

GENETIC DIVERSITY IN *PISUM SATIVUM* AND A STRATEGY FOR INDIGENOUS BIODIVERSITY CONSERVATION

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Abstract

Pisum sativum germplasm collected from Pakistan was evaluated for agronomic traits and analyzed for biodiversity within each district. The differences proved its validity in substantiating the postulated regions of diversity or gene centres. Migration of landraces into new regions, followed by some degree of contamination by mixture or out crossing with other landraces were observed that might be due to frequent exchange of germplasm or transportation of grains from one place to others. The areas with a high level of stress is expected to present tolerance to environmental stresses, but homogeneous mixtures that needs less extensive sampling for genetic resources conservation purposes. The study confirmed the existence of a wealth of phenotypic divergence in the local pea germplasm and identified pure-lines are suggested to be utilized in crop improvement through simple selection or using in hybrid program. The variation appears attributable to different districts without influence that how frequently area was explored. Further collecting missions to main pea areas with greater diversity could concentrate efforts on sampling as many geographically and ecologically distinct areas as possible, rather than collecting extensively from fields close to motorable roads. The germplasm with high mean values along with medium to high genetic variance should be exploited through simple selection.

Introduction

Pea (*Pisum sativum* L.) of the family Fabaceae comprises only two species *Pisum sativum* and *Pisum fulvum* (Smart, 1990). It is self pollinated, annual herb, stem weak, alternate leaves and terminal branched tendrils leaflets ovate or elliptic (Duke, 1981). Peas are cultivated for the fresh green seeds, tender green pods, dried seeds and foliage in the temperate region of the world and as winter crop in sub-tropics. Genetic improvement of crop is the main component of modern agriculture that can be achieved by utilizing diverse gene sources of species. However, the estimates of genetic diversity and relationship between germplasm collections are very useful for facilitating efficient germplasm collection and management. Unfortunately, legumes have received less attention from agriculture scientists and extension workers.

Plant germplasm is the genetic sources material used by plant breeders to develop new cultivars (Baranger, 2004). Morphological and agronomical traits as well as resistance to biotic and abiotic stresses that are known to individual accessions increase the importance of the germplasm. The economic value of a population is related to plant morphology, agronomic performance, seed quality and nutritional qualities. Efficient utilization of indigenous germplasm required knowledge of biodiversity of economic interest (Beer *et al.*, 1993). A successful collection strategy enables to assemble maximum diversity of any field crop but for this geo-botanical informations are pre-

requisite (Ghafoor *et al.*, 2001). Intra-specific variance has been long recognized by agricultural researchers engaged in various disciplines. The rate of loss in biodiversity has been studied thoroughly and tried to minimize by preserving in genebanks, material maintained by breeders, biologists and in herbariums. The results presented on genetic divergence in indigenous pea germplasm could be utilized for evaluation, investigating biodiversity, utilization and planning future exploration strategies. The major crops, *i.e.*, wheat, rice, chickpea and maize have been genetically eroded due to evolution of new cultivars, but the crop like pea in which low research priority delayed improvement can yet be collected but this should not be delayed any more due to impact of CBD. The present study was conducted to investigate the extent of intra-accession variation along with biodiversity for germplasm collected from 41 districts of Pakistan on the basis of agronomic performance that will help in developing future exploration methodologies to preserve maximum genetic diversity for future use of mankind.

Materials and Methods

One hundred and forty five accession were planted in an augmented designed with three checks (Mateor, Dasan and Rondo) after every ten rows under field conditions at Plant Genetic Resource Program (PGRP), National Agriculture Research Center (NARC), Islamabad (30° 42' N and 37° 08' E, 540 masl) during October 2003 and harvested in March 2004. The row length was 5m with one meter row spacing and intra row distance was kept at 15cm. The data were recorded for qualitative traits *i.e.*, flower color, fresh pod surface, fresh seed color, plant type, degree of curvature of pod, anthocyanin pigmentation on leaf margin, stem and on whole plant, cotyledon color, testa color of dry seeds hilum color and dry seed shape surface. Quantitative traits include vegetative (chlorophyll content, fresh pod length, fresh pod width, fresh pod thickness, fresh pod weight, seeds fresh pod⁻¹, locules fresh pod⁻¹), dry seed and pods (pod length, pod width, pod thickness, pod weight, seeds pod⁻¹, 100-seed weight) and yield components (pods plant⁻¹, biological yield plant⁻¹, grain yield plant⁻¹, harvest index). The data recorded on ten plant samples were averaged and analyzed for simple statistics including mean, standard deviation, variance, frequency distribution, and simple correlation coefficient using computer software MS EXCEL, Windows 98.

Further a set of 94 accessions collected predominantly from farmers fields in 41 districts all over the country was evaluated for agronomic traits to identify the potential sources for high yield potential and to make future germplasm collection strategy. Twenty plant samples were studied for intra-accessions diversity. The data thus recorded were analyzed for variance to investigate inter and intra-accessions variability along with average agronomic performance for individual accessions with deviation from central tendency using the methods discussed by Steel & Torrie, (1980) with the help of computer software MS Excel for Windows. On the basis of data, variance for each trait was computed and then expressed as percent of mean for each character. The variances thus calculated were aggregated for each set of accessions collected from a specific district, *i.e.*, 41 in present case. Deviation from central point of aggregated variance were taken as an indicator for genetic biodiversity and expressed in terms of percent plant samples differed *i.e.*, from none to 100 % that were depicted on the map along with total number of accessions collected from these districts. The total number of collected and preserved accessions in the genebank is slightly higher in some districts due to exclusion of germplasm from present study collected from markets.

Table 1. Frequency distribution of qualitative traits.

Traits	f	Percentage	Traits	f	Percentage
Flower color			Hilum color		
Purple	48	33.1	Creamy	66	45.52
White	97	66.9	Dark creamy	3	2.06
Anthocynin pigmentation			Light brown	5	3.44
Present	5	3.45	Brown	19	13.1
Absent	140	96.55	Dark brown	2	1.38
Vine type			Yellowish green	5	3.44
Erect	13	8.96	White	9	6.21
Prostrate	52	35.8	Black	36	24.83
Semi erect	80	55.17	Seed Surface		
Degree of curvature of pod			Smooth	110	75.86
Straight	124	85.52	Intermediate	28	19.31
Slightly curve	13	8.96	Wrinkled	7	4.83
Strongly curve	8	5.52	Seed shape		
Fresh pod surface			Round	56	38.63
Smooth	101	69.65	Oval	47	32.41
Rough	44	30.34	Irregular	20	13.79
Fresh seed color			Partial oval	11	7.59
Green	42	28.96	Tyre shaped	11	7.59
Light green	68	46.2	Cotyledon color		
Yellow green	35	24.14	Yellow	103	71.03
Dry seed testa color			Orange	38	26.21
Creamy	48	33.1	Green	4	2.76
Dark creamy	9	6.2	Spots on testa		
Green	26	17.93	Present	40	27.59
Olive green	20	13.79	Absent	105	72.41
Brownish green	7	4.83			
Yellowish green	3	2.07			
Light brown	1	0.69			
Brown	25	17.24			
Dark brown	5	3.45			
Black	1	0.69			

Results

Table 1 presents the results for frequency distribution of pea germplasm characterized for 12 plant traits. High variability was observed for dry seed and pod traits including seed testa colour, hilum colour and seed shape. The characters with discrete classes are important to use for gene tagging and varietal description as these are not influenced by environmental fluctuations. It is important to note that 38 accessions in the present study were with orange cotyledon and these could be exploited for production of dry seed either whole or split to substitute other winter pulses, especially lentil that is at serious risk due to abiotic stresses (Javaid *et al.*, 2002). Basic statistics including mean, standard error, standard deviation and coefficient of variability have been presented in the Table 2. High variation along with range was observed for most of the characters that indicated the scope of simple selection from this material to improve pea. On the basis of evaluation, promising genotypes have been identified and listed in the Table 3 and these are suggested to use in genetic improvement through selection or involving these in hybrid program for crop improvement.

Table 2. Basic Statistics for 17 quantitative traits in *Pisum sativum* germplasm.

Variables	Mean \pm SE	SD	CV	Range
<i>Vegetative Traits</i>				
Chlorophyll content	30.01 \pm 0.81	9.77	32.55	12.14 – 64.66
Fresh pod length (cm)	5.38 \pm 0.05	0.70	13.01	3.8 – 7.98
Fresh pod width (mm)	10.95 \pm 0.13	1.63	14.88	5.63 – 14.61
Fresh pod thickness (mm)	9.00 \pm 0.14	1.70	18.89	4.24 – 12.36
Fresh pod weight (g)	2.47 \pm 0.07	0.91	36.84	0.68 – 4.92
Seeds fresh pods ⁻¹	4.98 \pm 0.06	0.82	16.46	3.4 – 7.4
Locules in fresh pods	1.41 \pm 0.03	0.37	26.26	1.0 – 2.5
<i>Dry Seed and Pod Traits</i>				
Dry pod length (cm)	4.94 \pm 0.07	0.80	16.19	2.94 – 6.62
Dry pod width (mm)	9.76 \pm 0.14	1.69	17.31	5.53 – 12.95
Dry pod thickness (mm)	0.05 \pm 0.10	1.19	19.67	3.32 – 9.71
Dry pod weight (g)	0.65 \pm 0.02	0.29	34.61	0.15 – 1.51
Seeds pods ⁻¹	4.41 \pm 0.08	0.97	21.99	2.2 – 6.8
100 – seed weight	12.28 \pm 0.40	4.84	39.41	3.03 – 26.32
<i>Grain Yield Components</i>				
Pods / plant ⁻¹	31.91 \pm 1.87	22.46	20.38	4.25 – 179.33
Biological yield plant ⁻¹	46.16 \pm 1.64	19.75	22.78	9.13 – 103.25
Grain yield plant ⁻¹	13.65 \pm 0.77	9.29	28.06	1.52 – 43.13
Harvest Index	26.39 \pm 0.71	8.52	32.28	9.74 – 47.23

Table 3. Selection of superior cultivars for important quantitative traits in *Pisum sativum*.

Quantitative traits	Range	Selected genotypes
Chlorophyll contents	50 – 65	10611, 10639, 10645, 10695, 10696
Fresh pod length	6.01 – 8	11089, 11133, 11138, 10497 10607, 10608, 10610, 11078
Fresh pod thickness	11.01 – 13	10608, 10680, 10690, 10695, 10696, 11052, 11089, 11114, 11133
Fresh pod weight	4 – 5	10608, 10610, 10630, 11052, 11133
Seed fresh pod ⁻¹	6 – 8	10610, 10622, 10624, 10630, 11054, 11061, 11123, 11130, 11133
Pods plant ⁻¹	80 – 180	10622, 11116, 10476, 10479
Dry pod width	12 – 13	10608, 10628, 10631, 10678
Dry pod thickness	8 – 10	10608, 10609, 10672, 11052, 11065, 11095
Dry pod weight	1 – 2	10602, 10609, 11060, 11093, 11133, 11134
100 – seed weight	20 – 26	10603, 10609, 10623, 10625, 11083, 11089
Grain yield plant ⁻¹	30 – 43	10602, 10603, 10605, 10625, 10639 10640, 10644, 10673, 10687
Biological yield plant ⁻¹	90 – 104	10638, 10640, 11142
Harvest index	48 – 50	10603, 10607, 10609, 11052, 11094, 11100, 11114, 10479

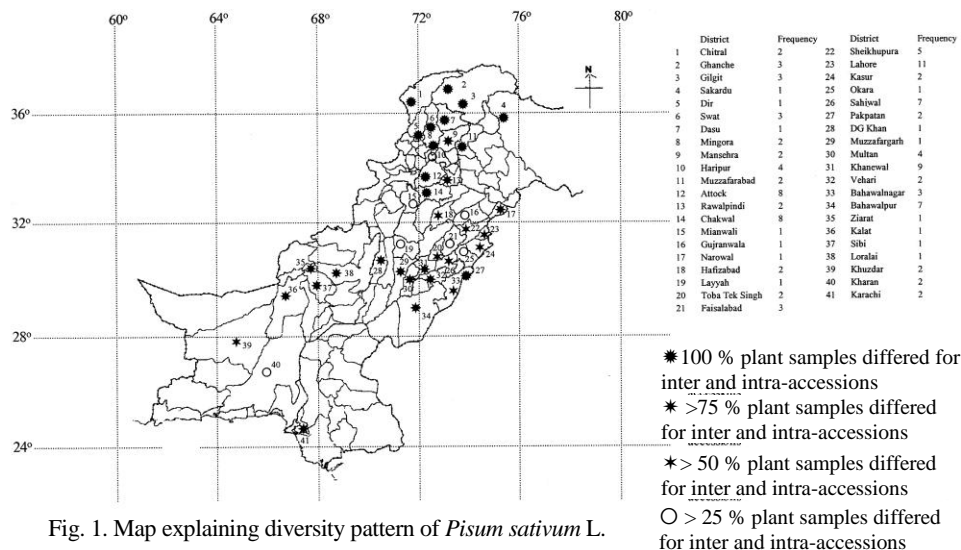


Fig. 1. Map explaining diversity pattern of *Pisum sativum* L.

Figure 1 presents the mode of diversity on the basis of agro-morphological traits of peas for inter and intra-accession variance based on 22 plant samples accessions⁻¹. Variance was categorized into four broader classes, i.e., < 25% plants differed for intra-accessions performance (low diversity), < 50% plants differed (medium diversity), < 75% plants differed (high diversity) and 100% plants differed (maximum diversity). It was observed that the areas with higher geographic diversity presented high genetic diversity. The material collected from high mountain from Northern Areas and NWFP gave the maximum biodiversity that was followed by the germplasm collected from Baluchistan, whereas the material collected from Central Punjab exhibited low to medium biodiversity. One explanation for this could be that these accessions might be pure-lines with common ancestors or material exchanges frequently within and between various districts. Up till now pea germplasm has been collected from 41 districts of Pakistan and this study indicated biodiversity related to various collection sites. Collection of pea germplasm started two decades ago that could assemble less than a hundred accession and these were mainly from high mountainous region. During 2002 and 2003, pea germplasm was collected from Central Punjab and NWFP that indicated less biodiversity. One of the reasons for this might be due to rapid adaptation of improved cultivars in the plains of Punjab.

Discussion

One of the approaches for building gene pool is to collect material from diverse geographical origins from proposed centres of diversity in individual samples. Representative samples from the complete geographical range of the crop species can help to ensure conservation of co-adapted gene complexes (Ghafoor *et al.*, 2003; Beuselinck & Steiner, 1992). Pea is an important winter vegetable that is cultivated throughout the country. Its germplasm has been collected from various parts of the country, but more emphasis have been given to collect the material from easily approachable areas closer to the motorable roads, that is reflected in the intra-accession variance in the present study. One of the problem in vegetable crops is that in most of the cases these are harvested as green vegetables and few farmers grow vegetables for seed production. Further it is observed that seed of most of the vegetable crops is being

imported from other countries and sometimes mixed by the enterprisers that cause genetic impurity that make biological tracking difficult. Further this situation leads a tremendous genetic erosion especially in vegetable crops. Although, germplasm collection from easily approachable areas is first priority due to threat of genetic erosion because advanced breeding material can adapt easily to the areas nearer to major towns (Bisht *et al.*, 1998, 1999). But, to fetch maximum genetic diversity, it is important to make a comprehensive plan for expeditions to have maximum area explored, especially interior territories and valleys between high mountains where maximum genetic diversity exists.

Variances of heritable quantitative traits provide an estimate of genetic diversity within and between collecting sites that is expressed by phenotypic and genotypic diversity for various legumes including lentil (Erskine & Muehlbauer, 1991); soybean (Perry & McIntosh, 1991), peas (Kumar & Raj, 1998; Amurrio, *et al.*, 1991, 1993, 1995), blackgram (Ghafoor *et al.*, 2003) and cowpea (Coulibaly *et al.*, 2002; Fall *et al.*, 2003). The differences according to geographical regions shown by the analysis of quantitative traits is useful in substantiating the postulated regions of diversity or gene centres (Smith *et al.*, 1995). Genetic diversity in the present study indicated the worth in examining the centre of genetic diversity in pea. Similarly it was observed that areas with greater environmental stresses represented high biodiversity even in the same vicinity as the case of Haripur District, where low variability might be due to vegetable growing culture in this area. Eight accessions, viz., 27028, 27029, 27038, 27040, 27042, 27047, 27083 and 27097 were observed high yielding that could be utilized for genetic improvement and these accessions are available in limited quantity to researchers. The rare alleles, each only occurring in one or two apparently random populations can be considered to be mutants, migration or the results of other coincidental events (Van Hintum & Elings, 1991).

Migration of landraces into new regions, followed by some degree of contamination by mixture or out crossing with other landraces can be expected in the country like Pakistan, where germplasm movement is not restricted from one area to other. The areas with a high level of stress are expected to present interesting tolerance to environmental stresses, but occurring in homogeneous mixtures, and hence, need less extensive sampling for genetic resources conservation purposes. The germplasm collected from areas of environmental stresses could provide a base material for developing cultivars tolerant to environmental stresses. Further collecting missions to main pea growing areas that has not been explored yet with greater diversity could concentrate efforts on sampling as many geographically and ecologically distinct areas as possible, rather than collecting extensively from fields close to motorable roads within individual province as already has been suggested by Pecetti *et al.*, (1992 & 1996) for tetraploid wheat. Collecting expedition to the areas where genetic erosion is expected in cowpea along with the areas where existing genetic diversity has not been yet gathered is suggested to explore for genepool building that is a pre-requisite for any successful breeding program.

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