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CHARACTERIZATION OF RESISTANCE RESPONSE OF GARDEN PEA (*Pisum sativum* L.) AGAINST POWDERY MILDEW (*Erysiphe pisi* DC) IN SUB-TROPICAL PLAINS OF INDIA

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SUMMARY

In field conditions, the level of plant responsiveness to powdery mildew under severe disease incidence was assessed during reproductive phase of the crop and expressed as Percentage Disease Index (PDI) and Disease Incidence (DI). For PDI severity index, 26.7% genotypes were observed to be resistant (21 highly resistant and 6 resistant) whereas, 9.9% were tolerant to powdery mildew pathogen. The results of PDI were highly correlated with obtained DI values (r = 0.95; P < 0.01). The genotypes VRPMR-9, VRPMR-11, Punjab-89, PMR-62 along with 17 other genotypes were found to be immune while maximum disease incidence was observed in genotype IC-36 (PDI = 87.3%). In the detached leaf assay, plants were evaluated for days to pustule formation. Detached leaf and field results were comparable except for genotypes JP-15, JP-501A/2, JP-825, Arka Ajit, VP-233 and Vasundhra. There was a highly significant and positive correlation between laboratory (detached leaf assay) and field condition results for powdery mildew resistance in garden pea (r = 0.83; P < 0.05) on the basis of PDI values. The overall results show the utility of detached leaf assay for screening garden pea for powdery mildew resistance. No disease reaction or slow disease development of powdery mildew in number of garden pea genotypes proved their potential for resistance breeding.

Key words: Detached leaf assay, field screening, garden pea, powdery mildew, resistance

Key findings: Immune genotypes for reaction to powdery mildew identified in this study can be utilized by breeders for resistance breeding program of garden pea.

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INTRODUCTION

Powdery mildew disease caused by the biotrophic ascomycete fungus *Erysiphe pisi* DC is most important disease affecting production of garden pea (*Pisum sativum* L.) in Punjab and north-western states of India (Geddes and Iles, 1991). The disease become prominent in climates with warm, dry days and cool nights (Sillero *et al.,* 2006) and limits the total yield, number of pods per plant, number of seeds per pod, plant height and number of nodes (Gritton and Ebert, 1975). The specific losses due to powdery mildew infection leads to 24-27% reduction in pod weight, 21-31% in pod number and 25-86% in total yield (Munjal *et al.*, 1963; Reiling 1984; Warkentin *et al.*, 1996; Nisar *et al.*, 2006). Various fungicides like

hexaconozole, penaconazole and wettable sulphur are used to control powdery mildew infection (Surwase, 2009) but the air borne nature and fast conidial multiplication renders fungicidal control ineffective. Moreover, indiscriminate use of these fungicides created resistance in pathogens and results in environmental threats affecting human and animal health (Singh and Singh, 1983). Thus the most efficient, economical and ecological strategy to mitigate the effect of this disease seems to be the use of resistant cultivars. Earlier worldwide attempts are made to locate the source of resistance to powdery mildew and different sources with complete or incomplete resistance to *E. pisi* have been described (Heringa *et al.,* 1969; Mathur *et al.,* 1992; Thakur *et al.,* 1996; Nisar *et al.,* 2006; Fondevilla *et al.,* 2007a). Three genes *er1*, *er2* (Heringa *et al.,* 1969) and *Er3* (Fondevilla *et al.,* 2007b) carrying resistance to powdery mildew have been identified. The presence of gene *er1* is reported in many pea accessions (Tiwari *et al*,, 1997a) but even with the absence of specific virulent races of *E. pisi*, breakdown in resistance against *er1* gene had been reported (Schroeder and Providenti, 1965; Tiwari *et al.,* 1997b). The expression of *er2* gene had found to be dependent on temperature, growing conditions (field vs. green house) and leaf age (Tiwari *et al.,* 1997a; Fondevilla *et al.,* 2006). The gene *Er3* is present in wild *Pisum* species, *Pisum fulvum* and was reported to be monogenic and dominant in resistance, expressed as post penetration hypersensitive response, however the interspecific crossing lead to sterile F_1 hybrids (Fondevilla *et al.,* 2007b and 2008). General experience shows that single gene resistance control is ephemeral because of the evolution of pathogen virulence. Pathogens such as the powdery mildew fungi pose a particular threat because they combine a regular but infrequent sexual cycle with high rates of asexual reproduction and spore dispersal. As far as cultivated *Pisum* is concerned, the phenomenon of resistance is specific to particular agro-climatological zones or environmental conditions. Therefore, new sources of host resistance to *E. pisi* are needed to control the powdery mildew disease in pea.

Considering the high potential of pea production in India, a study was conducted to identify and characterize the pea germplasm for its reaction to powdery mildew. The error free screening of pea germplasm for disease under natural conditions is difficult especially when the pathogen development is weather dependent especially temperature, as in the case of *E. pisi* (Banyal and Tyagi, 1997). Therefore, in this study, resistance against *E. pisi* was characterized under field and controlled conditions (detached leaf assay), in pea genotypes differing in resistance to *E. pisi*.

MATERIALS AND METHODS

Plant materials

One-hundred and one garden pea (*Pisum sativum* L.) germplasm comprising breeding lines, accessions and cultivated varieties were selected from germplasm collection of 200 lines on the basis of yield performance, which were collected from different agro-climatological zones of India or internationally with known and unknown reaction to powdery mildew for their use as test material in present investigation (Table 1). PB-89, Angoori and AP-3 are commonly grown varieties in India.

Field experiments

During the 2008-09 growing season, pea germplasm was sown at Vegetable Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana (30°55' N and 75°54' E) to identify the powdery mildew susceptible lines for use as spreader or infector lines and to determine the number of rows of a test lines that can be surrounded by susceptible lines to ensure high and uniform disease pressure on the test line plants (*data not shown*). The 2 cultivars, Lincoln and Bonneville were identified for use as spreader and as control in the experiment. Test lines were sown for 2 consecutive years during growing season in 2009-10 and 2010-11 on 12 November and 4

Table 1. List of garden pea germplasm used in the study.

No.	Genotypes	Area of Geographic Distribution/Source	No.	Genotypes	Area of Geographic Distribution/Source	No.	Genotypes	Area of Geographic Distribution/Source
1.	Arkel	Ludhiana	35.	JP 19	Jabalpur	69.	PMVAR 5	AICRP*
2.	AP-1	Kanpur	36.	JP 20	Jabalpur	70.	PHPMR 1	Hisar
3.	AP-3	Kanpur	37.	JP 62	Jabalpur	71.	VRP 5	Varanasi
4.	AC Tomour	USA	38.	JP 141	Jabalpur	72.	VRP 6	Varanasi
5.	Alaska	USA	39.	JP 625	Jabalpur	73.	VRP 7	Varanasi
6.	Arka Ajit	IIHR, Bangalore	40.	JP 825	Jabalpur	74.	VRP 22	Varanasi
7.	Angoori	Doctor Seeds Ltd.	41.	JPBB 4	Jabalpur	75.	VRP 4	Varanasi
8.	Aryaveer	KS Seeds Ltd.	42.	KS 257	Kalyanpur	76.	VRP 8	Varanasi
9.	Bilaspuri Lincoln	Bilaspur	43.	KS 205	Kalyanpur	77.	VRPMR 9	Varanasi
10.	CHP-I	Rahuri	44.	KS 268	Kalyanpur	78.	VRPMR 11	Varanasi
11.	C-96	New Zealand	45.	Kinnauri	H.P.	79.	VL 8	Almora
12.	C-308	New Zealand	46.	KS 210	Kalyanpur	80.	VP 5	Almora
13.	C-400	New Zealand	47.	LPF 48	Ludhiana	81.	VP215	Almora
14.	C-778	New Zealand	48.	PB. 87	Ludhiana	82.	VP 8902	Almora
15.	CHPMR-II	Ranchi	49.	PB. 88	Ludhiana	83.	VP 433	Almora
16.	CHP-II	Rahuri	50.	PB. 89	Ludhiana	84.	VP 434	Almora
17.	Darl 104	AICRP*	51.	PSM 3	Pantnagar	85.	Vasundhra	Tycoon Seeds Ltd.
18.	DGP 207	Durgapur	52.	PM 65	Pantnagar	86.	Sel-AB	Ludhiana
19.	DGP 19	Durgapur	53.	PM 69	Pantnagar	87.	UN-53-6	IARI, New Delhi
20.	DAP II	N/A	54.	PEW 9	USA	88.	KTP 8	N/A
21.	E-1	Ludhiana	55.	PMR 4	Pantnagar	89.	DPP 68	Palampur
22.	E-4	Ludhiana	56.	PMR 19	Pantnagar	90.	Tara	USA
23.	Garry Field	USA	57.	PMR 20	Pantnagar	91.	Little Marvel	USA
24.	GS 10	Golden seeds	58.	PMR 53	Pantnagar	92.	Prachi	Nath Seeds Ltd.
25.	HUVP 3	Varanasi	59.	PMR 62	Pantnagar	93.	RE 89	Hindustan Seeds
26.	HUVP 4	Varanasi	60.	PMR 69	Pantnagar	94.	NDVP 8	Faizabad
27.	IC 312269	NBPGR, Delhi	61.	PS 8	Hippar	95.	NDVP 10	Faizabad
28.	IP 3	Pantnagar	62.	PS 11	Hippar	96.	VP 233	Almora
29.	JP 501 A/2	Jabalpur	63.	PS 19	Karnal	97.	MA-6	Ludhiana
30.	JP 179	Jabalpur	64.	PS 24	Panchkula	98.	10-6-A	Ludhiana
31.	JM 1	Jabalpur	65.	PMVAR 1	AICRP*	99.	NDVP 104	Faizabad
32.	JM 5	Jabalpur	66.	PMVAR 2	AICRP*	100.	VP 316	Almora
33.	Jagatpur	Jagatpur	67.	PMVAR 3	AICRP*	101.	IC 36	Austria
34.	JP 15	Jabalpur	68.	PMVAR 4	AICRP*			

*AICRP: All India Coordinated Research Project (vegetables); N/A - Not available

November respectively at spacing of 60 x 10 cm in field comprising a row of 3.5 m length for each genotype. Each genotype was represented by 3 replications in a randomized block design. The susceptible varieties, Lincoln and Bonneville were placed at 5 rows interval among the test genotypes and a spreader row of Lincoln plants was placed around all sides to ensure high and uniform level of powdery mildew infection. Three rows of each control (Lincoln and Bonneville) were also planted on one side of experiment. The crop was sown with all standard agronomical practices but no fungicidal spray was given throughout the cropping season. Plants were irrigated once a week to increase humidity in order to provide a favorable environment for powdery mildew infection. The soil was Gangetic alluvial (entisol) with a sandy clay loam texture, pH 8.5, organic carbon 0.18%, available N: 240 kg ha^{-1}, available P: 13.6 kg ha^{-1} and available K: 75 kg ha^{-1} at the time of initiation of the experiment. Recommended doses of inorganic N as urea (110 kg $ha^{-1}year^{-1}$), inorganic P as P_2O_5 (62 kg $ha^{-1}year^{-1}$) were applied. Besides recommended N dose, N as urea @ 200 kg ha^{-1} in 4 equal splits was also applied to ensure heavy level of powdery mildew infection. Screening was carried out when the weather conditions were most suitable for disease development and disease was at peak period i.e. during pod development stage (in mid-February). Natural disease infection was recorded on all the genotypes for 2 years (2009-10 and 2010-11) following the 0-9 scale (Saari and Prescott, 1975).

Growth chamber experiments

The lines conferring resistance to powdery mildew under field conditions for 2 consecutive seasons were further taken for a detached leaf assay in plant growth chamber under controlled conditions of temperature and light. For the detached leaf assay, the fifth leaf from the third node below the apex from 40 day old greenhouse grown plants was excised and placed adaxial surface up on cotton sheets in petri dishes containing 6 ml of 5% sucrose solution as described by Warkentin *et al.* (1995). Inoculum was prepared by inoculating the disease free leaves of highly susceptible cultivar i.e. Lincoln (by dusting conidia onto detached leaves in petri dishes from young leaflets that were 50-100% covered with powdery mildew using camel hair brush). Finally the inoculation of test material was done using a settling tower to give a density of about 5 conidia/mm^2. After inoculation petri dishes were wrapped with para-film and incubated in growth chamber (COTTOR NSW) at day/night temperature of 16 ±2 ^oC/20 ±2 ^oC under a photoperiod of 16 h light and 8 h dark with light intensity of 140 µmol m^{-2} s^{-1} (supplied by high intensity fluorescent light) and 85% relative humidity in plant growth chamber. Petri dishes containing inoculated leaves of each genotype were arranged in the incubator in a completely randomized design with 3 replications (3 plates per genotype, 2 leaves per plate). Observations on inoculated detached leaves commenced 3 days after inoculation and continued for 12 days. The development of powdery mildew hyphae on leaves was assessed visually and under a dissecting microscope for recording, and scored using a 0-9 scale based on percentage of foliar area affected as PDI.

Disease assessments

The percentage of leaf area affected by powdery mildew was assessed visually on a 0 (resistant) to 9 (susceptible) following the 0-9 scale of Saari and Prescott (1975) (Table 2). Disease Incidence (DI) was calculated using the formula:

$$DI\ (\%) = \frac{\text{Number of infected leaves on the main branch}}{\text{Total number of leaves on the main branch}} \times 100$$

Percentage disease index (PDI) was calculated for each genotype using the formula:

$$PDI\ (\%) = \frac{\text{Sum of all ratings}}{\text{Maximum disease grade x Total number of observed plants}} \times 100$$

The various garden pea genotypes were classified for their reaction to powdery mildew on the basis of their PDI percent as: PDI 0% = highly resistant, PDI 0.1-10% = resistant, PDI 10.1-30% = moderately resistant, PDI 30.1-50%

Table 2. Scale used for scoring of powdery mildew infection.

Scale Used/leaf area affected	Remarks
0 = 0%	Absolutely free from any pustules of powdery mildew.
1 = 0.1-5%	One or 2 pustules on few leaves
2 = 5.1-10%	Few pustules on some leaves
3 = 10.1-17%	Few isolated pustules on most of the leaves
4 = 17.1-25%	Many pustules on most of the leaves
5 = 25.1-50%	Many pustules coalescing to each other
6 = 50.1-75%	Coalescing pustules on almost whole plant
7 = 75.1-90%	Almost uniform powdery growth covering leaves and pods
8 = 90.1-95%	Uniform powdery growth without any conspicuous pustules on the leaves, pods and stem
9 = 95.1-100%	Whole plant covered with powdery mass giving light greyish white appearance leading to premature drying of plants

= moderately susceptible, PDI 50.1-75% = susceptible, PDI 75.1-100% = highly susceptible.

Statistical analysis

Analysis of variance of all the parameters was performed using Excel software and the means were separated by Fisher's least significant difference at 5% level of significance. Because of heterogeneity in the variances was observed in the data from screening methods, the data were arcsine-square root transformed before analysis. Disease scores were averaged across the replicates, and seasonal results for each genotype and resulting mean scores were used in Pearson's correlation to compare qualitative and quantitative indices.

RESULTS

Field screening

The field screening for powdery mildew resistance for 2 years (2009-10 and 2010-11) under natural epiphytotic conditions revealed a uniform trend of powdery mildew infection for both the parameters with sufficient variation (PDI: 87.3-fold; DI: 95.6-folds) in disease reactions across the germplasm (Table 3). All of the 101 tested genotypes were significantly different ($P < 0.05$) for their reaction to *E. pisi*. The mean PDI was found to be 37.8% (Table 3), compared to a PDI of zero for Punjab-89, Angoori, Alaska, JP-141, PMR-62, VRP-22 and 15 other highly resistant genotypes (Table 4). Besides, 6 other genotypes namely VP-233 (PDI: 0.8), JM-5 (4.1), JP-501A/2 (4.9), E-4 (5.3), Vasundhra (8.3), JP-825 (8.8) gave resistant response to powdery mildew (Table 4). Therefore, among the germplasm sources, 20.7% of the genotypes were found to be highly resistant (PDI = 0) and 5.9% were found resistant (PDI = 1-10%) to *E. pisi* infection. The range of PDI of genotypes classified as resistant (PDI = 1-10%) had 4.2 to 47.3-folds lower disease reactions than the mean PDI value (37.8) of the population. The 10 other genotypes namely, GS-10, JP-179, JM-1, JP-19, JPBB-4, Kinnauri, PMR-20, PMR-53, VP-8902, DPP-68 were found to be moderately resistant with observed PDI ranges from 10.6-25.8% (Table 4). Out of the total genotypes evaluated, 64 genotypes i.e. 63.3% were found to be susceptible to powdery mildew with PDI ranges from 30.6 to 87.3%. Further among the susceptible genotypes, 23 lines were classified as moderately susceptible, 35 as susceptible and 6 as highly susceptible with PDI ranges of 30.6-49.7, 50.2-74.8 and 76.3-87.3% respectively. The cultivar IC-36 was found to have highest disease severity with PDI value of 87.3. This revealed that disease severity was very high in this genotype. Also, the disease severity was higher in 2009-10 than in 2010-11. Like PDI, DI

Table 3. Powdery mildew (*Erysiphe pisi*) assessment of garden pea germplasm under natural field condition.

Class	PDI (%)			DI (%)	
	Range	Mean	No. of genotypes	Range	Mean
Highly Resistant (HR)	Zero	0.0	21	Zero	0.0
Resistant (R)	0.8-8.8	5.4	6	3.8-23.6	16.6
Moderately Resistant (MR)	10.6-25.8	19.0	10	22.0-34.3	28.0
Moderately Susceptible (MR)	30.6-49.7	43.0	23	35.2-59.1	49.5
Susceptible (S)	50.7-73.9	67.0	35	58.0-80.1	73.2
Highly Susceptible (HS)	76.3-87.3	83.2	6	80.4-95.6	88.0
Accession mean		37.8			51.2
Checks	74.6-81.8	78.2	2	80.0-90.0	85
$LSD_{(0.05)}$		3.1			7.5
CV		31.0			26.3

Table 4. Assessment of garden pea genotypes on the basis of PDI.

Pathogen Reaction	Genotypes
Highly Resistant (Zero)	Alaska, ACTomour, Arka Ajit, Angoori, CHP-I C-96, C-778, DAP-2, HUVP-3,JP-15, JP-20, JP-141, JP-625, Punjab-89, PMR-4, PMR-62, PMVAR-1, VRP-22, VRPMR-9, VRPMR-11, KTP-8
Resistant (0-10%)	VP-233 (0.8), JM-5 (4.1), JP-501A/2 (4.9), E-4 (5.3), Vasundhra (8.3), JP-825 (8.8)
Moderately Resistant (10.1-30%)	JP-179 (10.6), JP-19 (13.5), JM-1 (16.9), GS-10 (17.3), VP-8902 (17.4), DPP-68 (19.7), JPBB-4 (21.5), PMR-53 (23.6), PMR-20 (23.9), Kinnauri (25.8)
Moderately Susceptible (30.1-50%)	PS-11 (30.6), PS-24 (32.2), CHPMR-2 (32.8), JP-62 (35.3), NDVP 10 (37.2), IC-312269 (38.0), PS-19 (41.0), VP-5 (42.3), CHP-2 (42.3), Prachi (42.4), KS-257 (43.1), Aryaveer (43.7), Bilaspuri Lincoln (46.0), C-400 (47.2), HUVP-4 (47.4), RE-89 (47.9), PMR-19 (48.0), KS-268 (48.1), VRP-8 (48.2), VP-316 (48.4), Punjab-88 (49.0), VRP-5 (49.7), C-308 (49.7)
Susceptible (50.1-75%)	Jagatpur (50.2), VRP-7 (50.7), Darl-104 (51.1), PMVAR-5 (52.2), IP-3 (52.3), Tara (53.8), PMVAR-3 (54.0), NDVP-8 (54.2), Garry Field (54.9), KS-205 (55.9), Little Marvel (56.4), AP-1 (56.7), PHPMR-1 (57.4), VP-215 (57.4), PS-8 (57.6), VP-434 (57.7), VL-8 (59.3), VP-433 (60.0), PMVAR-2 (60.4), Sel-AB (61.1), AP-3 (61.9), PEW-9 (62.0), KS-210 (62.0), Punjab-87 (62.7), VRP-6 (63.1), LPF-48 (63.8), UN-53-6 (63.8), PMVAR-4 (64.1), MA-6 (65.3), 10-6-A (68.1), PSM-3 (68.2), VRP-4 (73.9), NDVP-104 (74.3), DGP-207 (74.8)
Highly Susceptible (75.1-100%)	Arkel (76.3), PMR-69 (77.7), PM-69 (77.7), PM-65 (81.4), E-1 (83.7), DGP-19 (85.3), IC-36 (87.3)

Figures in parentheses are corresponding Percent Disease Index (PDI) values.

scores also yielded significantly different ($P < 0.05$) reactions between resistant and susceptible germplasm (Table 5). The correlation between mean values of PDI and DI for 2 seasons was found to be 0.95 ($P < 0.01$). Moreover, the trend of reactions of various genotypes to powdery mildew (PDI and DI) was very stable across seasons ($r = 0.81$; $P < 0.05$) indicating that we had obtained consistent results.

Detached leaf assay

In genotypes screened under detached leaf assay, no disease symptoms were observed for 7 days. The pustules of powdery mildew appeared on seventh day after inoculation. The disease progress reached maximum up-to the twelfth day of inoculation and thereafter no further development of any pustules was observed.

Table 5. Mean disease responses of garden pea genotypes to powdery mildew in field conditions.

Genotype	Disease Reaction		Rank based on PDI
	PDI (%)	DI (%)	
Highly Resistant			
C-96	0.0	0.0	1
Punjab-89	0.0	0.0	1
VRPMR-9	0.0	0.0	1
PMR-4	0.0	0.0	1
Resistant			
VP-233	0.8	3.8	22
JP-501A/2	4.9	12.4	24
JM-5	4.1	13.6	23
E-4	5.3	18.4	25
Moderately Resistant			
JP-179	10.6	22.0	28
JM-1	16.9	29.6	30
PMR-53	23.6	32.0	35
Kinnauri	25.8	34.3	37
Susceptible			
PS-11	30.6	35.2	38
IC 312269	38.0	44.4	43
HUVP-4	47.4	58.1	52
KS-205	55.9	65.7	70
UN-53-6	63.8	74.7	87
MA-6	65.3	75.0	89
PMR-69	77.7	86.0	96
IC-36	87.3	95.6	101

Scores are based on severity ratings for 10 plants entry^{-1}season^{-1} for 2 consecutive years. Values were back transformed by arcsine transformation. The results of some highly resistant and resistant; a few moderately resistant and most susceptible germplasm are presented.

Out of 27 resistant genotypes (under field conditions), 21 were found to be highly resistant under laboratory conditions with no disease symptoms, 4 as resistant and 2 as moderately resistant (Table 6). The genotypes JP-15, E-4 and JM-5 required more days ($P < 0.05$) for the appearance of disease symptoms as compared to JP-825 and Vasundhra, while Arka Ajit was found to be intermediate for days taken to symptom appearance (Table 6). The disease reaction of all the resistant and susceptible genotypes was mostly found consistent under field and laboratory conditions with the exception of JP-15 and Arka Ajit which were found highly resistant to powdery mildew in field conditions, but found resistant to powdery mildew under laboratory conditions with PDI values of 2.5 and 6.3 percent respectively. The genotypes JP-501A/2 and VP-233 showed opposite trends i.e. highly resistant under laboratory conditions and resistant under field conditions having PDI value of 4.97 and 0.80 respectively (Table 6). The genotypes 'Vasundhra' and 'JP-825' which were found resistant during field screening (PDI: 8.33 and 8.88 respectively) were found to have moderately resistant reaction under laboratory conditions with PDI values of 11.0 and 13.0 respectively (Table 6). Although the detached leaf assay was found to be more positively correlated with field screening on the basis of PDI ($r = 0.83$; $P < 0.05$) as compared to DI severity index ($r = 0.77$; $P < 0.05$).

DISCUSSION

Powdery mildew disease causes significant yield losses to crops (Ahmad *et al.*, 2001). Breeding garden pea for powdery mildew resistance requires appropriate disease screening methodologies.

Table 6. Disease development and comparative mean disease responses of selected pea germplasm sources (highly resistant + resistant) to *E. pisi* in laboratory and field conditions.

Genotypes	Detached Leaf Assay[a]			Field Evaluation[b]			
	Observations after inoculation	Symptom appearance	PDI[c] (%)	Reaction	PDI (%)	Reaction	DI[d] (%)
JP-15	9 days	Normal	2.5	R	0	HR	0
	12days	Symptoms					
JP-20	12 days	Normal	0	HR	0	HR	0
JP-141	12 days	Normal	0	HR	0	HR	0
JP-625	12 days	Normal	0	HR	0	HR	0
VRPMR-9	12 days	Normal	0	HR	0	HR	0
VRPMR-11	12 days	Normal	0	HR	0	HR	0
PMVAR-1	12 days	Normal	0	HR	0	HR	0
CHP-1	12 days	Normal	0	HR	0	HR	0
HUVP-3	12 days	Normal	0	HR	0	HR	0
Punjab-89	12 days	Normal	0	HR	0	HR	0
PMR-4	12 days	Normal	0	HR	0	HR	0
PMR-62	12 days	Normal	0	HR	0	HR	0
AC-Tomour	12 days	Normal	0	HR	0	HR	0
Alaska	12 days	Normal	0	HR	0	HR	0
VRP-22	12 days	Normal	0	HR	0	HR	0
Arka Ajit	7 days	Normal	6.3	R	0	HR	0
	9 days	Symptoms					
KTP-8	12 days	Normal	0	HR	0	HR	0
Angoori	12 days	Normal	0	HR	0	HR	0
C-96	12 days	Normal	0	HR	0	HR	0
C-778	12 days	Normal	0	HR	0	HR	0
DAP-2	12 days	Normal	0	HR	0	HR	0
JP 501A/2	12 days	Normal	0	HR	4.9	R	16.2
E-4	9 days	Normal	4.4	R	5.3	R	19.0
	12 days	Symptoms					
JM-5	9 days	Normal	3.0	R	4.1	R	15.5
	12 days	Symptoms					
JP-825	7 days	Symptoms	13.0	MR	8.8	R	23.6
VP-233	12 days	Normal	0	HR	0.8	R	3.8
Vasundhara	7 days	Symptoms	11.0	MR	8.3	R	21.0

Scores are based on severity rating for 10 plants^{-1}season^{-1}for 2 consecutive seasons.
[a] evaluated by inoculating the detached leaves under controlled environmental conditions; [b] evaluated under natural disease epiphytotic, when disease development was at peak during late season; [c] Percent Disease Index , [d] Disease Incidence

Most commonly, screening of genotypes has been conducted under field conditions; however, field screening has limitations, because it depends on natural occurrence of suitable environmental conditions and pathogen inoculum. In addition, field screening can usually be conducted only once in a year. Similarly, screening of genotypes under greenhouse conditions also requires proper conditions for disease development and only a limited number of genotypes can be evaluated due to space limitations. More often, these limitations restrict rapid progress in breeding for disease resistance. Direct inoculation of detached leaves in moist chambers is an assay may overcome the limitations associated with time and space dependency of field and greenhouse evaluations of genotypes for disease resistance. In this study, we showed that the detached-leaf assay is a reliable and rapid method to discriminate powdery mildew resistance in garden pea in the laboratory in a short period of time, while providing conditions for uniform inoculum levels. In a range of lines

with variable levels of resistance, infection parameters were similar in the detached-leaf assay and evaluation under natural disease epidemic in field conditions. Several germplasm and breeding lines resistant to powdery mildew were already identified using these screening methods (Pandey *et al.*, 1999; Singh 2001; Fondevilla *et al.*, 2006).

In this study, the field screening of numerous genotypes of garden pea identified complete resistance to powdery mildew in 21 genotypes and although incomplete but limited disease development to different degrees (up to 10 PDI value) in 6 other genotypes have also been found (Table 4), while 19 genotypes were found as immune (excluding JP-15, Arka Ajit, JP 501A/2 and VP-233 which showed variable results) and 6 as resistant as indicated by results drawn on the basis of artificial as well as natural disease screening (Table 6). The genotypes classified as resistant (with PDI value up to 5) is due to 1 or 2 infected plants, which may come through physical seed mixture during harvesting and threshing (Pandey *et al.*, 1999). However, lack of durable resistance is a problem for airborne fungal pathogens such as powdery mildew (Sillero *et al.*, 2006). Previously, Warkentin *et al.*, (1995) and Fondevilla *et al.* (2006) showed a relationship between powdery mildew development on intact plants and detached leaves supported by sucrose solution and benzimodazole agar respectively. Similarly in the results presented here, a close correlation was found between field screening and detached leaf assay (r = 0.83 for PDI; r = 0.77 for DI) method in 21 out of 27 genotypes revealing similar disease reactions i.e. highly resistant and resistant. Among the genotypes evaluated in this study, JP501A/2, NDVP-8 and PMR-20 were previously reported as resistant to pea powdery mildew by Pandey *et al.* (1999) for which they reported disease severity of 4.6, 45.6 and 16.9 respectively. Similarly the genotypes NDVP-10, AP-1, Punjab-87 and MA-6 were found to have same disease reaction as reported by Singh (2001) in the previous studies. Also, there exists difference in disease reaction of some genotypes *viz.* Arka Ajit, PMR-19 and VL-8 from previously reported studies, probably due to the effect of environment (Thakur *et al.*, 1996). PDI value range for moderately resistant group was also in agreement with Pandey *et al.* (1999). The PDI and DI severity index showed a high correlation value of 0.95 which was in agreement with the findings of Chattopadhyay *et al.* (2010). The high correlation values between PDI, DI and detached leaf assay showed a good agreement between the methods of disease assessment however, high positive correlation between natural and artificial screening for PDI (0.83) do not show the superiority of one method over the other however, the tests support the observation of a high correlation of powdery mildew response between natural and artificial inoculation when screening field pea germplasm (Davidson *et al.*, 2004). Dwarf plant type varieties were found to be more susceptible to powdery mildew than indeterminate or tall plant type varieties as reported by Gupta (1990) and Pandey *et al.* (1999). Similar results were found in this investigation as far as the plant type was concerned.

Our findings clearly showed that 19 (excluding 3 showing variable results in 2 systems of screening) garden pea genotypes are promising sources for powdery mildew resistance. At the same time, the single or multiple genes for resistance in the identified sources need further qualitative and quantitative genetic analysis. As 3 different genes along with markers linked to them, conferring resistance against *E. pisi* had already been identified (Ek *et al.*, 2005; Fondevilla *et al.*, 2008) but such results revealed the presence of weak or environment specific resistance in these genotypes.

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