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Multivariate analysis of various agronomic traits in grasspea (*Lathyrus* spp) germplasm

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ABSTRCT

Diversity among 368 accessions of grasspea (*Lathyrus sativus*) from diverse indigenous and exotic sources was assessed for various agronomic traits. Analysis of variance for quantitative traits revealed that all accessions were significantly different and a wide range of variability exists for most of the traits studied. Association studies revealed that seed yield had positive significant correlation with most of the traits studied. Cluster analysis using Wards method classified 368 populations into 19 distinct groupings. A large number of genotypes were placed in cluster I (36 genotypes) followed by cluster II and cluster III each with 34 accessions. The maximum inter-cluster distance was observed between clusters XII and XII indicating the possibility of high heterotic effect if the individuals from these clusters are cross-bred. The estimates of eigen value associated with the principal components and their respective relative and accumulated variances explained 49.77% of total variation in the first two components. The characters with highest weight in component first were seed yield/plant, pods/plant, biological yield and seeds/pod which explained 38.11% of total variance. The results of principal component analysis were closely in line with those of the cluster analysis. The study of diversity reflects that in the present set of germplasm , ample amount of genetic variation exists for seed yield and other yield components suggesting direct selection for seed yield and indirect selection based on other component traits for the development of high-yielding varieties in grasspea.

Key words: Genetic variation, *Lathyrus* spp, Principal component analysis, Quantitative traits

Lathyrus is the largest genus with 200 species and subspecies in tribe *viceae* of the family *Fabaceae* and worldwide cultivated as a food, feed and fodder crop (Hanbury *et al*. 2000, Biswas 2007). It was first domesticated about 8000 years ago in the eastern Mediterranean region and was cultivated in southern parts of Europe, North Africa and across Asia. It has different important traits like drought tolerance, resistance to insect-pests, adaptability to various types of soil and climatic conditions (Granati *et al.* 2003, Vaz Patto *et al*. 2006). In addition, it effectively nodulates with *Rhizobium leguminosarum* (Yadav and Bejiga 2006), leaving the soil enriched in nitrogen for the next season crop (Campbell *et al.* 1994). Its strong and penetrating root system allows growing in a wide range of soil types including very poor soils and heavy clays (Tiwaril and Campbell 1996), while its ability to utilize remnant water and soil nutrients makes the species adapted to low or zero inputs.

Lathyrus sativus (Grasspea) is an important crop of economic significance in India, Bangladesh, Pakistan and Ethiopia. It is also cultivated in Central, South and East Europe, Crete, Rhodes,Cyprus and in West Asia and North Africa. The seeds of grasspea contain 31% protein, 41% carbohydrate, 17% total dietary fiber (2% soluble and 15% insoluble), 2% fat and 2% ash, on a dry matter basis (Akalu *et al*. 1998).

Since it is a crop that can withstand extreme environmental conditions, it has been realized that grasspea can serve as a survival foodstuff in difficult situations. These abilities make it an interesting pulse crop and encourage for a thorough and extensive characterization of its germplasm. Unfortunately grasspea, together with many other pulse crops, belongs to the 'research orphan crops' and dearth of information is available on it, particularly at the level of genetic variability. Therefore, further addition of knowledge about genetic diversity and genetic resources of *Lathyrus* is needed. This can be done through studying the variation in various yield contributing traits among the *Lathyrus* population. Therefore, the aim of present investigation was

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characterization and clustering/grouping of diverse indigenous and exotic germplasm for their further utilization in *Lathyrus* improvement.

MATERIALS AND METHODS

The experimental material included 368 accessions from diverse exotic and indigenous sources with two checks BioL 12 and Pusa 24. The material included three species of grasspea (i) 358 accessions of *L. sativus*; (ii) 5 accessions of *L. aphaca* and (iii) 5 accessions of *L. cicera*. The study was conducted at Research Farm of Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India. The site of experiment falls under semi arid Northern plains on latitude of 26.28° North and longitude of 80.21° East and at an elevation of 152.4 meters above mean sea level. The soil textural class at the site was sandy loam. The accessions were grown in an augmented design (Federer 1956) with two intermittent checks after every twentieth row. Each accession was grown in two rows of four meter length and inter- and intra-row distances were kept at 60 cm and 10 cm, respectively. The recommended package of practices was followed to raise the healthy crop. Observations were recorded on five randomly chosen plants for twelve quantitative traits. All the characteristics were recorded according to the "Descriptors for *Lathyrus* spp" by IPGRI (2000). Analysis of variance (ANOVA) for augmented design for all traits was performed using statistical software, SAS, version 9.3. Different analysis were performed on mean data for each trait using different statistical software,viz. Principal component analysis (SAS version 9.3), Correlation (SPSS, ver.11), Biplot drawing ('R', versions 2.15) and Cluster analysis (SAS, version 9.3).

RESULTS AND DISCUSSION

Analysis of variance and correlation studies

Analysis of variance for quantitative traits revealed that the accessions were significantly different for the traits studied. This indicates that sufficient amount of variability among the accessions for all quantitative traits was present. Phenotypic and genotypic correlation coefficients among the twelve quantitative traits were also estimated (Table 1). It was observed that days to maturity had highly significant positive phenotypic and genotypic correlation with all the traits studied except biological yield and harvest index, respectively. Days to flowering showed positive significant genotypic correlation with days to maturity and seeds/pod. 100-seed weight had significant positive phenotypic association with all the traits except primary branches, pod/ plant and days to flowering. Seeds/pod exhibited highly significant positive phenotypic correlation with all the traits except days to flowering. Seed yield per plant showed high positive significant phenotypic and genotypic association with all the characters except days to flowering and days to flowering, 100-seed weight, respectively. Seeds/pod exhibited high positive significant genotypic correlation with all the traits except primary branches and 100 seed weight. The strong positive correlation of number of pods and seeds/pod with seed yield has previously been reported in grasspea (Kumar and Dubey 1996, 2001; Tadesse and Bekele 2002).

On the other hand, days to flowering had negative significant phenotypic and genotypic correlation with 100 seed weight and plant height, respectively suggesting that selection for early flowering could result in higher 100-seed

Table 1 Phenotypic and genotypic correlation among different yield contributing traits in grasspea

Character name	DF	DM	PH	PB	Pods/ plant	Seeds/ pod	100-SW	PL	PW	Yield/ plant	BY	HI
DF	**1.00**	0.27**	– 0.06	– 0.03	– 0.06	0.01	– 0.10*	0.01	0.05	– 0.09	– 0.08	– 0.04
DM	0.19**	**1.00**	0.25**	0.32**	0.28**	0.17**	0.13**	0.14**	0.15**	0.22**	0.06	0.32**
PH	– 0.15**	0.34**	**1.00**	0.11*	0.22**	0.15**	0.31**	0.21**	0.20**	0.34**	0.18**	0.36**
PB	– 0.10*	0.15**	0.08	**1.00**	0.29**	0.14**	0.05	0.05	0.14**	0.25**	0.18**	0.23**
Pods/plant	0.02	0.37**	0.24**	0.18**	**1.00**	0.59**	0.02	0.51**	0.57**	0.75**	0.65**	0.49**
Seeds/pod	0.14**	0.26**	0.14**	0.05	0.65**	**1.00**	0.15**	0.55**	0.57**	0.55**	0.49**	0.38**
100-seed	0.03	0.22**	0.16**	– 0.10*	0.00	0.04	**1.00**	0.14**	0.10*	0.25**	0.16**	0.25**
PL	– 0.02	0.18**	0.15**	0.01	0.49**	0.58**	0.01	**1.00**	0.72**	0.50**	0.43**	0.34**
PW	0.08	0.13*	0.11*	– 0.00	0.54**	0.56**	0.04	0.70**	**1.00**	0.52**	0.42**	0.37**
Yield/plant	0.01	0.24**	0.24**	0.12*	0.79**	0.58**	0.05	0.46**	0.47**	**1.00**	0.79**	0.69**
BY	0.03	0.27**	0.22**	0.16**	0.77**	0.54**	0.11*	0.42**	0.46**	0.87**	**1.00**	0.14**
HI	– 0.01	0.07	0.17**	– 0.00	0.42**	0.40**	– 0.05	0.33**	0.26**	0.66**	0.24**	**1.00**

Phenotypic correlation: above diagonal; Genotypic correlation: lower diagonal; *, ** Significant at P=0.05 and 0.01, respectively
DF, Days to flowering; DM, days to maturity; PH, plant height; PB, primary branches; PL, pod length; PW, pod width, BY, biological yield; HI, harvest index

weight and plant height. Biological yield and harvest index had high positive significant phenotypic and genotypic association with most of the characters. The values of phenotypic correlation were in accordance with genotypic correlation suggesting due contribution of genotype in the expression of various quantitative traits (Kumar and Dubey 2001, Dixit *et al*. 1996). The information generated would be helpful in understanding relationship between characters studied which, henceforth, may help in indirect selection in grasspea breeding programme.

Cluster analysis

To describe the genetic diversity and grouping based on similar characteristics cluster analysis has been widely used. Separate two-way cluster analysis (using Ward's methods) based on principal component was performed for grasspea accessions (Fig 1). Total nineteen clusters were formed and maximum numbers of 36 accessions were present in cluster I followed by cluster II and cluster III, each with 34 accessions. However, minimum numbers of accessions (7) were present in the cluster 13 and 17. The minimum inter cluster distance was 0.64 units between cluster XIII and XIV and maximum inter cluster distance was between cluster XII and XIII (24.03 unit). Accession from clusters which had more inter cluster distance could be crossed for creation of maximum variability for effective selection. The minimum intra cluster distance was found in cluster XIV (1.48 unit) having 25 accessions, whereas the maximum intra-cluster distance was in cluster XIII (21.09 unit) having 7 accessions (Table 2). The genotypes of cluster XIII were diverse indicating that there is opportunity of improvement through selection within cluster. For the ease of presentation of accessions through two way dendogram, the original dendogram produced for 368 accessions were redrawn using 76 accessions. These 76 accessions were selected from each of the 19 clusters through Probability Proportional to Size Simple Random Sampling (PPS SRS) technique so as to obtain a true representation of original dendogram (Fig 1). Two way dendogram showed grouping of accessions on the basis of all yield contributing traits. None of the clusters contained any genotype with all the desirable character which could be directly selected and utilized as a commercial variety. To obtain desirable plant types, recombination among genotypes of diverse clusters is necessary (Polignano *et al*. 2005).

Principal component analysis

In PCA analysis, the variance–covariance matrix was used to transform the entire attribute into a single index of similarity in the form of principal component, which represented 12 eigen value for 12 eigen vectors. The eigen value represent the variance of principal components and the cumulative per cent of the eigen values indicating percentage contribution to the total variance attributable to each principal components were estimated. According to eigen value and cumulative percent criterion, first seven principal components represent the data efficiently. The estimates of the eigen value associated with the principal component and their respective relative and accumulated variances explained 49.77 percentage of the total variation in the two first principal

Table 2 Inter and intra cluster distances

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
I	**3.19**	3.82	3.85	4.22	4.69	4.57	4.19	5.02	4.78	5.81	4.85	6.08	13.00	1.42	4.35	2.07	12.81	3.02	4.34
II		**3.43**	3.93	4.11	4.49	3.83	4.12	4.79	4.35	5.15	4.90	5.91	16.60	1.57	5.52	2.36	15.03	3.42	4.91
III			**2.95**	3.79	4.21	3.73	3.98	4.28	4.21	4.65	4.16	5.12	14.33	1.29	5.05	2.34	12.85	2.87	4.34
IV				**3.02**	4.00	4.10	4.33	4.14	4.50	4.87	4.80	5.25	16.62	1.43	5.87	2.43	15.02	3.08	5.09
V					**2.71**	4.41	5.41	4.57	3.88	4.25	4.79	5.02	19.92	1.63	6.98	3.07	19.21	4.29	6.34
VI						**2.82**	3.78	4.36	3.84	4.00	4.17	4.57	19.48	1.43	6.33	2.72	16.43	3.57	5.17
VII							**3.18**	5.25	4.96	5.63	4.52	5.92	17.05	1.46	5.08	2.53	13.31	2.98	3.81
VIII								**3.53**	4.11	3.91	4.36	4.99	18.93	1.61	6.72	2.44	18.71	3.62	6.21
IX									**3.04**	3.60	3.87	4.98	19.91	1.69	6.90	2.94	20.01	4.16	6.30
X										**2.89**	4.16	4.36	23.12	1.72	7.89	3.17	21.92	4.50	7.00
XI											**3.31**	5.35	18.89	1.65	6.29	2.85	18.34	3.65	5.43
XII												**2.71**	24.03	1.20	7.91	3.38	21.68	4.71	7.26
XIII													**21.09**	0.64	5.83	2.85	15.73	3.74	5.54
XIV														**1.48**	3.86	1.90	12.05	2.76	4.22
XV															**3.50**	2.15	11.28	3.02	4.05
XVI																**2.02**	10.72	2.12	3.80
XVII																	**16.12**	2.89	5.18
XVIII																		**2.68**	3.42
XIX																			**2.85**

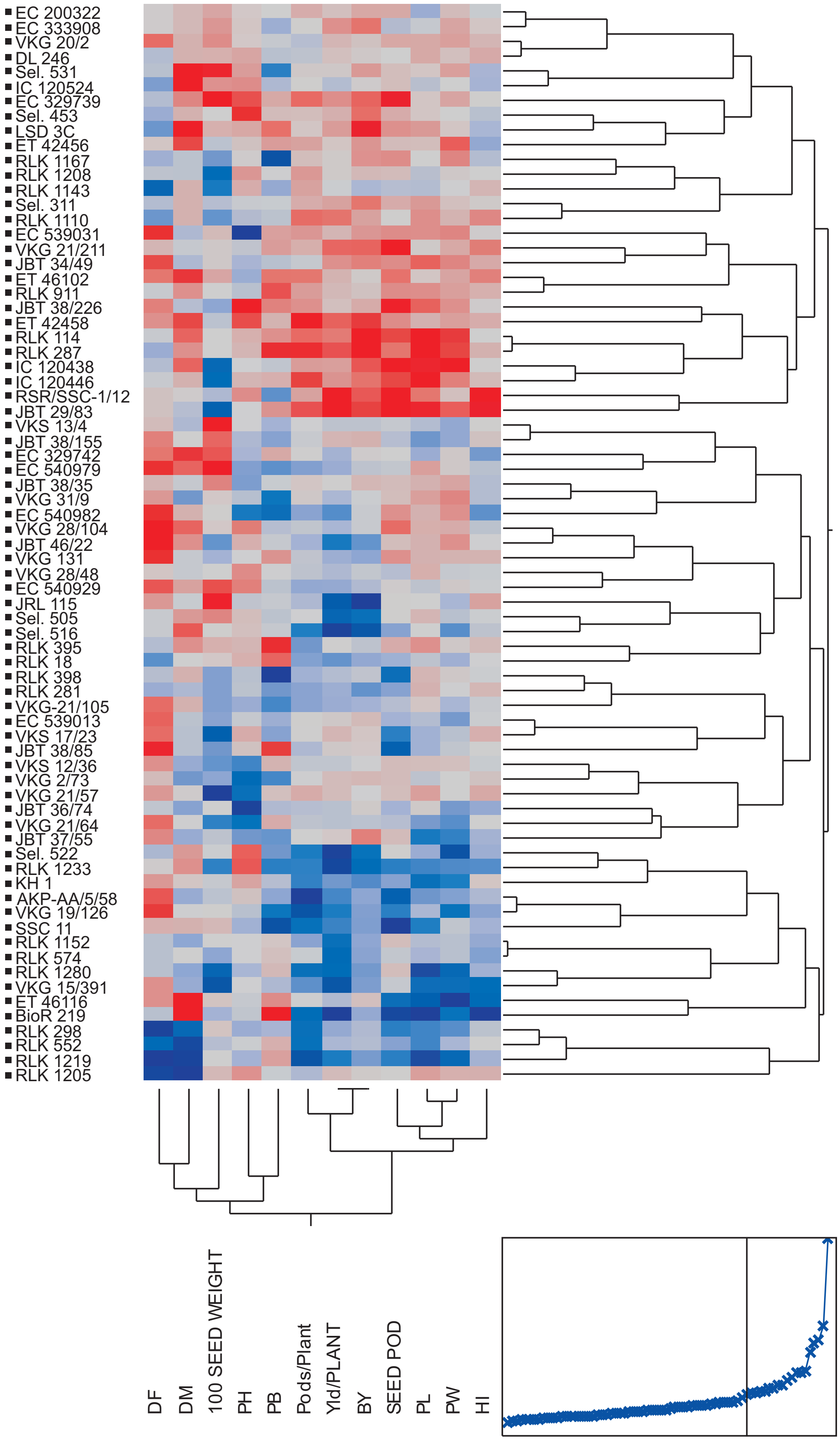

Fig 1 Dendogram showing 76 genotypes drawn 19 clusters using PPS SRS technique

Table 3 Eigen values, proportional and cumulative percent variation and Eigen vectors for 12 yield contributing traits

Principal component	Eigen value	Proportional Percent	Cumulative percent
1	4.57	38.106	38.11
2	1.40	11.661	49.77
3	1.20	10.005	59.77
4	1.10	9.143	68.92
5	0.91	7.586	76.50
6	0.82	6.848	83.35
7	0.64	5.304	88.65
8	0.50	4.181	92.83
9	0.40	3.296	96.13
10	0.27	2.248	98.38
11	0.18	1.535	99.91
12	0.01	0.086	100.00

components indicating a high degree of correlation among the characters for the accessions analyzed. Percentages of variation attributable to the first two components by decreasing order were 38.11% and 11.70%. Most of the variation was distributed up to the 7th principal component, being responsible for 88.65% of the relative variation that was observed (Table 3). By examining the eigen vectors of individual components, indication may be obtained about their level of association with the original traits. The characters with the highest weight in component first were seed yield/ plant, pods/plant, biological yield and seeds/pod, which explained 38.11% of the total variance. Component 2 was associated with days to maturity, 100-seed weight, plant height and primary branches which account for 11.70% of the variance. In the component 3, traits with highest weight were days to flowering, 100-seed weight, seeds/pod, and days to maturity which explained 10.05% of the total variance. Similarly, the characters with the highest weight in component 4 were primary branches, days to flowering and days to maturity (9.14%), component 5-harvest index, seed yield/ plant and days to flowering (7.59%); component 6-plant height and days to maturity (6.85%) and component 7-harvest index, primary branches and 100-seed weight (5.30%) (Table 4). It is interesting to note that yield and some yield contributing traits appear strongly in the first three components (Tavoletii and Capitani 2000, Polignano *et al*. 2005, Mustafa *et al*. 2007, Singh *et al*. 2012). The relationship between different yield contributing traits and accession behavior is plotted in the biplot graph (Fig 2). The biplot provides a useful tool for data analysis. If the angle and directions between vectors or lines which indicated yield contributing traits are less than 90°C, it represents a positive correlation and if the angle between the lines is more than 90°C, this indicates the correlation is negative. According to the biplot, there was positive correlation between most of the traits that appear in graph near to each other's confirming the simple correlation results.

Evaluation of lathyrus accessions showed a wide range of variation in various yield contributing traits. Significant correlations were observed among the traits studied. Seed yield had significant positive correlation with most of the traits suggesting indirect selection based on components traits will accelerate grasspea improvement programme. PCA analysis revealed significant variations among traits with seven major principal components explaining 88.65% of variations. Cluster analysis using Wards method classified the genotypes in 19 distinct clusters. Accessions from diverse group will maximize opportunities to obtain transgressive segregants as there is a higher chance from such genotypes

Table 4 Estimates of the weighting coefficient (eigen vector) associated with the principal components and different characters of grasspea

Character name	Eigen vectors											
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC9	PC10	PC11	PC12
DF	0.03	0.12	0.71	0.43	0.25	0.11	0.08	0.45	0.06	0.09	0.10	– 0.01
DM	0.19	0.58	0.16	0.17	– 0.02	0.31	– 0.02	-0.61	0.22	– 0.05	– 0.21	0.01
PH	0.15	0.47	– 0.34	– 0.29	0.13	0.47	– 0.20	0.51	– 0.13	0.02	0.03	– 0.01
PB	0.07	0.18	– 0.48	0.62	– 0.25	– 0.09	0.50	0.19	-0.01	0.01	– 0.01	-0.01
Pods/plant	0.42	0.02	– 0.05	0.13	0.02	– 0.10	– 0.20	– 0.15	– 0.05	– 0.26	0.82	0.00
Seed/pod	0.37	– 0.09	0.17	0.02	– 0.09	0.08	0.10	– 0.12	– 0.86	– 0.02	– 0.22	– 0.04
100-seed weight	0.04	0.49	0.20	– 0.47	– 0.08	– 0.52	0.44	0.07	– 0.03	0.00	0.10	– 0.01
PL	0.34	– 0.20	0.09	– 0.18	– 0.40	0.27	0.18	– 0.01	0.21	0.68	0.18	– 0.01
PW	0.34	– 0.18	0.16	– 0.13	– 0.44	0.12	0.05	0.25	0.30	– 0.62	– 0.23	0.00
YLD/plant	0.42	– 0.06	– 0.11	0.03	0.33	– 0.26	– 0.08	0.03	0.18	0.11	– 0.23	– 0.73
BY	0.38	0.07	– 0.06	0.12	0.02	– 0.43	– 0.40	0.13	0.08	0.21	– 0.28	0.58
HI	0.27	– 0.25	– 0.11	– 0.14	0.61	0.18	0.51	– 0.09	0.14	– 0.10	– 0.03	0.36

DF, Days to flowering; DM, days to maturity; PH, plant height; PB, primary branches; PL, pod length; PW, pod width; BY, biological yield; HI, harvest index

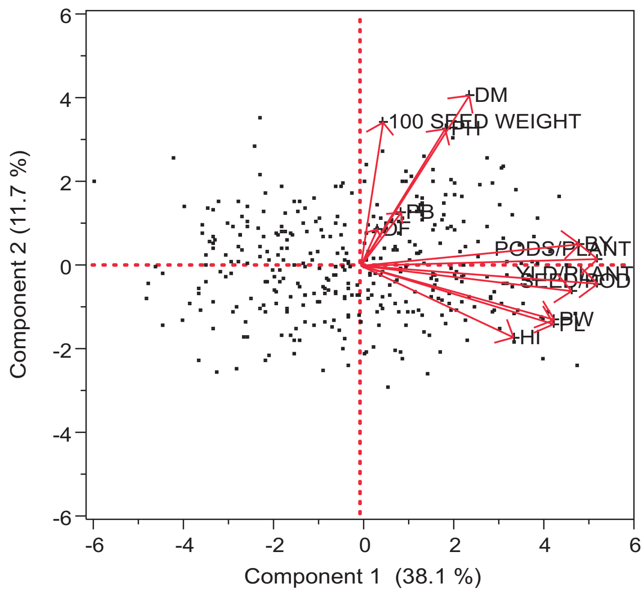

Fig 2 The biplot of 368 accession of grasspea based on PC1 and PC2

to contribute unique desirable alleles at various loci (Kanwal *et al*. 1983). The study of diversity reflected that ample amount of genetic variation exists for seed yield and other yield components in the set of germplasm. Hence, indirect selection for seed yield based on component traits may lead to create better genetic recombinants for improving yield *per se*. Such recombinants will further widen the *Lathyrus* gene pool. These results can now to be used by the breeders to develop high yielding *Lathyrus* varieties and new breeding protocols for *Lathyrus* improvement.

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