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Assessment of genetic diversity and trait association in grass pea using morphometrics, grain protein and seed ODAP content

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Abstract
Fifty grass pea (*Lathyrus sativus* L.) genotypes were evaluated for morphological variability, grain protein and ODAP content using principal component analysis. Cluster analysis and correlation coefficient were also enumerated. Plant height, primary branch number, pod per plant, seed per pod and 100 seed weight had the direct positive effect on yield per plant. Out of thirteen, five PCs exhibited more than 1 eigen value but the level of dissimilarity was low which indicates that the germplasm has narrow genetic base. The first five principal components were selected exhibiting 68.16% variation. The PC1, PC2, PC3, PC4 and PC5 had 20.47%, 15.72%, 12.62%, 9.78% and 9.57% variability respectively, among the genotypes for the traits under study. All the genotypes are grouped into five clusters using D^2 statistics. These results suggested that dry biomass yield, days of 50% flowering, soluble protein content and yield per plant were the most important characters in differentiating the genotypes.

Keywords: Genetic diversity, Grass pea, ODAP, Principal component analysis, Traits association

Introduction

Grass pea (*Lathyrus sativus* L.) belonging to the family Leguminosae/ Fabaceae and it is commonly known as lathyrus or *khesari* in India. It has been a traditional crop both for animal consumption as forage and grain, and for human consumption as a food grain. The main qualities of this grain legume consist of its sturdiness, drought tolerance, and adaptability to a wide range of soil types. According to Milczak *et al*. (2001), grass pea seeds were brought by Tatars and accompanied their lentil seeds, probably as a weed. Over the time, the crop found better growth and development conditions and consequently today as a dominant species is more popular than lentil. In Poland (Milczak *et al*., 2001), alike Italy (Tavoletti and Capitani, 2000; Polignano *et al*., 2005), Spain (De la Rosa and Martin, 2001), Slovak Republic (Benkova and Zakova, 2001) and Hungary (Lazanyi, 2000) it is one of the relatively infrequent grain legumes. Also, high protein content makes this species interesting as a forage crop (Polignano *et al*., 2003; Crin' o *et al*., 2004; Polignano, 2007). Although rich in protein, the utilization of grass pea grain is limited by the presence of water soluble, non-protein amino acid *â*-N-oxalyl diamino propionic acid (*â*-ODAP) which causes lathyrism in animal. It has been agreed by researchers that assessing the variability of grasspea for morphological and biochemical characters is highly important for the safeguard and utilization of these precious genetic resources. With this background grasspea genotypes were evaluated for yield, nutritional and morphological traits.

Materials and Methods

Fifty genotypes were selected from a collection of grasspea germplasm currently maintained at Bidhan Chandra Krishi Viswavidyalaya (BCKV), West Bengal. All the genotypes were grown in field located at Balindi Research Farm, BCKV, and using randomized complete block design with three replications. Observations on yield and yield attributing characters *viz*., X1: Plant height (cm), X2: Number of primary branches per plant, X3: Number secondary branches per plant, X4: Number of pods per plant, X5: Number of seeds per pod, X6: Fresh weight of biomass per plant(g), X7: Dry weight of biomass per plant(g), X8: Days to 50% flowering, X9: Days to maturity, X10: 100-seed weight (g), X11: Soluble protein content in seed (%), X12: Yield per plant(g), X13: ODAP content in seed (%) were recorded. In each plot, five random plants were tagged to record these observations. By taking the average, the mean value for the treatment was computed. The protein content was analyzed by Lawory's method using BSA as a standard protein. For the analysis of ODAP content we are using spectra-photometric methods developed by Rao *et al*. (1964).

The data collected from field experiment was analyzed through principal component analysis (PCA) (Ogunbayo *et al*., 2005). Cluster analysis was done using D^2 statistic. Principal component analysis on the basis of correlation was used for the determination of diversity in current germplasm.

Results and Discussion

Analysis of variance revealed highly significant genetic differences except the ODAP content among the genotypes evaluated for 13 quantitative traits. Highest variability is reported for plant height followed by fresh biomass yield and days to maturity (Table 1). Among the genotypes plant height ranged from 97.4 (BK-14-8-4) to 45.3cm (BK-14-1-3). Number of primary and secondary branches ranged from 5.18 (M-Local) to 2.5 (BK-12-4) and from 10.1 (BK-6-1) to 4.8 (Nirmal mutant), respectively. Genotype BK-24-1 had maximum number of pods per plant (44.97) while BK-7-12 had minimum (23.81). Genotype BK-7-12 had the maximum number of seeds per pod (4.03) while BK-38-1 had minimum (1.67). Among the genotypes BK-12-1showed maximum fresh biomass yield (65.48 g) followed by BK-212-5 (61.14) and BK-24-1 (60.51) while for dry biomass yield BK-12-1 had maximum (28.82 g) followed by BIOL-212 (26.23) and BK-212-5 (24.34). Similar type analysis was also done by Badre *et al*. (2012) and Sharma (2015) in legume crops.

Among the genotypes BK-12-1 showed maximum seed yield/plant (25.53 g) followed by BK-3-1 and BK-17 while BK-37-1 had highest 100 seed weight coupled with maximum soluble protein content (23.34%). Correlation of morphological traits was calculated by studying the data of germplasm lines of grass pea (Table 2). It revealed that plant height showed significant positive correlation with primary branches, seed per pod, fresh biomass yield, dry biomass yield, days to 50% flowering and ODAP content. While plant height had significant negative correlation with secondary branches, pod per plant, days to maturity, 100 seed weight, soluble protein content and seed yield. Number of primary branch per plant had significant positive correlation with biomass and days to 50% flowering. Seed yield had positive correlation with number of secondary branches per plant, pods per plant, 100 seed weight and soluble protein content. Similar correlations were found in a collection of Spanish faba bean germplasm (Suso *et al*., 1993). While ODAP content had the positive correlation with plant height, secondary branches, seeds per pod, biomass yield, days to maturity, 100 seed weight and

Table 1. Mean squares of quantitative characters of grass pea

Source of variation	df	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
Replication	2	12.08	0.34	5.08	2.51	0.20	14.83	3.31	30.09	2	0.22	5.94	44.75	0.28
Genotypes	49	420.63**	1.26**	4.45**	99.83**	0.67*	274.05**	66.03**	63.63**	80.27**	1.39*	18.72**	52.03**	0.005
Error	98	114.06	0.76	1.52	42.95	0.23	66.04	12.83	12.89	31.04	0.67	11.32	23.95	0.012

'*' indicates significance at p<0.05; '**' indicates significance at p<0.01

Table 2. Correlation matrix among the morphological characters of grass pea

	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	0.49	-0.122	-0.02	0.021	0.145	0.596**	0.243*	-0.013	-0.002	-0.033	-0.023	0.048
X2	1	-0.098	-0.147	-0.116	0.236*	0.353**	0.21	-0.264*	-0.041	-0.083	-0.139	-0.132
X3		1	-0.028	0.078	-0.168	-0.044	-0.273*	-0.094	0.143	-0.188	0.016	0.042
X4			1	-0.28	-0.301**	-0.19	0.047	-0.181	0.178	-0.086	0.838**	-0.041
X5				1	-0.005	-0.061	0.113	-0.008	-0.068	0.035	-0.226*	-0.272*
X6					1	0.41	-0.058	0.15	-0.265*	-0.045	-0.353**	0.062
X7						1	0.032	-0.135	0.084	-0.112	-0.119	0.227*
X8							1	-0.325**	-0.166	-0.142	-0.082	-0.135
X9								1	-0.087	-0.008	-0.069	0.131
X10									1	0.271*	0.521**	0.118
X11										1	0.047	-0.056
X12											1	0.039

seed yield per plant. It was found that yield per plant and ODAP content were positively correlated. So, selection of low ODAP content genotypes will result in reduced seed yield. Protein content had the positive correlation with yield but negative correlation with ODAP content. Selection on basis of protein content may give us high yielding and low ODAP content genotypes.

Out of thirteen, five principal components (PCs) exhibited more than one eigen values and showed about 68.163% of variability so these five were given due importance for further explanation (Table 3). The PC1 had 20.47%, PC2 showed 15.72%, PC3 exhibited 12.62%, PC4 showed 9.78% and PC5 showed 9.57% variability among the genotypes for the characters under study. Eigen value and variance associated with each principal component, decreased gradually and stopped at 0.069 and 0.53% respectively. The PC1 was more related to fresh biomass yield, dry biomass yield, plant height and number of primary branch as it was cleared from the values of Table 4 for PC1. Poor in yield per plant, pod per plant, ODAP content, soluble protein content, 100 seed weight and secondary branch number per plant but positive effect of yield contributing traits so it must be considered. In the PC2 yield per plant and dry weight of biomass were given due importance. The PC3 exhibited positive effects for dry biomass yield and days to maturity. It showed maximum variation for these characters but poor in days to 50% flowering, seed per pod, pod per plant and primary branches per plant. In the PC4 soluble protein content, 100 seed weight and seed per pod were given importance due to its positive effect. The PC5 was more related to secondary branches per plant. From first four PCs it was cleared that among all the 13 variables, Dry biomass yield had high weight age value and pod per plant had lowest value. From this study it is cleared that a good hybridization breeding program can be initiated by the selection of genotypes from the PC1 and PC2. In a similar study by Tavoletti and Capitani (2000) in grass pea accessions, collected in the Marche region of Italy, the accumulated variation in the first 3 principal components was 91.74%. This high difference in percentage of variation could be explained by the different provenance of accessions.

Table 3. Eigen values and % total variance for first five PCs

Component	Total	% of Variance	Cumulative %
1	2.66	20.47	20.47
2	2.04	15.72	36.19
3	1.64	12.62	48.81
4	1.27	9.78	58.59
5	1.24	9.57	68.16

Table 4. Principal components (PCs) for thirteen characters in 50 germplasm of grass pea

	Eigen vectors				
Variables	1	2	3	4	5
x12	-0.764	0.517	0.074	-0.038	-0.085
x4	-0.683	0.494	-0.125	-0.325	-0.083
x6	0.633	-0.04	0.31	-0.123	-0.174
x7	0.561	0.523	0.382	0.132	0.15
x1	0.486	0.623	0.081	0.127	-0.021
x2	0.524	0.533	-0.125	0.098	0.059
x13	-0.012	0.102	0.667	-0.211	0.071
x8	0.248	0.333	-0.645	-0.168	-0.114
x9	0.017	-0.439	0.477	-0.179	-0.311
x11	-0.149	-0.075	0.082	0.675	-0.549
x10	-0.475	0.349	0.288	0.573	0.057
x5	0.192	-0.346	-0.396	0.441	0.166
x3	-0.198	-0.152	0.145	0.154	0.852

A principal component plot showed that variables are super imposed on the plot as vectors (Fig. 1). Distance of each variable with respects to PCI and PC2 showed the contribution of this variable in the variation of germplasm. It showed that as a whole dry weight of biomass, plant height, primary branch number and fresh weight of biomass contributed towards the variability in the germplasm. While in PCI only most of the yield contributing traits were responsible for the variability which were good for further use of this germplasm in breeding programs.

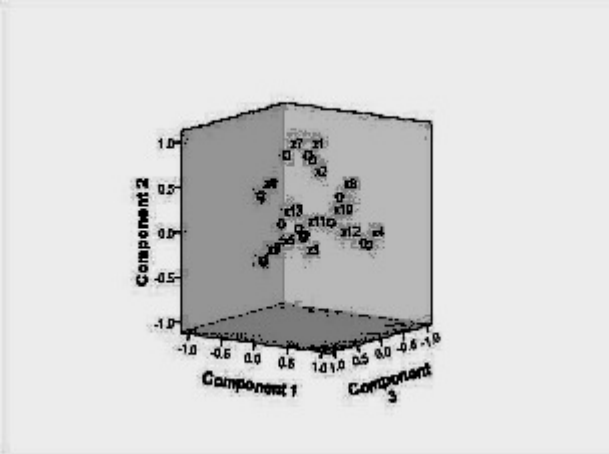

Fig 1. Component plot of grass pea germplasm lines

The entries in this study were grouped into five clusters based on D^2 statistic analysis. D^2 analysis done with 12 characters except ODAP content due to its non-significant F value. Cluster 1 consisted of seven genotypes, cluster 2 of 30, cluster 3 of 2, clusters 4 of 3 and cluster 5 of 8 accessions (Table 5). Mean value for each cluster revealed that accessions in cluster 1 had higher in plant height, primary branches, dry biomass yield and days to 50% flowering but lower in maturity. Selection from this cluster is suitable for early mature genotypes which are used as forage crops due to its higher dry biomass yield. Accessions grouped in cluster 2 had highest yield per plant. Genotypes in cluster 3 were highest in days to maturity, 100 seed weight and soluble protein content but lower in pods per plant, days to 50 % flowering and yield per plant. Cluster 4 had highest mean for pods per plant, seed per pods and fresh biomass yield and lowest for plant height, primary branches, secondary branches, dry biomass yield, 100 seed weight and soluble protein. Cluster 5 contained accessions that contained lowest means for seed per plant and fresh biomass yield. It showed highest value for secondary branches per plant.

According to Euclidean distances (D^2) among clusters (Fig. 2), the five clusters were statistically different from each other. The most similar clusters were on the one hand, cluster 1 and 2 (5.51units) and on the other hand, cluster 2 and 3 (5.57units).

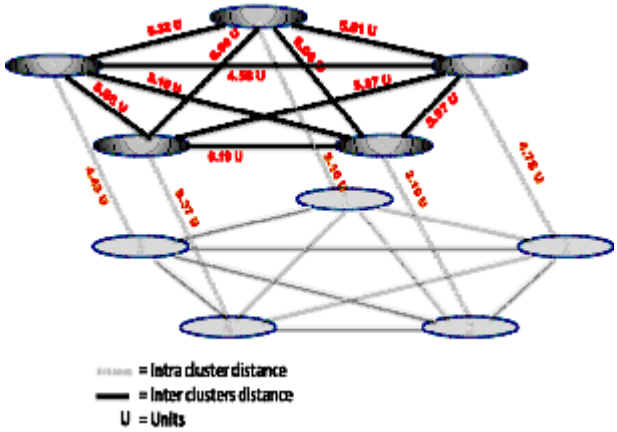

Fig 2. Diagram showing (D^2) intra and inter-cluster distances of 50 genotypes of grass pea

The largest inter cluster distance (6.90 units) was between cluster 1and 4. This result showed that plant height, primary branches, dry biomass yield and days to 50% flowering are the most important characters in differentiating the genotypes. Cluster 2 and 5 have the lowest inter cluster distance (4.58 units). This analysis helps to define groups of genotypes that were significantly different from each other for characters of interest. By observing mean values of all the 5 clusters, it was cleared that for high yield, selection of genotypes from cluster 2 and cluster 4 will be useful. They had comparatively highest mean values for most of the yield contributing traits.

Table 5. List of germplasm according to their cluster

Clusters	Genotypes list
1	BK-212-5, BK-24-1 , BIOL-212 , BK-14-8-4 , Nirmal mutant, BK-41-1 and BK-25-5
2	BK-231 , BK-17-6, BK-123-1, P-24 , BK-14-1-3 , BK-26-3, BK-8-1, BK-12-1, BIOLXNIRMAL, BK-7-12 , BK-3-1, BK-38-1, BK-12-4, BK-22-4, BK-37-1, BK-29-3, BK-8, BK-6-1, BIO-12, BK-17, BL-2-17 , BK-33-3, BK-20-3, BK-5-1 W+B, BK-30-1 , Nirmal, BK-18-5 , BK-9-3, BK-40-1, M-Local and BK-11-3-1.
3	BK-21-2-2 and BK-14-8-6
4	BK-12-1, BK-12-3-3 and BK-24-1
5	BK-5 , BK-3-2 , BK-CB-4, Nirmal-5, BK-15-5-1, BK-12-6 , BK-18-8-2 and BK-3-1

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