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Kumari ShubhaScientist, Germplasm Evaluation
Division, ICAR-NBPGR, New
Delhi, India**Vikender Kaur**Scientist, Germplasm Evaluation
Division, ICAR-NBPGR, New
Delhi, India**Shri Dhar**Principal Scientist, Division of
Vegetable Science, ICAR-IARI,
New Delhi, India

Genetic diversity assessment in garden pea (*Pisum sativum* L.) germplasm through principal component analysis

Kumari Shubha, Vikender Kaur and Shri Dhar

Abstract

The present study was undertaken to evaluate thirty garden pea genotypes for ten quantitatively yield related traits and *Fusarium* wilt response. Analysis of variance of present study indicates that there is lots of variation with reference to traits of garden pea genotypes which has to be quantified for using in pea improvement programme. *Fusarium* wilt response of different genotypes reported range from 6.5-95.25%. The PCA used to eliminate the redundancy in data set revealed that all the 10 quantitatively measured traits including *Fusarium* wilt response have been loaded on first five components. Based on Ward's cluster analysis, the genotypes were grouped into three genetically diverse clusters.

Keywords: Garden pea, principal component analysis, yield, *Fusarium* wilt, ward's cluster analysis

1. Introduction

Garden pea (*Pisum sativum* L.) is a cool season vegetable crop of the family Fabaceae. It is one of the major, highly valued and extensively cultivated legume crops throughout the world. Globally, it is grown in approximately 5.5 million hectares per year and ranks as the third most important grain legume crop after soybean and common bean (Rana *et al.*, 2017) [14]. Apart from nutritional qualities such as dietary fibres, protein, and micro- and macronutrients (Messina 1999) [12], it also revitalizes soils by fixing atmospheric nitrogen, which offers the benefit of low inputs and sustainable organic farming. The crop is normally grown in the field during mid-October to November in northern plains of the India. Early sown crops (September sowing) fetch high return but the crop is more vulnerable to *Fusarium* wilt (*Fusarium oxysporum* f.sp. *pisi*) due to prevailing high temperature and humidity. *Fusarium* wilt gets more importance in this area due to changing climate state. Rising temperature promote this disease. Therefore it is prime concern to develop variety have high levels of resistance to wilt and agronomic superiority. Morphological traits are mostly influenced by environmental factors, but the importance of these traits cannot be underestimated for analysing the diversity of a crop species as they are the primary constituents of diversity studies. Many earlier researchers have utilized morphological variation to precisely estimate characteristic variability for drawing inferences in pea (Smykal *et al.* 2008; Cupic *et al.* 2009; Sarikamis *et al.* 2010; Gixhari *et al.* 2014) [19, 4]. Knowledge of genetic diversity's extent and the identification, differentiation and characterization of genotypes and populations, respectively, provides an informative tool for the detection of duplicates in the collection, effective extension and better characterization and use in breeding (Hornokova *et al.*, 2003) [8]. In order to benefit transgressive segregation, genetic distance between parents is necessary (Joshi *et al.*, 2004) [10]. The higher genetic distance between parents, the higher heterosis in progeny can be observed (Joshi and Dhawan, 1966; Anand and Murty, 1968) [9, 1]. Similarity/dissimilarity indices and pattern of relationships obtained through genetic diversity and PCA are useful to evaluate potential breeding value of germplasm through traits loaded on various components (Keneni *et al.* 2005) [11]. The present investigation was conducted on thirty genotypes of garden pea (*Pisum sativum* L.) to categorize on the basis of yield contributing traits, *Fusarium* wilt response. The information generated in our study will be useful to optimize the management of pea germplasm and in choosing diverse parental genotypes for future breeding programmes in the pea.

Correspondence**Kumari Shubha**Scientist, Germplasm Evaluation
Division, ICAR-NBPGR, New
Delhi, India

Material and Methods

The present experiment was conducted in research farm of the Division of Vegetable Science, Indian Agricultural Research Institute (IARI), New Delhi India. Thirty lines were evaluated for diversity analysis in field during 2012-13. *Fusarium* wilt screening was done in two consecutive years 2012-13 and 2013-14. The experiment was carried out in a randomized

block design, with thirty treatments, three replications. Seeds of each genotype were sown in first week of October at a spacing of 40 cm × 5 cm apart in 5m² plot size keeping three rows of 4.2 m length each. The crop was fertilized with farmyard manure (10 tonnes/ha) and NPK (20:60:40 kg/ha), followed by recommended cultural practices.

Table 1: List of table of variety/ genotype, their source under study

S. N	Variety/ Genotype	Sources	S.N	Variety/ Genotype	Sources
1	Arkel	IARI, New Delhi	16	GP 473	IARI, New Delhi
2	Pusa Pragati	IARI, New Delhi	17	VL-10	VPKAS, Almora
3	AP 3	IIPR, Kanpur	18	GP-48	IARI, New Delhi
4	VRP-6	IIVR, Varanasi	19	GP-904	IARI, New Delhi
5	PM 65	GBPUA&T,	20	GP-906	IARI, New Delhi
6	PSM 4	GBPUA&T,	21	Arka Ajit	IHR, Bangalore
7	GP 55	IARI, New Delhi	22	GP-910	IARI, New Delhi
8	GP 6	IARI, New Delhi	23	GP-912	IARI, New Delhi
9	GP 17	IARI, New Delhi	24	GP 901	IARI, New Delhi
10	VL-7	VPKAS, Almora	25	GP-914	IARI, New Delhi
11	VP-248	VPKAS, Almora	26	GP-917	IARI, New Delhi
12	VP-525	VPKAS, Almora	27	GP-918	IARI, New Delhi
13	VP-625	VPKAS, Almora	28	VP-266	VPKAS, Almora
14	GP-914	IARI, New Delhi	29	VP-233	VPKAS, Almora
15	GP-1101	IARI, New Delhi	30	GP-1102	IARI, New Delhi

Plant characters and data recording

Agro-morphological observations were recorded for nine yield and yield contributing traits *i.e* day to 50% flowering (D50%F), node bearing first flower (NBF), plant height (PH), number of pod per plant (NPP), pod length (PL), number of seed per pod (NSP), average pod weight (APW), shelling percent (SP) and green pod yield (YLD). Pea genotypes were screened for their level of resistance against *Fusarium* wilt under field conditions in wilt sick plot at Division of Vegetable Science, IARI, New Delhi during cool winters of 2012-13 and 2013-14. The difference between the initial and final plant populations of different genotypes was used to calculate the percent disease intensity, which was characterized as per Charchar and Kraft (1989) [3].

Statistical analysis

Data represent the mean of three replicate samples for each genotype were subjected to analysis of variance as per Gomez and Gomez (1983) [7] for randomized block design. The

Principal Component Analysis based on Pearsons correlation matrix cluster analysis and heat map were performed using a demo version of XLSTAT-Pro (Addinsoft). Correlation biplots of traits were generated on which genotypes were superimposed.

Results

Analysis of variance

The analysis of variance indicated that there was lots of variation exists with reference to different traits among the garden pea genotypes (Table 2). The variance due to treatments (genotypes) were found highly significant for days to 50% flowering (D50F), plant height (PH), number of pod per plant (NPP), shelling percent (SP) and green pod yield (YLD) while significant (*P < 0.05) for node bearing first flower (NBF). However, the traits pod length, number of seed per pod and average pod weight showed non-significant results.

Table 2: Analysis of variance of yield and yield contributing traits of garden pea

Source of variation	d.f	Mean sum of square								
		D50%F	NBF	PH	NPP	PL	NSP	APW	SP	YLD
Replication	2	251.22	0.528	119.35	35.39	0.165	1.68	0.251	18.78	207.29
Treatment	29	584.65**	7.199*	342.94**	7.42**	1.147*	1.29	0.270	41.10**	534.52**
Error	58	19.60	1.906	23.18	3.17	0.180	0.445	0.199	8.30	127.70

(**P < 0.01, *P < 0.05)

Principal component analysis

The principal component analysis (PCA) was performed to assess the variation considering nine yield and yield related traits. Based on factor loadings of these traits, first four components of PCA explained 73.99% of the total variation (Table 5). The first principal component (PC1) was contributing 33.43% of the total variation including number of seed per pod and average pod weight had positive support whereas days to 50% flowering negative involvement.. The principal component second (PC2) accounted for 19.35% of total variation and the variables, node bearing first flowering, plant height, number of pod per plant and yield per plant

included. The third principal component (PC3) explained 12.35% of total variation with positive association by shelling percent. The fourth component, accounting for 8.84% of the total variation, included *Fusarium* wilt response. A 2DA scatter bi-plot of the component scores has been plotted (Fig.1) based on first two components. The plot grouped the samples according to their phenotypic resemblance. For example, line GP-55 reported highest values for the plant height and node bearing first flower and uniquely placed in upper left plane while GP-6 reported highest value for days to 50% flowering and distinctively placed in lower left plane. Similarly genotype GP-910 and GP-912 observed for number

of pod per plant, average pod weight yield per plant and placed close together in the upper right plane. These results demonstrated that number of pod per plant; average pod weight and yield per plant are highly positively correlated.

Similarly plant height and node bearing first flower were highly positively correlated and as a result, these yield contributing traits led to the highest loading factors in this PCA analysis.

Table 3: First four components from the PCA analysis of 9 quantitative yield traits and one *Fusarium* wilt response in 30 Garden pea genotypes

Traits	F1	F2	F3	F4
D50F	-0.774	-0.162	-0.103	-0.106
NBF	-0.476	0.543	0.509	-0.001
PH	-0.438	0.642	0.366	0.212
NPP	0.086	0.753	-0.098	-0.184
PL	0.563	0.106	-0.182	-0.004
NSP	0.862	-0.207	0.166	-0.100
APW	0.776	0.382	0.106	0.010
SP	0.394	-0.190	0.673	-0.528
YLD	0.452	0.628	-0.468	-0.145
FW	0.546	-0.069	0.281	0.696
Eigen value	3.344	1.936	1.236	0.885
Variability (%)	33.437	19.356	12.356	8.846
Cumulative %	33.437	52.793	65.149	73.995

Day to 50% flowering (D50F), node bearing first flower (NBF), Plant height (PH), Number of pod per plant (NPP), pod length (PL), number of seed per pod (NSP), Average pod

weight (APW), shelling percent (SP) and green pod yield (YLD)

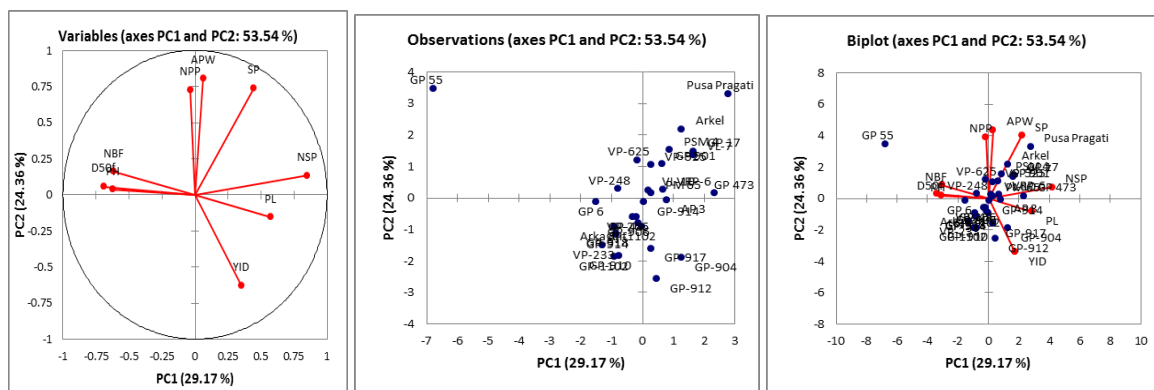


Fig 1: PC1:PC2 plot showing relationship among agro-morphological traits, 2. PC1:PC2 plot showing relationship among thirty garden pea genotypes 3. Biplot for agro-morphological traits activity of thirty garden pea genotypes

Clustering

Ward’s hierarchical cluster analysis was used to see patterns of clustering between the garden pea genotypes. The Euclidean distance was used as a measure of genetic distance. Results shown as Dendrogram) indicated that the different grouping of the varieties by dissimilarity. Using dissimilarity level, thirty pea genotypes based on nine yield contributing traits and *Fusarium* wilt response were classified into three groups. The dendrogram of thirty genotypes showed three clusters (Fig. 2). Cluster I included highest number of genotypes (15) followed by cluster III (13) while Cluster II

have two genotypes. From clustering pattern it was observed Cluster I comprises of 15 early maturing and high yielding genotypes (Arkel, Pusa Pragati, AP 3, VRP-6, PM 65, PSM 4, VL-7, VL-10, GP-904, GP-906, Arka Ajit, GP-910, GP-918, VP-266). All these genotypes are highly susceptible to *Fusarium* wilt. Cluster II included two genotype (GP-6, GP-55) which are distinct genotypes based on *Fusarium* wilt response, late in flowering behavior, maturity time and yield response whereas Cluster III consisted 13 genotypes which are medium in maturity, high yielding and moderate resistant to *Fusarium* wilt.

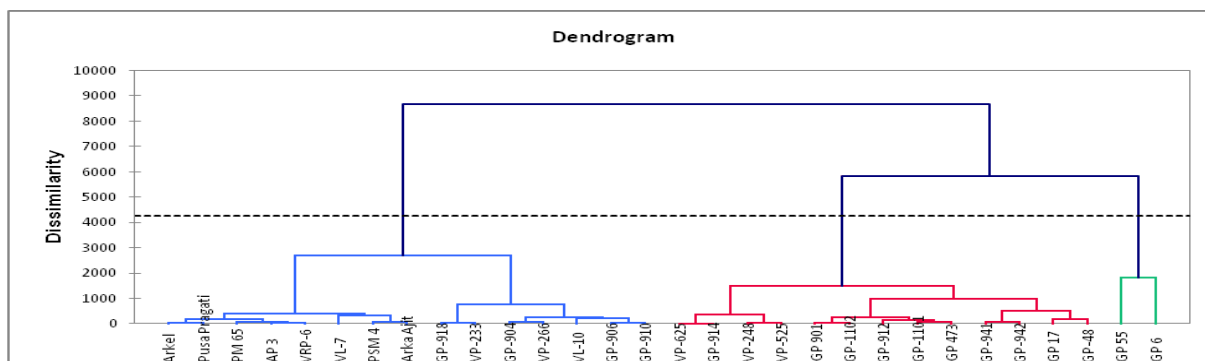


Fig 2: Dendrogram of 30 garden pea genotypes grouping in three clusters.

Fusarium wilt response

The variable reaction of genotypes against *Fusarium* wilt was observed and recorded as wilting percentage (Fig. 3). All the test entries recorded significantly lower disease intensity as compared to the susceptible check Arkel. The line GP-6 recorded the maximum resistant reaction to *Fusarium* as it exhibited the lowest wilting of 6.25% which was followed by the GP-942 (7.50%) and GP-17 (12.00). The line GP-55 showed comparatively slightly high wilting percentage (8.75%) in 2013-14. According to disease severity method, three genotypes were recorded as highly resistant (GP-6, GP-55, and GP-942), four resistant (GP-17, GP-473, GP-48, GP-941), three moderately resistant (EC-677215, EC-677211,

EC-677212), eight susceptible (EC-677213, VP-248, VP-525, VP-625, GP-914, VP266, GP-945, GP-1102) and sixteen were highly susceptible varieties (Arkel, Pusa Pragati, AP-3, VRP-6, EC-677214, EC-677216, Arka Ajit, GP-943 VL-7, VL-10, GP-916, GP-918, GP-917, GP-904, GP-906, GP-910). It is interesting to note that all the agronomic superior commercial varieties (Arkel, Pusa Pragati, AP-3, VRP-6, VL-10 and VL-7) are highly susceptible while highly resistant lines like GP-6 and GP-55 are agronomical poor in performance. Therefore repeated backcross breeding with agronomical superior lines is only scope to utilize resistant gene (s).

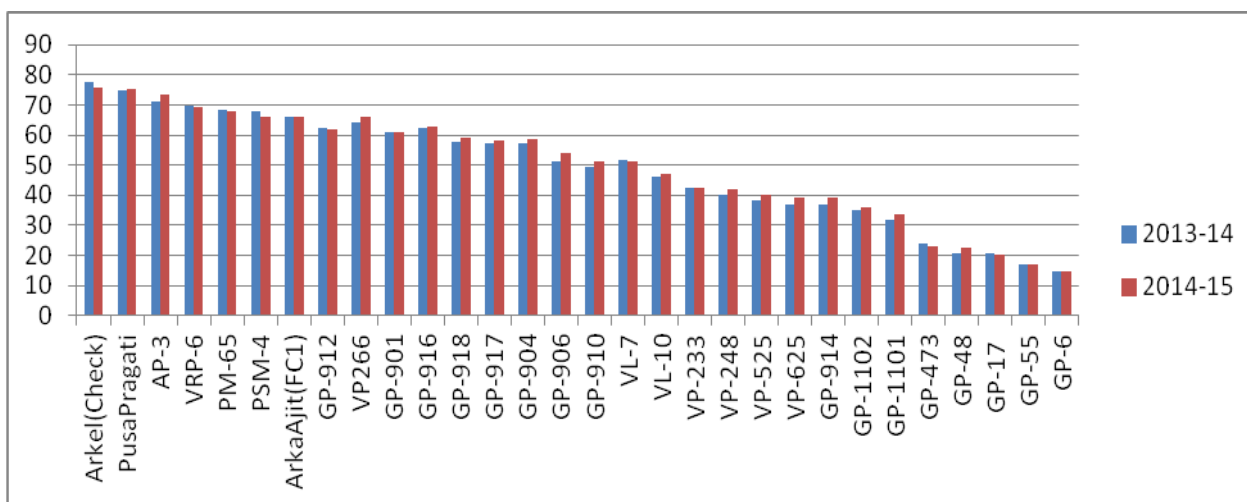


Fig 3: Wilt incidence percentage respond by 30 genotypes in two consecutive years

Discussion

Pea is an important multipurpose crop grown for its fresh succulent vegetables or dried pulse due to its high protein content in the cool temperate zones and tropical highlands of the world (Shubha *et al.* 2015) [18]. The analysis of genetic diversity in set of garden pea genotypes conserved and maintain is important for interpret nature and extent of variability, genetic relationship between traits for the management and use of germplasm (Rana *et al.* 2013) [13].

PCA showed that some traits had the highest loadings in the first two components. These traits included number of seed per pod, average pod weight, node bearing first flowering, plant height, number of pod per plant and yield per plant. These results indicate that such traits are not only useful for the assessment of diversity, but also for characterization of garden pea germplasm. Cluster analysis of the 30 genotypes of garden pea was based on 10 quantitative morphological traits using Tocher's method (Rao, 1952) [15], which was found to be most suitable for classifying them into separate and distinct clusters. Cluster analysis clearly classify 30 genotypes in three distinct class based on yield response, flowering behavior, maturity time and *Fusarium* wilt response. In cluster I consist 15 early maturing and high yielding genotypes (Arkel, Pusa Pragati, AP 3, VRP-6, PM 65, PSM 4, VL-7, VL-10, GP-904, GP-906, Arka Ajit, GP-910, GP-918, VP-266) however all these genotypes are highly susceptible to *Fusarium* wilt. Cluster II included two genotype (GP-6, GP-55) which are distinct genotypes based on highly resistant to *Fusarium* wilt response, late in flowering behavior, moderate to low yield potential, small pod and seed/ pod and late maturity time whereas Cluster III consisted 13 genotypes which are altogether different from

genotypes of other cluster due to medium maturity, high yielding, large attractive pod and genotypes fallen in this category are moderate resistant to highly resistant to *Fusarium* wilt. It was observed that highly resistant genotypes (GP-55, GP-6) present in this set germplasm but they have poor agronomic background (small pods, late maturity and less number of seed per pod) carrying several undesirable gene. However, dominant nature of *Fusarium* wilt resistant gene was reported (shubha *et al.*, 2016) [17]. Therefore repeated backcross breeding with agronomical superior lines is only scope to utilize resistant gene (s). Beside identification of resistance source it is good to obtain added information on the extent of genetic diversity and agronomic performance of resistant germplasm accessions (Ghafoor *et al.* 2005; Smy'kal *et al.* 2008; Ceyhan *et al.* 2008) [5, 19, 2]. The genotypes, which have high levels of resistance and agronomic superiority may reduce the time taken to eliminate the undesirable gene through repeated back crossing by plant breeders (Rana *et al.* 2013) [13].

Conclusion

The study revealed the existence of adequate genetic variation in given set of lines. The pea lines, which were collected from different collection zones were found to cluster together, indicating that differences in eco-geographic origins may not necessarily guarantee existence of genetic diversity in pea genotypes. Beside this, focus was also given to evaluate agronomic performance of lines along with their response to *Fusarium* wilt. Result revealed that most of the lines which were high yielding and superior pod quality (Arkel, Pusa Pragati, AP-3, VRP-6, VL-10 and VL-7) were highly susceptible to *Fusarium* wilt. It was also identified that GP-17

and GP-473 were resistant as well as superior in agronomic performance can be use in wilt affected area. GP-17 is extra early in pod bearing and ready to harvest in 40 days after showing. Highly resistant germplasm lines GP-6 and GP-55 are agronomical poor in performance however repeated backcross breeding with agronomical superior lines is only scope to utilize resistant gene (s).

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