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Assessment of genetic diversity of garden pea (*Pisum sativum*) as perspective to isolate horticulturally desirable transgressive segregants

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ABSTRACT

Genetic restructuring of germplasm is essential to identify the potential genotypes for use in future breeding programmes. An attempt was made to determine the degree of divergence among 41 diverse genotypes of garden pea (*Pisum sativum*) (23 isolated from induced mutations, 12 through hybridization followed by selection and six other released varieties) by following multivariate analysis. These genotypes were evaluated in Randomized Complete Block Design with three replications in winter season during 2009-10 and 2010-11. A considerable genetic diversity was observed and the genotypes were arranged into 8, 13 and 5 clusters in 2009-10, 2010-11 and pooled over years, respectively with maximum genotypes in cluster I. DPPMR-09-1, DPP-25G and DPPM 64 exhibited consistency in clustering pattern over the years. Harvest index, total biomass and 100-seed weight contributed maximum towards total genetic divergence. To conclude, genotypes Punjab 89, DPPM 74, Palam Priya, DPPM 64, DPPM 73, DPPMFWR 4, DPPMFWR 30-2 and DPP 25G offer promise as a breeding stock for utilization in hybridization as parents for the isolation of transgressive segregants in garden pea.

Key words: Clusters, Genetic divergence, Genotypes, *Pisum sativum*

Garden pea (*Pisum sativum* L.), a member of *Papilionaceae* family, is a rich source of health building substances, viz. proteins, vitamins, minerals, and also lysine (a limiting essential amino acid in cereals). It is a leading vegetable crop in the north-western Himalayan region of India comprising the states of Himachal Pradesh, Jammu & Kashmir and Uttarakhand. Owing to diverse agro-climatic conditions in Himachal Pradesh, the crop is grown year round as an off-season cash crop during summer in the high altitude areas and in low and mid hills during winter which in turn results in high remuneration to the growers (Sharma *et al.* 2010 b). Accordingly, pea holds a very coveted position in Himachal Pradesh by covering more than one-fourth of the total area under vegetable crops and thus garden pea ranks first in acreage (21.74 thousand hectares) with an annual production of 237.28 thousand tonnes (Anonymous 2010).

High yield, long and dark green pods, sweetness and resistance to pests and diseases are the main criteria which are taken into consideration by the breeders for genetic improvement of garden pea. Despite continuous breeding efforts, its average yield is low due to genetic drift in the cultivars and development of the new pathogen races (Bhardwaj 2011). Age old varieties like Azad P-1, Lincoln, Arkel etc. are still preferred by the growers due to desirable horticultural traits though the varieties have become vulnerable to a plethora of biotic and abiotic stresses.

The improvement potential of any crop is proportional to the magnitude of genetic variability in the germplasm (Singh *et al.* 2009) which provides the possibility to improve the yield and quality through strategic breeding programme. Therefore, genetic restructuring of pea germplasm is the first step to identify the potential genotypes for use in breeding programme. The multivariate analysis is a powerful tool for determining the degree of divergence among genotypes in the population and nature of forces operating at different levels (Coelho *et al.* 2007). The inclusion of diverse parents in hybridization programs serves the purpose of combining desirable genes to obtain desirable recombinants (Ceolin *et al.* 2007). The assessment of genetic divergence helps in reducing the number of breeding lines from the large germplasm and the progenies derived from diverse parents are expected to show a broad spectrum of genetic variability and provide better scope to isolate superior recombinants. Keeping this in view, study was undertaken to gather information on genetic divergence of 41 genotypes of garden

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pea developed through mutation and hybridization. The information shall enable the breeders to make informed decisions about suitable parents while planning breeding programme for high yield along with desirable horticultural traits.

MATERIALS AND METHODS

The present investigation was undertaken at the Experimental Farm, Department of Vegetable Science and Floriculture, CSK HPKV, Palampur (1 290.8 m above mean sea level, with latitude 32°6' N, longitude 76°3' E). The soil of experimental field was clay loam with pH 5.7. The experimental material comprised 41 genotypes of garden pea which includes 23 isolated from induced mutations, 12 through hybridization followed by selection and six released varieties. The experiments were laid out in Randomized Complete Block Design with three replications for two consecutive years (November to April 2009-10 and 2010-11). These genotypes (Table 1) were sown in two rows each of 4 m length at inter and intra-row spacing of 45 cm and 10 cm, respectively. Soil was once ploughed using a 3-disc tractor and twice using a 7-disc tractor followed by power tiller. The seeds were sown at a depth of 4-5 cm. The fertilizers were applied at the time of sowing @ 50 kg N, 60 kg P_2O_5 and 60 kg K_2O/ha. Weeds were controlled with Pendimethalin @ 1 kg a.i./ha as pre-emergence application at the initial stages of growth followed by two manual weedings at 40 and 60 days after sowing. The irrigation was applied at 15 days interval depending upon the requirement twice at vegetative growth stage followed by critical stages of flowering and pod development.

The observations were recorded on randomly selected ten plants of each genotype from each replication for 23 traits, viz. days to first flower, first flower node, days to 50% flowering, days to first picking, number of branches, internodal length (cm), nodes/plant, plant height (cm) at final harvest, pod length (cm), seeds/pod, shelling (%), pods per plant, pod yield/plant (g), total biomass (g), seed yield/plant (g), harvest index (%), 100-seed weight (g), total soluble solids (°brix), ascorbic acid (mg) [Titration method, AOAC (1970)], protein content (%) [Microkjeldahl Method (AOAC 1990)], total sugars (%) [Dubois *et al*. 1956], reducing sugars (%) [Miller 1972] and starch content (%) [Sadasivam and Manickam 1996]. The data collected were subjected to multivariate analysis utilizing Mahalanobis D^2 statistic as suggested by Mahalanobis (1936) and Rao (1952) using statistical software WINDOSTAT 8.0 developed by Indostat Services. Genotypes were grouped into various clusters following Tocher's method as suggested by Rao (1952).

RESULTS AND DISCUSSION

The garden pea genotypes were significantly different for almost all the characters during both the years and pooled over years with few exceptions, viz. days to first picking in the year 2010-11 (Table 2) and first flower node, number of branches, shelling (%), total sugars and reducing sugars in pooled environments. There were significant genotypic × environmental interactions for yield and yield related traits.

Table 1 Pedigree and breeding method of the genotypes

Genotypes	Pedigree and breeding method used
DPPMFWR 1	Arkel (EMS 0.3 %)
DPPMFWR 2	Azad P-1 (EMS 0.2 %)
DPPMFWR 3	Arkel (EMS 0.3 %)
DPPMFWR 4	Arkel (EMS 0.3 %)
DPPMFWR 5	Azad P-1 (200Gy)
DPPMFWR 8	Azad P-1 (150 Gy)
DPPMFWR 11	Arkel (EMS 0.3 %)
DPPMFWR 12	Arkel (EMS 0.3 %)
DPPMFWR 20	Azad P-1 (200 Gy)
DPPMFWR 27	Arkel (EMS 0.3 %)
DPPMFWR 29	Arkel (EMS 0.3 %)
DPPMFWR 30-1	Arkel (EMS 0.3 %)
DPPMFWR 30-2	Arkel (EMS 0.3 %)
DPPM 1	Azad P-1 (200 Gy)
DPPM 22	Arkel (EMS 0.3 %)
DPPM 64	Arkel (200 Gy)
DPPM 65	Azad P-1 (200 Gy)
DPPM 72	Azad P-1 (200 Gy)
DPPM 73	Azad P-1 (200 Gy)
DPPM 74	Azad P-1 (200 Gy)
DPPM 07-4	Azad P-1 (75Gy)
DPPM 07-9	Azad P-1 (EMS 0.3 %)
DPPM 07-30	Arkel (75 Gy)
DPPMR 09-1	Hybridization followed by selection
DPPMR 09-2	Hybridization followed by selection
DPPMR 09-3	Hybridization followed by selection
DPPMR 09-5	Hybridization followed by selection
DPPMR 09-9	Hybridization followed by selection
DPP 25G	Hybridization followed by selection
DPP 89	Hybridization followed by selection
DPP 100	Hybridization followed by selection
DPP 168	Hybridization followed by selection
DPP 3-1	Hybridization followed by selection
DPP 11-2	Hybridization followed by selection
DPP 17-2	Hybridization followed by selection
VP 215	Hybridization followed by selection (Almora)
Green Pearl	Introduction by Nunhems
Lincoln	Introduction
Azad P-1	Hybridization followed by selection (Kanpur)
Palam Priya	Hybridization followed by selection (Palampur)
Punjab 89	Introduction

*Genotypes 1-23 developed through mutation (^{60}Co gamma cell and Ethyl methane sulphonate-EMS, Sharma *et al.* 2010 a), 24-35 through hybridization followed by selection and 36-41 are released varieties

Table 2 Analysis of variance for different characters in garden pea during the years 2009-10, 2010-11 and pooled over years

Characters	Mean sum of squares										
	Replication		Genotype (G)		Error		Genotype (G)	Environment (E)	G× E	Error	F-Test (Homo geniety test) 60
	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11	Pooled over years				
	df	4	40	80			40	1	40		
Days to first flower	29.20	14.44	9.90*	34.58*	4.16	8.61	32.87*	15648.13	11.61*	6.39	4.28*
First flower node	0.07	10.41	0.64*	2.79*	0.24	0.90	1.97	0.03	1.46*	0.57	13.50*
Days to 50% flowering	129.82	37.69	36.98*	38.50*	5.97	7.37	62.40*	4122.00	13.09*	6.67	1.52
Days to first picking	26.81	70.63	23.30*	13.83	6.55	9.04	28.60*	8301.00	8.55	7.79	1.90*
Number of branches	0.07	0.46	0.12*	0.82*	0.03	0.11	0.55	61.09	0.39*	0.07	13.44*
Internodal length (cm)	1.60	0.03	1.80*	2.25*	0.09	0.10	3.04*	7.68	1.01*	0.09	1.23
Nodes/plant	2.95	9.95	2.86*	6.25*	1.52	0.20	6.37*	512.30	2.74*	0.86	57.75*
Plant height (cm)	52.15	17.71	274.51*	290.04*	9.88	8.79	470.00*	1615.75	94.56*	9.33	1.26
Pod length (cm)	1.05	1.82	4.64*	3.75*	0.13	0.29	7.89*	1.56	0.50*	0.21	4.98*
Seeds/pod	0.68	1.29	2.55*	2.69*	0.14	0.20	4.60*	6.81	0.64*	0.17	2.04*
Shelling (%)	17.14	46.55	27.30*	17.13*	5.46	5.31	26.69	112.63	17.74*	5.39	1.05
Pods/plant	6.34	3.42	16.42*	34.64*	1.90	1.28	37.15*	1358.72	13.90*	1.59	2.20*
Pod yield/plant (g)	21.76	2.94	296.77*	676.69*	15.43	16.01	759.10*	20509.84	214.37*	15.72	1.07
Total biomass (g)	1.63	9.20	183.49*	127.57*	5.99	3.81	221.58*	830.50	89.48*	4.90	2.47*
Seed yield/plant (g)	0.30	1.29	29.22*	23.99*	0.72	1.30	43.84*	105.71	9.37*	1.01	3.25*
Harvest index (%)	3.46	23.65	137.07*	154.13*	0.22	13.36	179.82*	31.22	111.39*	6.79	3687.81*
100-seed weight (g)	11.56	0.98	24.44*	18.95*	1.41	1.79	39.14*	197.64	4.25*	1.60	1.61
Total soluble solids (°brix)	10.31	5.68	2.80*	1.55*	1.19	0.22	3.06*	46.29	1.29*	0.70	29.34*
Ascorbic acid (mg)	17.27	1.09	3.14*	12.56*	0.28	1.12	14.13*	2.45	1.57*	0.70	16.02*
Protein content (%)	1.67	23.09	7.93*	11.76*	0.29	2.19	16.56*	112.13	3.12*	1.24	57.14*
Total sugars (%)	5.07	3.53	1.50*	1.74*	0.08	0.15	1.74	0.60	1.50*	0.11	3.52*
Reducing sugars (%)	0.35	0.10	0.84*	0.57*	0.06	0.14	0.59	2.72	0.82*	0.10	5.44*
Starch content (%)	9.71	4.95	34.17*	36.97*	2.83	3.52	44.08*	23.83	27.07*	3.17	1.55

*Significant at P = 0.05

Table 3 Performance of top 8 genotypes of garden pea for yield and yield related traits in comparison to recommended check varieties in pooled years

Genotypes	Days to flowering	Days to first picking	Pod length (cm)	Seeds/ pod	Shelling (%)	Pods/ plant	Plant height (cm)	Pod yield/ plant (g)	Seed yield/ plant (g)
DPPMFWR 11	89.50	124.00	8.61	6.81	47.08	12.80	66.39	54.42	9.96
DPPMFWR 12	93.17	126.00	8.07	6.18	48.93	13.79	54.67	45.30	10.23
DPPMFWR 30 -2	96.50	125.67	8.44	5.50	43.29	9.96	55.12	41.33	11.81
DPPM 64	91.67	121.33	13.35	8.37	45.95	8.79	69.58	57.33	12.66
DPPM 72	96.00	125.17	9.75	6.70	44.98	13.06	71.99	53.57	10.60
DPPM 73	95.17	125.17	9.84	7.09	46.65	10.38	72.84	41.67	12.33
DPPM 74	94.33	124.00	8.51	6.18	45.87	16.52	59.02	66.25	16.14
DPPM 07-4	92.67	124.17	9.96	6.61	43.87	11.00	71.59	54.22	14.14
Lincoln (C)	95.00	125.17	8.09	5.95	43.30	9.89	56.28	38.52	7.31
Azad P-1 (C)	94.17	126.50	8.38	6.17	46.34	10.80	65.89	45.67	9.84
Palam Priya (C)	97.00	125.67	8.17	6.08	44.47	13.71	57.19	53.83	11.90
Punjab-89 (C)	88.67	119.00	10.40	8.04	47.00	10.80	59.28	56.45	12.95
CD (P = 0.05)	2.96	3.20	0.52	0.47	2.66	1.44	3.50	4.54	1.15

The results showed genetic variability for these characters and also indicated the need for multi-location trials to identify the superior genotypes. On the basis of average performance over the years, DPPM 74 significantly resulted in maximum pods/plant, pod yield and seed yield over all the checks (Table 3). In addition, DPPM 64, DPPMFWR 11, DPPM 72 and DPPM 07-4 for pod yield and that of DPPM 64, DPPM 73 and DPPMFWR 30-2 for seed yield were statistically at

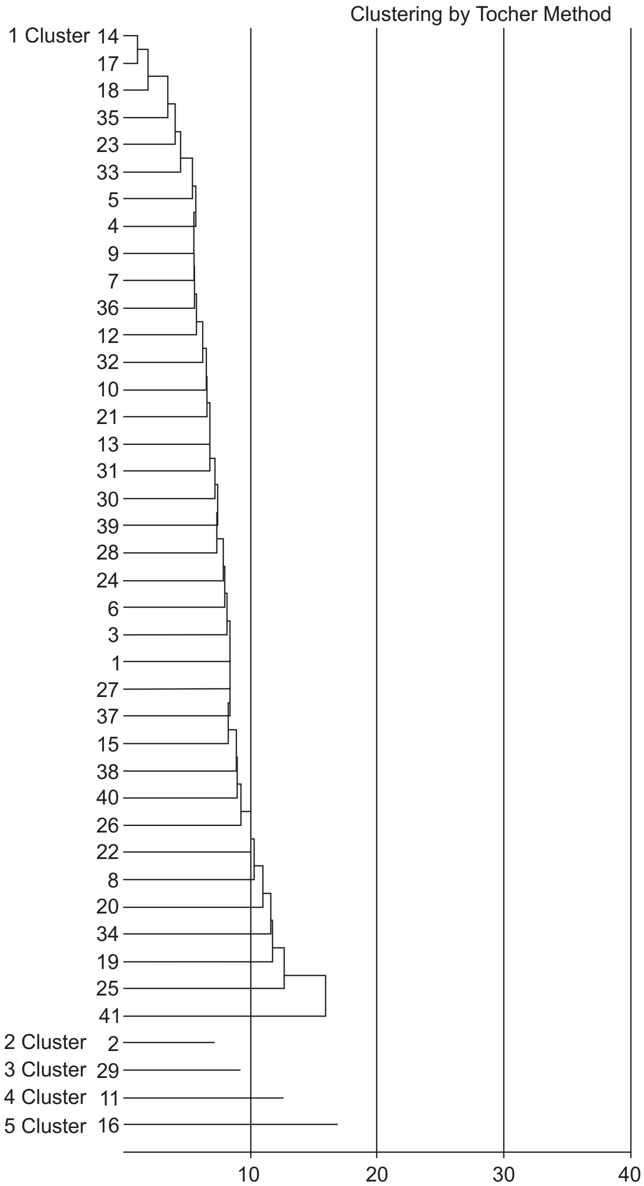

Fig 1 Grouping of 41 garden pea genotypes using D^2 cluster analysis

par with the best check variety Punjab 89. Also, genotype DPPM-07-4 significantly outperformed Punjab-89 for seed yield. Consumers prefer well filled long and green pods and accordingly the genotype DPPM 64 had significantly the highest pod length in comparison to all other genotypes including checks while DPPM 64 and check Punjab 89 contained significantly maximum number of seeds/pod.

The multivariate analysis (D^2) arranged 41 genotypes into 5 clusters in pooled over years following Tocher's procedure (Fig 1). Different clustering patterns using different genetic material were also reported by Yadav *et al.* (2009) and Devi *et al.* (2010) in pea. The cluster I was the largest comprising of 90.24 % genotypes. Four clusters namely, II (DPPMFWR 2), III (DPP 25G), IV (DPPMFWR 29) and V (DPPM 64) were monogenotypic, i.e. having only one genotype indicating that these genotypes diverged most from others. Singh and Mishra (2008) also observed similar results from their studies. The clustering of the genotypes was random which indicated no parallelism between clustering pattern and geographical diversity rather genetic constitution of the genotypes influenced the clustering pattern (Kumar *et al.* 2007).

The intra-cluster distance was observed to be the highest in cluster VI, IV and I in 2009-10, 2010-11 and pooled over years, respectively (Table 3). Sharma *et al.* (2009) also observed maximum intra-cluster variation among genotypes in their studies. Since the intra-cluster distance was low, the chances of developing good segregants by hybridization among parents within cluster would be low. Therefore, it is logical to attempt crosses between genotypes falling in different clusters based on inter-cluster distance.

The inter-cluster distance ranged from 6.04-37.63, 7.18-16.43 and 3.02-8.35 during 2009-10, 2010-11 and pooled over years, respectively (Table 4). The maximum inter-cluster genetic divergence was recorded between clusters V and VIII during 2009-10, between clusters XI and XII during 2010-11 and between clusters III and V in pooled over years suggesting wide diversity among genotypes of the two clusters due to different genetic constitution. Therefore, genotypes within clusters might not be selected rather selected from different clusters for further hybridization among themselves. The inter-cluster proximity was the maximum between clusters II and III, clusters III and V and clusters II and III during 2009-10, 2010-11 and pooled over years, respectively. This clearly indicates that the genotypes included in the clusters with high inter-cluster distance showed sufficient genetic diversity and selection of parents from these diverse clusters would be useful in hybridization programme for improving yield and other desirable horticultural traits of garden pea. The crosses involving the diverse genotypes would be expected to manifest maximum heterosis and release of desirable transgressive segregants (Singh and Mishra 2008 and Yadav *et al.* 2009).

A high mean for important traits is fundamental for the selection of superior material, since it is important not only to account for the performance but also their genetic divergences (Palomino *et al*. 2005). The composition of cluster means for different characters showed considerable differences among the clusters for each character. Cluster V observed to be the most imperative with the highest cluster means for majority of the traits namely, pod length, seeds/pod, shelling percentage, pod yield/plant, total biomass, seed yield/plant, harvest index, 100-seed weight, ascorbic acid, protein content and total sugars. Hence, different clusters of genotypes on the basis of means revealed divergence for different characters. The results obtained indicated that in order to get segregating population with possibilities of superiority upon the parents from bi-parents crosses, the

Table 4 Average intra and inter-cluster distances in garden pea germplasm over the years and pooled over years

Clusters	I	II	III	IV	V	VI	VII	VIII
				2009-10				
I	11.05 (3.32)	12.84 (3.58)	13.83 (3.72)	13.56 (3.68)	18.20 (4.27)	17.04 (4.13)	13.11 (3.62)	24.29 (4.93)
II		0.00 (0.00)	6.04 (2.46)	6.20 (2.49)	9.77 (3.13)	23.53 (4.85)	11.22 (3.35)	32.29 (5.68)
III			0.00 (0.00)	7.62 (2.76)	9.52 (3.09)	24.34 (4.93)	11.68 (3.42)	32.68 (5.72)
IV				0.00 (0.00)	9.94 (3.15)	24.89 (4.99)	13.88 (3.73)	32.55 (5.71)
V					10.39 (3.22)	29.40 (5.42)	16.21 (4.03)	37.63 (6.13)
VI						11.95 (3.46)	19.03 (4.36)	15.90 (3.99)
VII							0.00 (0.00)	28.29 (5.32)
VIII								0.00 (0.00)

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
							2010-11						
I	7.60 (2.76)	9.68 (3.11)	8.79 (2.96)	8.97 (2.99)	8.88 (2.98)	10.59 (3.25)	8.80 (2.97)	9.24 (3.04)	9.39 (3.06)	10.85 (3.29)	10.31 (3.21)	10.86 (3.30)	11.94 (3.46)
II		0.00 (0.00)	8.81 (2.97)	11.27 (3.36)	8.21 (2.87)	15.75 (3.97)	13.10 (3.62)	7.27 (2.70)	10.76 (3.28)	12.02 (3.47)	9.56 (3.09)	15.45 (3.93)	15.80 (3.97)
III			0.00 (0.00)	8.21 (2.87)	7.18 (2.68)	11.92 (3.45)	10.55 (3.25)	11.15 (3.34)	12.38 (3.52)	11.51 (3.39)	12.72 (3.57)	12.32 (3.51)	16.32 (4.04)
IV				8.40 (2.90)	10.03 (3.17)	12.33 (3.51)	10.64 (3.26)	10.06 (3.17)	11.36 (3.37)	10.45 (3.23)	12.55 (3.54)	12.15 (3.49)	15.36 (3.92)
V					0.00 (0.00)	10.93 (3.31)	9.99 (3.16)	10.22 (3.20)	11.71 (3.42)	13.57 (3.68)	12.81 (3.58)	11.07 (3.33)	15.18 (3.90)
VI						0.00 (0.00)	7.76 (2.79)	15.27 (3.91)	13.24 (3.64)	14.75 (3.84)	15.13 (3.89)	7.99 (2.83)	12.35 (3.51)
VII							0.00 (0.00)	11.74 (3.43)	10.97 (3.31)	12.43 (3.53)	12.07 (3.47)	10.74 (3.28)	9.99 (3.16)
VIII								0.00 (0.00)	9.24 (3.04)	10.99 (3.32)	9.16 (3.03)	14.66 (3.83)	13.64 (3.69)
IX									0.00 (0.00)	9.72 (3.12)	10.48 (3.24)	13.64 (3.69)	10.04 (3.17)
X										6.94 (2.63)	12.80 (3.58)	15.87 (3.98)	15.03 (3.88)
XI											0.00 (0.00)	16.43 (4.05)	11.24 (3.35)
XII												0.00 (0.00)	14.82 (3.85)
XIII													0.00 (0.00)

Clusters	I	II	III	IV	V
			Pooled over years		
I	3.08 (2.30)	4.16 (1.75)	5.10 (2.04)	4.72 (2.26)	5.30 (2.17)
II		0.00 (0.00)	3.02 (1.74)	5.06 (2.25)	7.74 (2.78)
III			0.00 (0.00)	5.51 (2.35)	8.35 (2.89)
IV				0.00 (0.00)	7.80 (2.79)
V					0.00 (0.00)

Values in bold figures are intra-cluster distances; Values in parenthesis are $\sqrt{D^2} = D$ values genotypes more productive of each clustering should be intercrossed for improving green yield (Ceolin *et al.* 2007).

The contribution of individual character toward diversity (Fig 2) showed that 100-seed weight, pod length and ascorbic acid were the most important characteristic for divergence. Therefore, these traits could also be used as parameters in selecting genetically diverse parents for hybridizing to create variability in the population. On the other hand, earlier reports revealed that early yield/plant (Gupta and Singh 2006), plant height (Kumar *et al.* 2007) and pods/plant (Sharma *et al.* 2009) contributed maximum towards total genetic divergence.

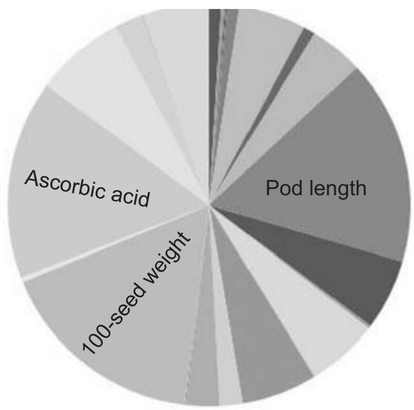

Fig 2 Contribution (%) towards genetic divergence of different characters in pooled years

It is imperative to identify the clusters with the highest genetic diversity. This should be followed by identification of the genotypes in each cluster which are having higher expression for the traits and also complementing for the important traits with the genotypes of the diverse cluster. Accordingly, genotypes namely Punjab 89, DPPM 73, DPPM 74, DPPMFWR 4, DPPMFWR 30-2, Palam Priya and Azad P-1 from cluster I showed higher expression for fresh pod and seed yield along with desirable yield characteristics. These genotypes can be used to complement the important traits with the genotypes of the diverse cluster with maximum inter cluster distance, e.g. DPPMFWR 2, DPP 25G, DPPMFWR 29 and DPPM 64. These genotypes, therefore, will show greater potentiality as a breeding stock and could be used as parents in hybridization for expecting transgressive segregants for further exploitation in garden pea improvement programme (Kumar *et al*. 2007).

It can be concluded that superior lines may be obtained in segregating population from the combinations Palam Priya × DPPM 64/DPP 25G, Punjab 89/DPPM 73 × DPPM 64/ DPPMFWR 29, Azad P-1 × DPPM 64/DPP 25G and DPPMFWR 30-2/DPPMFWR 4 × DPPM 64/DPP 25G.

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