
Dry pod width	7.7	5.7	13.42	10.5	0.122	1.51	2.271
Dry pod thickness	7.7	3.3	11.0	6.0	0.083	1.03	1.066
Dry pod weight	1.5	0.1	1.652	0.7	0.022	0.27	0.071
Biological yield/plant	161.0	9.0	170.0	62.5	2.573	31.83	1012.978
Grain yield/plant	47.0	2.0	49.0	13.2	0.717	8.84	78.063
Harvest index	112.4	2.6	115.0	24.9	1.623	20.07	402.947
Number pods/plant	191.5	3.5	195.0	42.4	2.185	27.03	730.674
100 seed weight	20.1	3.2	23.27	12.3	0.312	3.86	14.863
50%germination	11.0	15.0	26.0	19.0	0.202	2.50	6.242
Days to first flower	98.0	52.0	150.0	76.4	1.293	16.00	255.916
Flowering duration	30.0	6.0	36.0	17.3	0.541	6.69	44.750

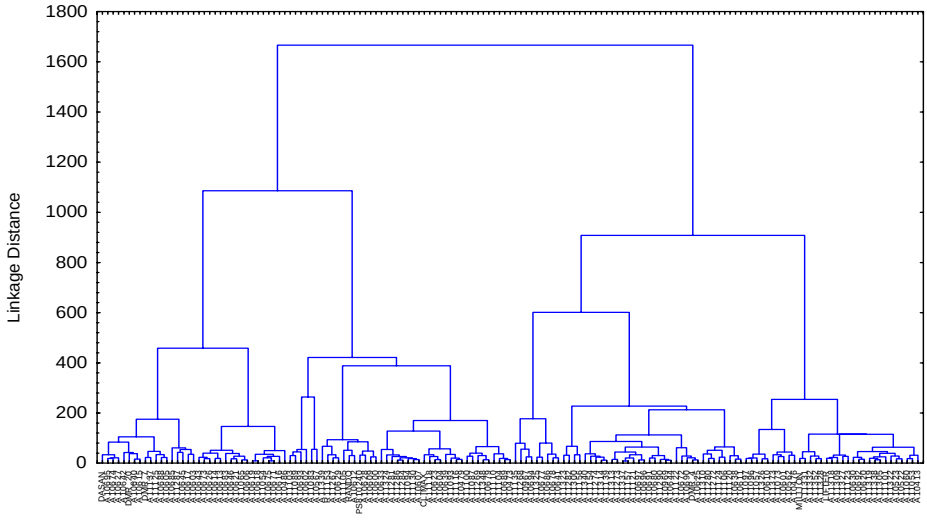

Fig. 1. Dendrogram of 153 genotypes of *Pisum sativum* based on quantitative traits.

Cluster analysis on the bases of SDS–PAGE markers: The results of the cluster analysis (Ward's method) are presented in the Fig. 2 on the bases of linkage distance. The cluster diagram revealed two major groups. Groups A consist of one cluster of only two accessions 10643, 10638 and group B consists of two sub groups B1 and B2. Further the B1 comprised of two clusters one having the 33 accessions and other contain 25 accessions. B2 also comprise of two clusters one contains 34 accessions and the other cluster contains 62 accessions.

Table 2. Correlation coefficient among 19 quantitative traits in local and exotic germplasm.

	SL	FPL	FPW	FPT	FPWe	NOS/P	NOL/P	DPL	DPW	DPT	DPWe	BY	GY	HI	NOP/P	100SW	50%G	TF
FPL	0.04																	
FPW	0.13	0.60**																
FPT	0.14	0.57**	0.59**															
FPWe	0.03	0.78**	0.63**	0.80**														
NOS/P	-0.01	0.33**	0.05	0.00	0.28**													
NOL/P	-0.05	0.39**	0.07	-0.11	0.15	0.67**												
DPL	-0.07	0.55	0.35	0.43	0.49	0.14	0.23											
DPW	0.17	0.21**	0.38**	0.39	0.29**	-0.07	-0.05	0.49**										
DPT	-0.03	0.20	0.17*	0.34**	0.32**	0.06	-0.10	0.38**	0.25**									
DPWe	0.01	0.22**	0.22**	0.43**	0.35**	0.01	-0.12	0.53**	0.39**	0.55**								
BY	0.29**	-0.07	-0.08	-0.04	-0.04	0.09	-0.12	-0.20	-0.05	0.04	0.00							
GY	0.19	0.02	0.05	0.19	0.12	0.04	0.00	0.07	0.18	0.11	0.26**	0.32**						
HI	-0.05	0.08	0.11	1.14	0.11	-0.03	0.18	0.20**	0.02*	0.02	0.17*	-0.38	0.56**					
NOP/P	0.18	-0.11	-0.09	-0.02	-0.06	-0.11	-0.15	-0.19	-0.02	0.04	0.01	0.68**	0.29**	-0.12				
100SW	0.05	0.31**	0.43**	0.55**	0.47**	-0.11	-0.14	0.32**	0.30**	0.38**	0.45**	0.01	0.33**	0.28**	-0.01			
50%G	0.04	-0.05	-0.08	-0.20	0.17	-0.09	0.02	-0.03	-0.02	-0.12	-0.08	0.02	0.05	0.10	0.01	-0.09		
DTF	0.00	-0.12	-0.05	-0.23	-0.19	0.12	0.12	-0.07	-0.06	-0.15	-0.14	0.08	-0.06	-0.11	0.09	-0.20	0.07	
FD	0.02	0.09	0.09	0.09	0.10	0.06	0.08	0.02	0.07	0.07	-0.08	-0.09	-0.02	0.01	-0.16	0.08	0.03	-2.26

*= Significant, **= Highly significant

Stem length= SL; Fresh pod length= FPL; Fresh pod width= FPW; Fresh pod thickness= FPT; Fresh pod weight= FPWe; Number of seeds/pod= NOS/P; Number of locules/ pod= NOL/P; Dry pod length= DPL; Dry pod width= DPW; Dry pod thickness= DPT; Dry pod weight= DPWe; Biological yield= BY; Grain yield= GY; Harvest index= HI; Number of pods/ plant= NOP/P; 100= seed weight= 100SW; 50% Germination= 50%G; Days to flowering= DTF

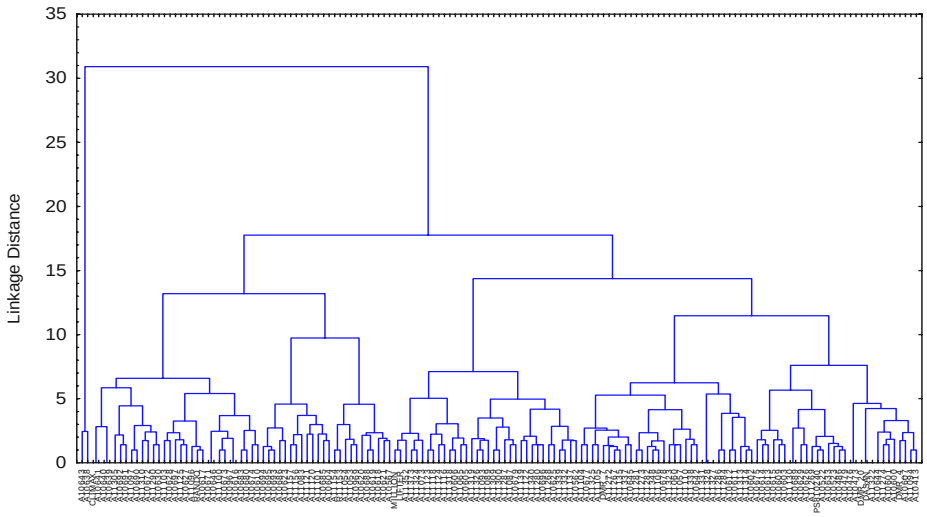

Fig. 2. Dendrogram of 153 genotypes of *Pisum sativum* based on SDS-PAGE.

Discussion

Quantitative trait: Quantitative traits of economic important that had variability, which can be used for improvement of the crop, are considered for evaluation. Quite often, the quantitative traits are economically important (Amurrio *et al.*, 1995). To overcome this large scale germplasm acquisition, inter specific hybridization is suggested to improve these important traits (Khan *et al.*, 1994). Many researchers like Donald (1962), Lal (1967), Singh (1977), Sing *et al.*, (1980) and Khan & Malik (1989) have already suggested selection on the basis of best performance. Stem length showed a high degree of variability in the pea germplasm for stem length, same results were reported by Mishra, (1979) and Mehrani (2002). Fresh pod length showed a narrow variance. These results were supported by the work of Turi (2004) while Gritton (1980) and Duke, (1981) reported that pod length range from 4 to15cm. Fresh pod thickness, fresh pod width revealed high degree of diversity in the present set of germplasm. This can contribute for further evaluation and breeding programs, (Hazra *et al.*, 1999). The fresh pod weight, number of locules per pod results were against the results given by Turi. (2004) due to exotic nature of germplasm in the same climate. The Dry Pod Width, Dry Pod Thickness Dry pod length, Dry Pod Weight, Number of Seeds per Pod had high value of variance indicates that the diversity in number of seeds per pod is present which can contribute to improvement of crop. Same results were observed by Turi. (2004), for another set of pea germplasm showing the consistency of the trait in the germplasm of pea. Number of pods per plant show highest degree of diversity and can be used directly for the improvement of the crop. Same results were reported by the work of Turi (2004) and Mehrani. (2002). Showing the consistency of the diversity of the trait in the pea germplasm. While Duke. (1981) reported that 100 seed could weigh from 10-36g. This is mainly due to the climatic and soil condition of the experimental area. These results were supported by the work of Turi (2004). Mehrani (2002) for the germplasm of pea. Grain yield have a high degree of diversity and can contribute to the improvement of the crop and selection

(Javaid *et al.,* 2002). Harvest Index Indicates high degree of diversity. Selection of high harvest index may have value for improving grain yield of cereal crops (Vogel *et al.,* 1963; Rostelle & Frey, 1975). Days to flowering showing high degree of diversity for flowering initiation this contribute most towards genetic divergence (Mishra. 1979). Exotic germplasm are mostly late in flowering than local germplasm.

Correlation analysis: As pea is a multipurpose crop, from correlation of the traits contributing to yield specific lines from the entire germplasm can be selected for specific purpose. The correlation coefficients were computed among all the quantitative traits. Harvest index has a highly significant positive correlation with grain yield, 100-seed weight and a positive significant correlation with dry pod weight Rangaiah & Nehru. (1998) reported the same results which shows the increase of grain yield with increase in harvest index. Grain yield has a highly significant positive correlation with number of pod/plant, 100-seed weight, biological yield, dry pod weight and positive significant correlation with fresh pod thickness same results were reported by Mehrani. (2002) which proved the consistency of the association. Biological yield has a positive highly significant correlation with grain yield, number of pods/plant.100-seed weight has a positive highly significant correlation with fresh pod length, fresh pod thickness, fresh pod width, fresh pod weight, dry pod length, thickness, width, weight, grain yield and harvest index. Mehrani. (2002) reported same results. Malik *et al.,* (1987) and Ghafoor. (1993) also reported positive correlation of grain yield with biological yield, which proved the complete association of the two traits. Day to flowering showed no positive correlation with any any of the traits which is against the results of Hazra *et al.,* (1999). This is mainly due to the exotic nature of the germplasm and its cultivation in the new environment. Number of pods per plant has highly significant positive correlation with Hundred seed weight, biomass grain yield and harvest index. Klysha (1988) presented information on the basis of a study of 16, 18 and 19 varieties in 1983, 1984 and 1985 respectively about yield correlation in peas. Seed yield was positively correlated with number of pods per plant.100seed weight has highly significant positive correlation with biomass, grain yield and harvest index. Seed yield was positively correlated with number of pods per plant. Biomass has highly significant positive correlation with grain yield and harvest index. Grain yield has highly significant positive correlation with harvest index.

SDS-PAGE based genetic diversity: According to the results revealed by SDS-PAGE, there was low degree of heterogeneity in the major protein bands although significant variations were there in the minor bands. The uniformity in major bands among various accessions indicates that the genes coding these proteins are conserved. Quantitative difference is also evident among the common bands, which is considered to be due to difference in dosage of the genes coding them (Gardiner & Forde 1992).

In the present study the results show low variation because most of the proteins bands are common to all accessions. Therefore diverse accessions based on SDS-PAGE are suggested to be acquired from various sources, preferably from center of diversity to build a broad based gene pool with maximum variability. Further for better management of gene bank, a precise comprehensive knowledge of agricultural and biochemical data (Proteins and DNA) is essential to eliminate duplicates, which will ultimately help in making core collection of pea germplasm. Cluster analysis based on the SDS- PAGE is not as of those of quantitative and qualitative traits.

Cluster analysis on the bases of SDS–PAGE markers: The dendrogram as a whole revealed very low genetic distance at protein level reflects the similarity of genes responsible for the seed storage proteins. Mehrani (2002) also reported the same results. Most of the accessions were in the same cluster shows the low diversity at genomic level for seed proteins. These results were confirmed by the work of Mehrani (2002), Raymond *et al.,* (1991) and deVroes, 1996).

Cluster analysis on the bases of quantitative traits: This shows that the accessions in one cluster are mostly identical and have less diversity. The high genetic distance of the accessions on the basis of quantitative traits revealed the diversity in the germplasm of *Pisum sativum*, which can be used for the improvement of crop.

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