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Principal component analysis for quantitative traits and powdery mildew resistance in pea (*Pisum sativum* L.)

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ABSTRACT

Powdery mildew (PM) caused by *Erysiph episi* is an important fungal disease of pea. Resistance to PM is known to be governed by two independent recessive genes *e1* and *er2* in cultivated peas and by a dominant gene *Er3* in wild peas (*Pisum fulvum* L.). Identification of new resistance sources and introgression of these gene(s) into varietal development is one of the most effective methods to control the disease. In the present study, 50 pea genotypes comprising vegetable and field peas were screened in the field during crop seasons 2012-13 and 2013-14 for seven quantitative characters including, days to 50% flowering, days to pod formation, plant height (cm), number of pods per plant, number of seeds per pod, 100-seed weight (g) and disease severity (%). Estimates of correlation coefficients indicated highly significant but negative associations between AUDPC with days to flowering ($r = -0.448$, $p < 0.001$) and 100-seed weight ($r = -0.622$, $p < 0.001$).Days to pod formation was significantly associated with days to 50% flowering ($r = 0.79$, $p < 0.0001$). Multivariate and regression analysis found, 100-seed weight and days to 50% flowering were the most reliable traits for selection of resistant genotypes. Some potent Indian PM resistant vegetable (VRPMR-9, VRPMR-10, VRPMR-11 and Arka Ajeet) and field (HUDP-5, JP-4, PMR-45 II and VRP-343) pea genotypes have been identified in the present study, that would be used in PM resistance breeding programs.

Keywords: AUDPC, field pea, stepwise regression, trait association, vegetable pea

Pea powdery mildew (PM) caused by *Erysiph episi* is a major fungal disease causing upto 50% reduction in yield by affecting quantity and quality of green pods and dry seeds (Ek *et al*. 2005). It is an obligate parasite invading epidermal cells and it is unique as its haustoria are restricted only to the epidermal cells devoid of chloroplasts and the development of the pathogen solely depends on the photosynthetic activity of the underlying mesophyll cells (Carver and Jones 1988). PM is mainly prevalent in tropical and sub tropical regions characterized by warm humid weather conditions. The disease is more prevalent at flowering and pod formation stage (Ghafoor *et al*. 2012). The initial symptoms appear as white powdery mycelia and spores on leaf and stem surfaces. With the advancement of the disease, entire aerial portion of the pea plant are covered with white floury patches. Although disease is reported to be controlled by chemical (Rajappan and Yesuraja 2000), but genetic resistance is the most effective, economic and environment friendly method to control powdery mildew disease (Cao *et al*. 2011). Identification of resistance sources and their incorporation into modern cultivars remains the most effective method of controlling the disease (Katoch *et al*. 2010).

Since, most of the available resistant sources showed variable reaction therefore there is a need to identify/ reaffirm some potent PM resistant genotypes through stringent selection criterion. Keeping in view, the present study was undertaken to quantify the variability for PM resistance in field and vegetable peas, so that the information generated could be used in selection of genotypes with high level of resistance and there subsequent use in pea PM resistance breeding programs.

MATERIALS AND METHODS

Plant material: Fifty pea genotypes (Table 1) were screened under field conditions in a randomized block design with three replications during the cropping season of 2012-13 and 2013-14 at the Agricultural Research Farm, Banaras Hindu University, Varanasi, India. Each genotype was grown in a two meter long row with inter and intra-row spacing of 30 and 10 cm, respectively. One row of PM highly susceptible pea genotype 'PG-3' was planted after every five rows of test genotypes. Five plants were randomly chosen from test genotype in each replication and observations were recorded on seven quantitative characters *viz*., days to 50% flowering (days), days to pod formation (days), plant height (cm), number of pods per plant, number of seeds per pod, 100-seed weight (g) and disease severity (%).

Assessment of powdery mildew severity: Fifty pea genotypes were inoculated at the flowering stage with the pathogen causing powdery mildew of pea (*E. pisi*) by tapping the conidia from the leaves on the young disease free leaves of pea plant during evening time (Lim1973). Severity of disease was scored visually on individual plants using a 0 to 9 scale based on percent of foliage covered with hyphae (Warkentin *et al*.1995), when PM severity on the susceptible genotype PG-3 reached to 20%.Warkentin *et al.* (1995) suggested that scores of 0-40 AUDPC (0-4

disease severity score) are considered resistant and those having 50-90 AUDPC (5-9 disease severity score are considered as susceptible ones. Five plants from each row were tagged before the expected appearance of disease. Powdery mildew severity was recorded at seven different dates at an interval of two days. These values were then used to calculate the AUDPC as per the formula given by Shaner and Finney (1977):

$$AUDPC = \sum [(Y_i + Y_{i+1})/2] (t_{i+1} - t_i)$$

Where, Y_i is the disease level at time t_i and

$(t_{i+1} - t_i)$ is time (days) between two disease scores.

Data analysis: Analysis of variance (ANOVA) for different parameters was performed using PROC GLM of SAS (version 9.2; SAS Institute Inc. Cary NC 2010). Correlation coefficients among different parameters were calculated using PROC CORR function of SAS. Principal component analysis was performed by PROC PRIN COMP of SAS. In addition, stepwise multiple regression analysis was conducted using AUDPC as response variable and the other six quantitative traits *i.e.,* day to flowering, days to pod formation, plant height, number of pods per plants, number of seeds per pod, 100-seed weight, as predictor variables.

RESULTS AND DISCUSSION

In India, pea powdery mildew usually appears during January to the end of February when the crop is in the flowering or pod formation stage when coincides with a warmer and humid weather, which favors growth, reproduction and spread of the powdery mildew pathogen. The losses caused by powdery mildew in pea are more when the crop is grown for seed purpose as it reduces both the number and size of seeds (Mert- Turk *et al*. 2008) because disease severity level increases towards maturity (Ahmad *et al*. 2001).

Variability of different phenotypic traits: In the present study, a considerable range of variations was found for AUDPC and other quantitative traits among the fifty pea genotypes (Table 1). The days to 50% flowering was shortest in VRP 130 (50.25) and was longest in EC 313635-II (80.50) whereas days to pod formation ranged between 60.75 (VRP 249; a PM susceptible genotype) to 88.00 (KPMR 536; a PM resistant genotype). Plant height of the genotypes ranged from 25.50 (PMR 44) to 111 cm (EC 324108 II). The mean number of pods per plant ranged from 2.25 in the case of PM susceptible genotype VRP 249 to 26.00 in a PM resistant genotype EC 328747, whereas the number of seeds per pod ranged from 2.15 (EC 322748 I; PM susceptible genotype) to 7.67 (EC 341753 II; PM moderately susceptible genotype). The 100-seed weight varied from 11.32 g in susceptible genotype EC- 341753 -I to 23.00 g in resistant genotype KPMR- 642. The AUDPC varied from 88.97 in susceptible genotype (AP-1) to 2.45 in resistant genotype (HUDP-5). Kujur *et al.* (2014) performed multivariate analysis among 191 pea genotypes for yield traits and reported that a mean pods per plant 17.92 varies from 10 to 26.67 whereas, 100-seed weight varied from 14.32 to 26.69g with an overall mean of 19.59g.

In the present study, wide variation among the pea genotypes for PM resistance suggested genetic makeup of genotypes owing to different resistance alleles (Banyal *et al.* 2005, Fondevilla *et al.* 2007; Ghafoor *et al.* 2012). Among 50 genotypes evaluated in the present experiment, 22 were powdery mildew resistance (d"40 AUDPC), whereas 28 were powdery mildew susceptible (>60 AUDPC). AUDPC values of 50 pea genotypes showed wide range of variation (6.70 to 88.80) and clearly separated the pea genotypes with high level of resistance. AUDPC has been used successfully to evaluate the progress of disease on different crops (Jeger *et al.* 2001, Skelsey *et al.* 2014). In general, the genotypes with higher AUDPC (more susceptible) had lower 100-seed weight(HSW)*e.g.*, AP-1 (AUDPC 88.97 and HSW 16.0 g), EC-32874 II (AUDPC 86.67 and HSW 12.57 g), EC-322745 (AUDPC 84.40 and HSW 12.12 g) and *vice-versa i.e.*, genotypes with lower AUDPC (more resistant) showed higher 100-seed weight e.g., KPMR-642 (AUDPC 11 and HSW 23 g), JP-4 (5.5 AUDPC and HSW 20.10 g) and PMR-38 (AUDPC 21.5 and HSW 21.17 g when the 100 seed weight of susceptible genotypes were compared with resistant genotypes, 21.55 % of reduction in seed weight was observed in case of susceptible genotypes on mean basis.

Interrelationships and multiple regression analysis: Estimates of correlation coefficients among seven quantitative traits are presented in Table 2. Days to pod formation was significantly associated with days to 50% flowering ($r = 0.79$, $p < 0.0001$). Number of pods per plant was significantly associated with 100-seed weight ($r = 0.35$, $p < 0.01$). Similarly, number of seeds per pod was negatively associated with two yield attributes namely, days to flowering ($r = -0.31$, $p < 0.01$) and days to pod formation ($r = -0.30$, $p < 0.01$). However, negative but highly significant associations were found between AUDPC with days to flowering ($r = -0.448$, $p < 0.001$) and test weight ($r = -0.622$, $p < 0.001$). Kujur *et al.* (2014) also reported a significant positive correlation of pods per plant with plant height and 100-seed weight.

Stepwise regression of AUDPC with other six quantitative characters contributing to yield was estimated in 50 pea genotypes considering AUDPC as dependent variable and other six characters as estimator variables. Days to 50% flowering (DTF) alone explained approximately 39% ($R^2 = 0.386$) of variation in AUDPC, while days to 50% flowering (DTF) and 100-seed weight (HSW) together explained 55% ($R^2 = 0.547$) of variation for predicting AUDPC. Analysis indicated that DTF and HSW play significant role in deciding AUDPC in pea as predictor variables. The model fitted for AUDPC in this study is as follows:

Table 1. Mean of seven quantitative traits in 50 pea genotypes.

S. No.	Genotype	Type of pea	DF	DP	PH	NOP	NOS	HSW	AUDPC
1.	EC-324705	field	61.00	79.00	75.05	6.85	3.62	17.37	81.55
2.	EC- 322745	field	66.25	78.00	55.15	15.62	3.40	12.12	84.40
3.	EC- 324107	field	64.50	78.00	54.77	4.25	3.30	12.95	75.60
4.	EC- 313635 I	field	65.75	79.25	80.50	12.57	4.00	11.67	69.27
5.	JP-625	field	61.50	80.00	88.78	12.25	3.38	13.12	66.45
6.	KPMR- 642	field	66.75	80.00	72.25	4.87	3.35	23.00	11.00
7.	EC- 341753 II	field	65.75	82.00	72.45	9.37	7.67	11.32	72.45
8.	Kashi Shakti	vegetable	59.50	71.00	42.00	5.52	5.17	18.25	88.08
9.	EC- 324108 II	field	60.00	79.25	111.00	8.00	3.15	12.90	82.60
10.	IPF-9728	field	63.00	76.25	79.75	10.20	3.32	14.12	67.85
11.	EC- 328747	field	66.00	77.25	91.50	26.00	3.52	11.47	83.27
12.	EC-328752 II	field	67.00	80.00	90.25	8.60	2.37	13.90	77.20
13.	PC- 531	vegetable	62.50	79.75	69.47	2.75	3.90	14.70	60.03
14.	KPMR-497	field	78.50	84.25	31.67	7.00	2.87	16.80	17.00
15.	KPMR- 557	field	71.75	77.00	67.85	8.25	3.40	19.65	17.50
16.	JP-4	field	65.25	77.25	88.05	7.00	3.82	20.10	5.50
17.	EC-262157	field	70.75	79.00	61.90	13.00	2.82	15.60	73.50
18.	VKG- 28157	field	71.25	79.00	56.50	7.15	3.40	16.15	12.75
19.	VRPMR- 11	vegetable	65.50	75.75	63.82	3.90	4.00	15.25	7.25
20.	EC- 318760	field	67.25	77.00	35.45	6.30	2.27	14.52	81.47
21.	EC- 328742	field	67.75	77.00	68.75	11.25	2.85	14.32	85.37
22.	KPMR- 516	field	69.50	78.25	66.25	6.80	3.05	18.92	18.50
23.	KPMR-619	field	71.50	81.00	53.85	12.50	4.20	19.17	16.35
24.	PMR-45	field	68.50	75.25	42.45	16.75	3.57	13.37	74.17
25.	VRPMR-9	vegetable	77.25	84.00	69.52	4.00	3.37	14.82	18.25
26.	VRPMR-10	vegetable	79.50	86.75	37.25	9.25	3.67	17.92	12.25
27.	HUDP-5	field	69.00	77.00	35.90	9.75	3.35	18.25	2.45
28.	DDR-56	field	69.50	78.00	50.65	4.50	3.35	19.30	4.25
29.	EC-318760	field	69.50	80.25	28.47	10.37	3.27	13.67	71.37
30.	EC- 328742 II	field	62.50	77.25	75.15	16.00	4.60	12.57	86.67
31.	EC- 328773 II	field	69.00	77.50	81.87	19.75	3.52	12.50	75.85
32.	AP-1	vegetable	64.75	81.25	65.00	2.50	4.00	16.00	88.97
33.	Pant Upahar	vegetable	65.50	79.75	42.62	8.25	3.55	16.95	59.27
34.	EC-328753	field	69.75	81.00	41.40	5.75	2.85	14.70	75.85
35.	VRP-12-1	vegetable	66.75	77.25	49.85	6.15	3.35	17.20	86.85
36.	VRP-130	vegetable	50.25	64.75	54.75	8.10	4.17	16.80	83.07
37.	ArkaAjeet	vegetable	69.75	77.50	55.00	9.50	3.27	16.95	12.50
38.	EC-324121 II	field	69.75	77.25	54.97	13.00	3.30	17.85	9.50
39.	PMR-45 II	field	69.75	78.25	83.32	9.87	3.00	17.62	6.50
40.	IPF-400	field	72.00	80.75	66.50	8.90	3.12	20.95	11.50
41.	EC-322748 I	field	63.00	81.00	50.15	9.25	2.15	20.02	83.85
42.	KPMR-526	field	79.50	88.00	36.22	3.00	2.25	16.92	16.00
43.	IPF-17	field	70.00	81.25	71.50	9.70	2.87	21.20	13.25
44.	PMR-44	field	71.75	81.00	25.50	6.75	4.45	19.20	23.75
45.	PMR-38	field	71.25	81.25	70.97	9.20	3.32	21.17	21.5
46.	EC-209105	field	78.25	87.25	64.90	12.50	2.87	14.22	73.72
47.	EC- 313635 II	field	80.50	87.50	85.77	2.75	3.07	12.32	63.42
48.	VRP-3	vegetable	63.00	66.00	53.40	8.00	4.47	17.50	83.65
49.	VRP-343	vegetable	63.00	65.50	58.87	5.65	4.45	17.65	6.75
50.	VRP- 249	vegetable	50.50	60.75	32.15	2.25	4.07	16.90	64.70
	Min.		50.25	60.75	25.50	2.25	2.15	11.32	2.45
	Max.		80.50	88.00	111.0	26.00	7.67	23.00	88.97
	Mean		67.65	78.37	61.22	8.83	3.52	16.24	49.70
	LSD (0.05)		2.22	0.48	0.92	3.06	0.75	1.23	2.60

DF= days to 50% flowering; DP= days to pod formation; PH= plant height (cm); NOP= number of pods per plant; NOS= number of seeds per pod; HSW= 100-seed weight (g) and AUDPC= area under disease progress curve.
Data averaged over two years (2012-13 and 2013-14) of pea powdery mildew screening under field conditions.

AUDPC = 296.11 + (-6.62 × 100-seed weight) + (-2.03 × Days to 50% flowering)

Principal component analysis: Relationships between different quantitative traits were revealed by Principal Component Analysis (PCA). For each trait, factor loading of more than 0.56 was considered as significant (Table 3). According to principal component analysis, the four principal components (PC) exhibited more than 0.80 Eigen

Table 2. Correlation coefficient among seven quantitative traits in 50 pea genotypes.

Trait	DF	DP	PH	NOP	NOS	HSW
DP	0.79***					
PH	-0.10	0.11				
NOP	0.03	0.01	0.25			
NOS	-0.31*	-0.30*	0.01	0.01		
HSW	0.08	-0.07	-0.26	0.35*	-0.14	
AUDPC	-0.44**	-0.17	0.12	0.24	0.09	-0.62***

***significant at p < 0.0001; **significant at p < 0.001; *significant at p < 0.01.

DF= days to 50% flowering; DP= days to pod formation; PH= plant height (cm); NOP= number of pods per plant; NOS= number of seeds per pod; HSW= 100-seed weight (g) and AUDPC= area under disease progress curve.

Table 3. Eigen value, cumulative variance and scores of the four major factors obtained from the PCA of seven quantitative traits performed on 50 pea genotypes.

Variable	PC1	PC2	PC3	PC4
Eigen value	2.30	1.79	0.89	0.84
% cumulative variance	32.98	58.55	71.40	83.42
Days to 50% flowering	0.80**	0.46	-0.07	0.25
Days to pod formation	0.63**	0.44	-0.07	0.19
Plant height	-0.26	0.48	0.71**	-0.21
Number of pods per plant	-0.31	0.58**	0.21	-0.02
Number of seeds per pod	-0.46	-0.27	0.24	0.79**
100-seed weight	0.56	-0.64**	0.27	-0.20
AUDPC	-0.74**	0.32	-0.43	-0.11

**Significant at p < 0.001 (Significant factor loading was observed above 0.56).

value and explained about 83.42% of total variation, and hence were taken into consideration. First principal component (PC1) was the most important and explained about 32.98% of total variance. Important loading factors for PC1 were days to 50% flowering, days to pod formation and AUDPC with loading value of 0.80, 0.63 and -0.74, respectively. PC1 was positively influenced by days to 50% flowering and days to pod formation whereas, negatively influenced by AUDPC. The second PC contributed 25.57 % of the variation among the genotypes. PC2 was positively defined by number of pods per plant and negatively influenced by 100-seed weight. The third principal component (PC3) accounted for 12.94% of total variance and was positively influenced by plant height (loading value 0.71). The fourth principal component (PC4) accounted for 12.02% of total variation explained and positively defined by number of seeds per pod. A number of workers have been done PCA in peas and other related legumes for yield and component traits (Jha *et al.* 2012; Parihar *et al*. 2013; 2014, Kujur *et al.* 2014). Parihar *et al*. (2014) revealed significant variation among traits studied where seven major principal components explained about 90% of total variation in field peas. Similarly, Kujur*et al.* (2014) reported that three principal components (PCs) explaining 67.88% of the total variation for seven quantitative traits (plant height, pods per plant, test weight, yield per plant, lodging score, stem diameter and linear stem density) in 191 diverse pea germplasm.

CONCLUSION

On the basis of principal component analysis of quantitative traits and powdery mildew resistance in pea, the present study identified a number of field (*e.g.*, HUDP-5, JP 4, PMR 45 II and VRP 343) and vegetable pea (*e.g.*, VRPMR 9, VRPMR 10, VRPMR 11 and Arka Ajeet) genotypes which are resistant to PM as well as potent in other yield attributing traits and thus, would be used in PM resistance breeding programs

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