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# Screening of pea germplasm for resistance to powdery mildew

**J. C. Rana · D. K. Banyal · K. D. Sharma · Manish K. Sharma · S. K. Gupta · Satish K. Yadav**

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**Abstract** Powdery mildew caused by *Erysiphe pisi* DC results heavy losses in the yield and quality of pods and seeds of pea crop. Germplasm comprising 701 accessions of garden and field pea originating from 60 countries were screened for powdery mildew resistance under natural epiphytotic conditions and 64 accessions found resistant in field screening for 2 years at one location were further screened both in field at two locations and artificially in laboratory to four isolates. The information was also obtained on the amount of genetic diversity and agronomic superiority in resistant accessions. Fifty-seven accessions showed resistant reaction for 3 consecutive years in field screening but only 14 accessions originating from 10 countries showed resistant reaction in laboratory screening against the four most prevalent isolates of *E. pisi* collected from different places in the area of experiment. Germplasm lines showed both complete and incomplete levels of resistance and variable reactions to different isolates. There was sufficient genetic diversity and agronomic superiority in the resistant accessions e.g. EC598655, EC598878, EC598704, IC278261, and IC218988, which may serve as useful genetic material to plant breeders for breeding pea varieties for powdery mildew resistance and high yield.

**Keywords** Agronomic traits · Genetic diversity · *Erysiphe pisi* · Pea · Powdery mildew

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## Introduction

Pea (*Pisum sativum* L.) belongs to family Fabaceae is an important multipurpose crop grown for green pods and grains in the cool temperate zones and tropical highlands of the world (Ali et al. 1994; Azmat et al. 2010). Generally, pea is grown in winter season in the Indian plains but it is an important summer (off-season) crop in the high hills (Rana et al. 2010; Bala et al. 2011). Powdery mildew of pea caused by

*Erysiphe pisi* DC is one of the most serious diseases resulting 25–50 % losses in yield and quality worldwide (Munjal et al. 1963; Singh et al. 1978; Warkentin et al. 1996; Katoch et al. 2010). Majority of the pea varieties grown here have found susceptible to powdery mildew. However, there are germplasm lines which have shown resistance to powdery mildew but most of them lack agronomic superiority (Rana and Gupta 1993b; Ghafoor et al. 2005). Therefore, it is necessary to identify germplasm lines, which may have genes both for disease resistance and agronomic superiority (Tiwari et al. 2004; Zong et al. 2008; Bing et al. 2011). This may help conventional plant breeders to overcome the difficulties faced due to linkage drag while transferring powdery mildew resistance from germplasm lines having poor agronomic performance.

The present experiment was designed to identify the germplasm lines resistant to powdery mildew disease and to assess the amount of genetic diversity and agronomic superiority present in the germplasm being conserved at Regional Station of National Bureau of Plant Genetic Resources, Shimla, Himachal Pradesh. The north-west Indian Himalayan region is one of the hot spots for the occurrence of powdery mildew. The isolates of *E. pisi* occurring here have high pathogenic variability because the fungus goes under sexual reproduction. In contrast, sexual stage i.e. cleistothecia formation does not take place in subtropical and tropical parts and fungus reproduce asexually, thus have low variability (Pal et al. 1980; Banyal et al. 2005). We assume that the information generated in this experiment could greatly assist in the conservation of pea germplasm and its efficient utilization in pea breeding programs.

## Materials and methods

The germplasm used in this experiment consisted of 701 accessions representing 60 countries (Table 1). Of these, 316 were indigenous collections (IC) means collected from different parts of India and 385 exotic collections (EC) procured from other countries. Based on usage, the germplasm were classified as—124 accessions of vegetable pea, 523 of pulse pea (erect plant type, white and brownish white seed color and bold seed size) and 54 accessions of field pea (spreading plant type, small pods and seeds and brown seed color). The material was evaluated in Augmented Block Design (Federer 1956) along with six standard check varieties viz. Lincoln and Azad pea as susceptible and HFP4, DMR11, DMR7 and Rachna as resistant to powdery mildew.

### Powdery mildew screening

The entire collection of 701 accessions was screened for powdery mildew resistance under natural epiphytotic conditions at Shimla in 2008 and 2009 and at two locations i.e. Shimla and Solan in the 2010. Geographically, Shimla is located at 31°06′N and 77°13′E at 6,800 ft. while Solan at 30°51′N and 77°10′E at 4,500 ft. Keeping in view the severity of disease that remain very high every year (Fig. 1), we did not use plant-to-plant artificial inoculation in the first year and only infector rows of check varieties were planted in each block consisted of 20 accessions to ensure uniform speared of disease. However, germplasm accessions grown in the 2nd and 3rd year were artificially inoculated by tapping conidia from heavily infected plant parts. The accessions showed resistance for 2 years in the field were further screened under artificial conditions against four most prevalent isolates of *E. pisi* using detached leaf technique (Banyal and Tyagi 1998) along with susceptible and resistant check varieties in the Plant Pathology Laboratory of Himachal Pradesh Agricultural University, Palampur. The four isolates viz. *rangway*, *trilokinath*, *stingri* were collected from the temperate climate while *kangra* from the sub-temperate climate of the north-west part of Indian Himalaya. The leaves along with petiole were detached from 15 to 30 days old seedlings of each accession and floated on tap water in petri dishes (Fig. 1). We added 50 ppm benzimidazole in the water to enhance longevity of the detached floating leaves and inoculated them with isolates of pathogen in five replications per accession for each isolate. The development of powdery mildew disease on intact plants and detached leaves floated on sucrose and benzimidazole medium has been found similar (Warkentin et al. 1995; Fondevilla et al. 2006). The inoculated leaves were incubated in the growth chamber at 22 ± 1° at 16/8 h day/night cycle. The disease reaction was recorded on 0–4 scale (Table 2) based on the infected foliage area, macroscopic and

**Table 1** Country of origin for 701 accessions used in the study

| Country | No. of accessions |
|---|---|
| Afghanistan | 13 |
| Albania | 2 |
| Australia | 7 |
| Austria | 2 |
| Brazil | 2 |
| Bulgaria | 3 |
| Canada | 2 |
| China | 11 |
| Costa Rica | 2 |
| Cyprus | 1 |
| Czech Republic | 12 |
| Denmark | 5 |
| Ecuador | 2 |
| Estonia | 1 |
| Ethiopia | 25 |
| Finland | 5 |
| France | 11 |
| Germany | 22 |
| Greece | 10 |
| Guatemala | 3 |
| Guinea | 1 |
| Honduras | 1 |
| Hong Kong | 1 |
| Hungary | 21 |
| Idaho | 1 |
| India | 316 |
| Iran | 10 |
| Israel | 3 |
| Italy | 1 |
| Japan | 4 |
| Kazakhstan | 1 |
| Kenya | 1 |
| Latvia | 1 |
| Lebanon | 1 |
| Macedonia | 2 |
| Malaysia | 4 |
| Mexico | 3 |
| Nepal | 6 |
| Netherland | 14 |
| New Zealand | 5 |
| Nigeria | 1 |
| Norway | 1 |
| Pakistan | 9 |
| Paraguay | 1 |
| Peru | 3 |
| Poland | 17 |
| Romania | 1 |
| Russia | 15 |
| Rwanda | 1 |
| Serbia and Montenegro | 4 |
| Spain | 10 |
| Sweden | 11 |
| Syria | 7 |
| Thailand | 1 |
| Turkey | 23 |
| Uganda | 1 |
| United Kingdom | 18 |
| Ukraine | 2 |
| United States of America | 35 |
| Yemen | 2 |

microscopic density of mycelia and sporulation at 9 days interval (Pal et al. 1980; Banyal and Tyagi 1998; Banyal et al. 2005).

## Agronomic evaluation

The data on morphological traits viz. growth habit, pod shape, flower colour, seed colour, seed surface, days to 50 % flowering, no. of primary branches/plant, pod length, no. of pods clusters/plant, no. of pods/plant, no. of seeds/pod, 100-seed weight (g) and seed yield/plant (g) were determined for two years on 64 accessions found resistant in field screening. The data were analyzed for mean, variances, correlations, regression, genetic diversity to find out genetic similarity/dissimilarity and principal component analysis (PCA) using the statistical software SYSTAT-12. Phenotypic and genotypic coefficients of variation (PCV and GCV) were computed (Burton 1952) and categorized the range as per Sivasubramanian and Madhavamenon (1978). Heritability was estimated (Lush 1940) and further classified into low, medium and high (Robinson 1966) while genetic advance estimated as per Johnson et al. (1955). The significance was assessed at the 5 % probability level.


**Fig. 1** Screening to powdery mildew using detached leaf technique and reaction type

## Results

### Screening under natural epiphytotic conditions

Out of 701 accessions screened at Shimla, 310 accessions showed heavy infection at second scoring while 251 accessions showed late infection but it reached to highest level (score 3 and 4) towards maturity in the first year itself. We eliminated these 561 susceptible accessions from further screening. In the 2nd year, remaining 140 accessions were grown more closely in 14 blocks consisted of 10 accessions each and leaves were inoculated artificially; 86 accessions were scored susceptible and 64 resistant. In the 3rd year, out of 64 accessions evaluated at two locations viz. Shimla and Solan, 57 accessions were scored resistant, and seven accessions viz. IC280357, IC311061, IC342025, IC342040, IC394027, IC469142, and IC469150 as susceptible (Table 3). We observe uniform spread of disease varying from 1 to 100 % on different accessions. There was no ambiguity in identifying powdery mildew resistant and susceptible plants because screening was done under heavy infection and in some case leaves, pods, and stems were covered with white powdery mass including tissue necrosis beneath turning black in susceptible plants (Fig. 1).

### Screening under artificial conditions

The same set of 64 accessions was screened artificially in the pathological laboratory to four isolates of *E. pisi* described above. The infection behavior of accessions was different to each isolates, showing both complete and incomplete levels of resistance. Out of 64 accessions, 14 accessions viz. IC208366, IC208378, IC218988, IC267142, IC278261 from India, EC381866-Ethiopia, EC598816-Turkey, EC598655-Czech Republic, EC598535-Iran, EC598729-Germany, EC598704-Sweden, EC598757-Poland, EC598538-United States of America, and EC598878 from Ecuador showed resistant reaction while seven accessions showed susceptible reaction to all the isolates (Table 3). Remaining 43 accessions showed variable disease reaction to different isolates. Individually, 45 accessions were resistant and 19 susceptible to *rungway*; 40 resistant and 24 susceptible to *trilokinath*; 38 resistant and 26 susceptible to *stingri* and 21 resistant and 43 susceptible to *kangra*. While in combination, eight accessions were resistant to *rungway/trilokinath*; six to *rangway/stingri*; three to *trilokinath/stingri*; one to *trilokinath/kangra*; nine to *rangway/trilokinath/stingri*; two to *rangway/stingri/kangra*; one to *rangway/trilokinath/kangra* and one to *trilokinath/stingri/kangra*.

We also observed that some accessions showed resistant reaction to only one isolates and susceptible to remaining three isolates. For instance, IC311067, IC311068, IC311070, IC394030, EC598845 were found resistant to *rungway*, IC311065, IC394020, IC469157 to *trilokinath*; IC218985, IC394029, EC381860 to *stingri* and IC342025 and IC381054 to *kangra*. Among check varieties, HFP4 showed

**Fig. 2** Powdery mildew disease severity and infection level in the field

**Table 2** Disease score, corresponding reaction and description for powdery mildew in pea

| Disease score | Reaction | Description |
|---|---|---|
| 0 | R | No mycelium growth |
| 1 | R | Sparse mycelium growth with very little sporulation |
| 2 | R | Slight growth of mycelium is evident macroscopically. Microscopically slight to moderate growth of mycelium with conidiophores of the fungus |
| 3 | S | Moderate growth of mycelium is evident macroscopically. Microscopically moderate development of mycelium with moderate to heavy sporulation is seen |
| 4 | S | Abundant growth mycelium is evident macroscopically. Microscopically abundant development of mycelium with heavy to very heavy sporulation is visible |

resistant reaction and Lincoln and Azad pea susceptible to all the four isolates while DMR7 was susceptible to *rangway* and Rachna and DMR11 to *kangra*.

## Statistical analysis

### Mean, variance, heritability and genetic advance

The frequency distribution of 701 accessions based on qualitative traits showed growth habit as erect (75), semi-erect (615), spreading (11); flower colour—white (415), whitish blue (87), pink (30), purple (169); pod thickness—thin (219), medium-thick (446) and thick (36); seed surface—smooth (529), wrinkled (172), and seed colour—white (96), creamy white (234), greenish white (121) greenish brown (72), green (124) and brown (54). The analysis of data for quantitative traits showed wide range of variability among the accessions. The mean performance of 32 resistant accessions (not given for all) was found at par with standard check varieties. For instance, EC598655, EC598878, EC598704, IC278261, IC218988 showed agronomic superiority for multiple traits like pod length (>7.50 cm), pods clusters/plant (>15), pods/plant (>30), 100-seed weight (>18.0 g) and seed yield/plant (30 g). Phenotypic and genotypic coefficients of variance were high for primary branches, pods clusters/plant, pods/plant, 100-seed weight and seed yield/plant (Table 4). The heritability was found high for all the traits and it was ranging from 70.59 % for clusters/

**Table 3** Disease reaction of 64 accessions and six check varieties of pea to powdery mildew (*Erysiphe pisi*)

| S. no. | Accessions | Infection type recorded in field screening | | Infection types recorded in laboratory screening with different isolates | | | |
|---|---|---|---|---|---|---|---|
| | | Shimla | Solan | *Rangway* | *Trilokinath* | *Stingri* | *Kargra* |
| Germplasm accessions | | | | | | | |
| 1. | IC208366 | 0 (R) | 0 (R) | 1 (R) | 1 (R) | 1 (R) | 2 (R) |
| 2. | IC208378 | 0 (R) | 0 (R) | 1 (R) | 1 (R) | 1 (R) | 1 (R) |
| 3. | IC208385 | 1 (R) | 2 (R) | 4 (S) | 1 (R) | 1 (R) | 3 (S) |
| 4. | IC209114 | 2 (R) | 2 (R) | 1 (R) | 1 (R) | 3 (S) | 4 (S) |
| 5. | IC218982 | 2 (R) | 2 (R) | 2 (R) | 2 (R) | 1 (R) | 3 (S) |
| 6. | IC218985 | 2 (R) | 2 (R) | 3 (S) | 3 (S) | 2 (R) | 4 (S) |
| 7. | IC218988 | 0 (R) | 0 (R) | 1 (R) | 1 (R) | 2 (R) | 1 (R) |
| 8. | IC219027 | 2 (R) | 2 (R) | 1 (R) | 3 (S) | 1 (R) | 2 (R) |
| 9. | IC267138 | 0 (R) | 1 (R) | 2 (R) | 1 (R) | 1 (R) | 4 (S) |
| 10. | IC267142 | 0 (R) | 1 (R) | 1 (R) | 1 (R) | 1 (R) | 1 (R) |
| 11. | IC267152 | 1 (R) | 2 (R) | 1 (R) | 1 (R) | 1 (R) | 4 (S) |
| 12. | IC267156 | 2 (R) | 2 (R) | 1 (R) | 3 (S) | 2 (R) | 1 (R) |
| 13. | IC267165 | 2 (R) | 2 (R) | 1 (R) | 4 (S) | 2 (R) | 4 (S) |
| 14. | IC267181 | 0 (R) | 0 (R) | 2 (R) | 1 (R) | 1 (R) | 3 (S) |
| 15. | IC278261 | 0 (R) | 0 (R) | 2 (R) | 2 (R) | 2 (R) | 1 (R) |
| 16. | IC418020 | 1 (R) | 2 (R) | 2 (R) | 1 (R) | 3 (S) | 3 (S) |
| 17. | IC342734 | 1 (R) | 2 (R) | 2 (R) | 1 (R) | 2 (R) | 4 (S) |
| 18. | IC280357 | 3 (S) | 3 (S) | 4 (S) | 3 (S) | 3 (S) | 4 (S) |
| 19. | IC291541 | 2 (R) | 2 (R) | 1 (R) | 1 (R) | 3 (S) | 3 (S) |
| 20. | IC296678 | 2 (R) | 2 (R) | 1 (R) | 1 (R) | 3 (S) | 4 (S) |
| 21. | IC310833 | 1 (R) | 2 (R) | 1 (R) | 1 (R) | 1 (R) | 4 (S) |
| 22. | IC311055 | 2 (R) | 2 (R) | 1 (R) | 1 (R) | 4 (S) | 4 (S) |
| 23. | IC311060 | 2 (R) | 2 (R) | 3 (S) | 1 (R) | 2 (R) | 3 (S) |
| 24. | IC311061 | 3 (S) | 3 (S) | 3 (S) | 3 (S) | 4 (S) | 3 (S) |
| 25. | IC311065 | 2 (R) | 2 (R) | 4 (S) | 1 (R) | 4 (S) | 4 (S) |
| 26. | IC311067 | 2 (R) | 2 (R) | 2 (R) | 4 (S) | 4 (S) | 4 (S) |
| 27. | IC311068 | 3 (S) | 2 (R) | 2 (R) | 4 (S) | 3 (S) | 4 (S) |
| 28. | IC311069 | 0 (R) | 1 (R) | 1 (R) | 1 (R) | 2 (R) | 4 (S) |
| 29. | IC311070 | 1 (R) | 2 (R) | 1 (R) | 4 (S) | 4 (S) | 4 (S) |
| 30. | IC328701 | 2 (R) | 2 (R) | 2 (R) | 2 (R) | 4 (S) | 3 (S) |
| 31. | IC342025 | 3 (S)) | 3 (S) | 3 (S) | 4 (S) | 4 (S) | 2 (R) |
| 32. | IC342037 | 0 (R) | 0 (R) | 1 (R) | 1 (R) | 1 (R) | 3 (S) |
| 33. | IC342040 | 3 (S) | 3 (S) | 3 (S) | 4 (S) | 4 (S) | 3 (S) |
| 34. | IC381054 | 2 (R) | 2 (R) | 3 (S) | 3 (S) | 3 (S) | 2 (R) |
| 35. | IC394020 | 2 (R) | 2 (R) | 3 (S) | 1 (R) | 3 (S) | 4 (S) |
| 36. | IC394027 | 3 (S) | 3 (S) | 4 (S) | 3 (S) | 3 (S) | 4 (S) |
| 37. | IC394029 | 2 (R) | 2 (R) | 4 (S) | 4 (S) | 2 (R) | 4 (S) |
| 38. | IC394030 | 1 (R) | 2 (R) | 2 (R) | 3 (S) | 4 (S) | 3 (S) |
| 39. | IC394032 | 1 (R) | 1 (R) | 1 (R) | 3 (S) | 2 (R) | 3 (S) |
| 40. | IC469142 | 3 (S) | 3 (S) | 3 (S) | 3 (S) | 4 (S) | 4 (S) |
| 41. | IC469150 | 3 (S) | 3 (S) | 4 (S) | 4 (S) | 4 (S) | 4 (S) |
| 42. | IC469157 | 2 (R) | 2 (R) | 4 (S) | 1 (R) | 3 (S) | 3 (S) |

**Table 3** continued

| S. no. | Accessions | Infection type recorded in field screening | | Infection types recorded in laboratory screening with different isolates | | | |
|---|---|---|---|---|---|---|---|
| | | Shimla | Solan | *Rangway* | *Trilokinath* | *Stingri* | *Kargra* |
| 43. | EC334160 | 2 (R) | 2 (R) | 4 (S) | 4 (S) | 1 (R) | 4 (S) |
| 44. | EC381866 | 0 (R) | 0 (R) | 1 (R) | 1 (R) | 1 (R) | 1 (R) |
| 45. | EC507770 | 1 (R) | 1 (R) | 1 (R) | 1 (R) | 3 (S) | 3 (S) |
| 46. | EC507771 | 2 (R) | 1 (R) | 3 (S) | 2 (R) | 4 (S) | 2 (R) |
| 47. | EC598825 | 1 (R) | 1 (R) | 1 (R) | 3 (S) | 1 (R) | 2 (R) |
| 48. | EC598816 | 1 (R) | 0 (R) | 2 (R) | 1 (R) | 1 (R) | 1 (R) |
| 49. | EC598832 | 2 (R) | 2 (R) | 1 (R) | 3 (S) | 1 (R) | 3 (S) |
| 50. | EC598841 | 2 (R) | 2 (R) | 3 (S) | 1 (R) | 1 (R) | 2 (R) |
| 51. | EC598588 | 1 (R) | 2 (R) | 1 (R) | 2 (R) | 2 (R) | 3 (S) |
| 52. | EC598655 | 1 (R) | 1 (R) | 2 (R) | 2 (R) | 1 (R) | 1 (R) |
| 53. | EC598535 | 1 (R) | 2 (R) | 1 (R) | 1 (R) | 2 (R) | 2 (R) |
| 54. | EC598729 | 1 (R) | 1 (R) | 2 (R) | 1 (R) | 2 (R) | 2 (R) |
| 55. | EC598843 | 1 (R) | 1 (R) | 2 (R) | 2 (R) | 3 (S) | 1 (R) |
| 56. | EC598845 | 2 (R) | 2 (R) | 2 (R) | 3 (S) | 3 (S) | 4 (S) |
| 57. | EC598710 | 1 (R) | 1 (R) | 1 (R) | 3 (S) | 2 (R) | 3 (S) |
| 58. | EC598704 | 0 (R) | 0 (R) | 2 (R) | 1 (R) | 1 (R) | 1 (R) |
| 59. | EC598757 | 1 (R) | 0 (R) | 1 (R) | 2 (R) | 1 (R) | 2 (R) |
| 60. | EC598538 | 0 (R) | 0 (R) | 2 (R) | 1 (R) | 1 (R) | 1 (R) |
| 61. | EC598844 | 1 (R) | 2 (R) | 3 (S) | 2 (R) | 1 (R) | 4 (S) |
| 62. | EC598878 | 0 (R) | 1 (R) | 2 (R) | 2 (R) | 1 (R) | 1 (R) |
| 63. | EC598744 | 2 (R) | 2 (R) | 1 (R) | 3 (S) | 1 (R) | 3 (S) |
| 64. | EC598537 | 0 (R) | 1 (R) | 2 (R) | 1 (R) | 3 (S) | 4 (S) |
| Check varieties | | | | | | | |
| 1. | Lincoln | 4 (S) | 4 (S) | 4 (S) | 4 (S) | 4 (S) | 4 (S) |
| 2. | Azad pea | 4 (S) | 4 (S) | 4 (S) | 4 (S) | 4 (S) | 4 (S) |
| 3. | DMR7 | 1 (R) | 2 (R) | 3 (S) | 2 (R) | 1 (R) | 1 (R) |
| 4. | DMR11 | 1 (R) | 1 (R) | 2 (R) | 1 (R) | 1 (R) | 3 (S) |
| 5. | Rachna | 1 (R) | 2 (R) | 1 (R) | 1 (R) | 2 (R) | 4 (S) |
| 6. | HFP4 | 0 (R) | 0 (R) | 1 (R) | 1 (R) | 1 (R) | 1 (R) |

plant to 95.55 % for days to flowering. The genetic advance was low for days to flowering, pod length, and seeds/pod and high for rest of the traits.

## Correlations and regression

The matrix developed for correlation coefficients showed significant positive correlation of seed yield/plant with pods cluster/plant, pod length, pods/plant, seeds/pod and 100-seed weight and negative correlation with days to flowering. Pod length had negative correlation with days to flowering, primary branches, pods clusters/plant, and pods/plant while positive correlation with seeds/pod and 100-seed weight. Primary branches showed positive correlation with pods clusters/plant, pods/plant and negative with pod length and 100-seed weight. Pods clusters/plant showed very high positive correlation with pods/plant and seed yield/plant but negative correlation with 100-seed weight. Pods/plant showed negative correlation with 100-seed weight. Regression analysis performed for seed yield/plant versus other traits showed that seeds/pod, pods/plant and 100-seed weight had highest direct contribution towards seed yield/plant

**Table 4** Statistical parameters of genetic variability in 64 accessions pea

| Traits | Range | Mean + SE | Variance (*P*) | Variance (G) | PCV (%) | GCV (%) | Heritability (%) | Genetic advance (%) |
|---|---|---|---|---|---|---|---|---|
| Days to flowering (days) | 60–120 | 85.8 ± 1.5 | 166.2 | 158.4 | 15.1 | 14.6 | 95.2 | 29.4 |
| No. of primary branches | 1.5–8.5 | 4.2 ± 0.2 | 2.7 | 1.9 | 39.3 | 32.8 | 70.5 | 56.5 |
| No of clusters/plant | 6.5–41.0 | 19.2 ± 0.8 | 53.5 | 42.9 | 38.1 | 34.1 | 80.2 | 62.9 |
| Pod length | 3.5–8.5 | 5.9 ± 0.1 | 1.3 | 1.3 | 19.5 | 19.1 | 95.5 | 38.4 |
| No of pods/plant | 11–59 | 32.8 ± 1.3 | 118.4 | 102.4 | 33.1 | 30.8 | 86.4 | 58.9 |
| No of seeds/plant | 3.0–7.5 | 5.2 ± 0.1 | 1.1 | 1.0 | 20.7 | 19.3 | 87.7 | 37.1 |
| Seed weight | 5.6–28.9 | 13.4 ± 0.6 | 30.4 | 28.3 | 40.9 | 39.5 | 93.1 | 78.5 |
| Seed yield/plant | 10.1–45.6 | 23.3 ± 8 | 55.7 | 49.7 | 31.9 | 30.1 | 89.9 | 58.6 |

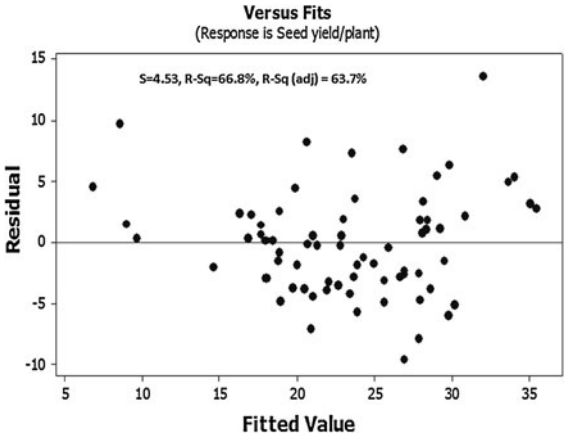


**Fig. 3** Scatter plot of residuals versus fitted values drawn through regressions analysis

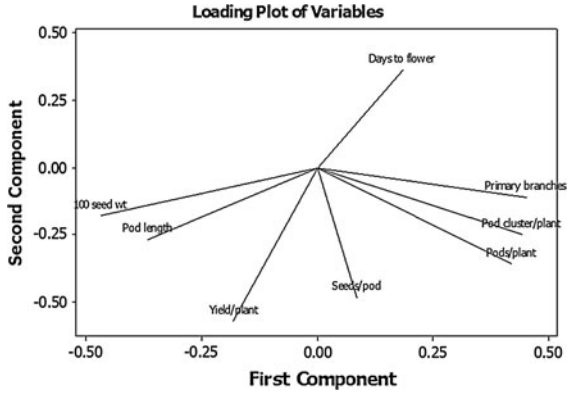


**Fig. 4** Biplot of different variables loaded on PC1 and PC2

while pod length, which had positive correlation but showed negative direct effect. Similarly, primary branches have non-significant positive correlation but highest negative effect. The plot of residual versus fitted values showed that residuals of majority of the accessions bounce randomly around 0 line forming horizontal band (Fig. 3). This suggests that the variances of the error terms are equal, relationship among accessions is linear and there are no outliers.

## Principal component and diversity analysis

The PCA used to eliminate redundancy in the data set revealed that two principal components (PC1) and (PC2) accounted for 79 % of the total variability observed among the pea germplasm evaluated. PC1 accounted for 48 % of variation, was loaded on primary branches, pods clusters/plant, pods/plant while PC2 accounted for 31 % of variation, was loaded on 100-seed weight, pod length, seeds/pod and seed yield/plant (Fig. 4). Dendrogarm produced to analyze the genetic distance between different accessions grouped 64 accessions and six check varieties into four clusters and each cluster have 14, 17, 20 and 19 accessions (Fig. 5). The cluster distances among four clusters were 25.36 between CI and C2, 32.84 (CI–CIII), 27.04 (C1–CIV), 17.5 (CII–CIII), 32.34 (CII–CIV) and 24.34 between CIII and CIV. The pattern of clustering was irrespective of the origin/place of collection of germplasm as accessions from different countries were grouped into different clusters except cluster 4, which has only one exotic accession.

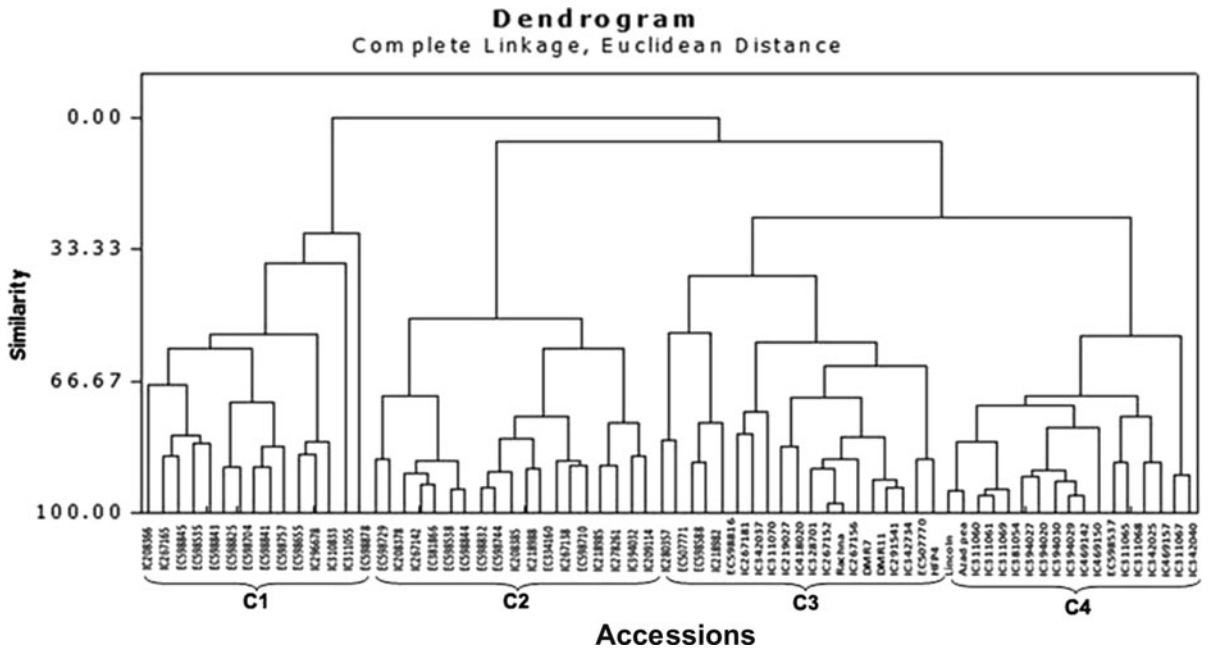


**Fig. 5** Dendrogram depicting genetic distance and clustering pattern among 64 germplasm accessions and 6 check varieties of pea

## Discussion

The losses caused by powdery mildew in pea are more when the crop is grown for seed purpose because disease severity level increases towards maturity (Munjal et al. 1963; Rana and Gupta 1994, 1995; Ahmad et al. 2001). The screening for disease resistance in field at different locations and for more number of years exposed the germplasm to many uncharacterized pathogen populations while under controlled environmental conditions to most prevalent isolates of that region (Kumar and Singh 1981; Singh et al. 1983; Tiwari et al. 1997b; Thomas and Kenyon 2004). These conditions helped us in avoiding the ambiguities resulting from the influence of environmental factors on the expression of diseases and we assume that resistant and susceptible reactions of accessions are based on plants with actual genetic resistance to the fungus *E. pisi*.

The variable reaction of accessions against different isolates suggests the difference in virulence levels of isolates and in the genetic makeup of resistance genes in the accessions (Saxena et al. 1975; Singh et al. 1983; Rana and Gupta 1994; Banyal et al. 2005; Nisar et al. 2006'; Fondevilla et al. 2007). Different sources of complete and incomplete resistance have been described in pea due to presence of two genes namely *er-1* that can bring about full resistance while *er-2* provides only leaf resistance (Heringa et al. 1969; Sharma 1992; Thakur et al. 1996). Other studies have also found gene *er-1* more stable and effective against powdery mildew than gene *er-2* which has proved to be less stable and ineffective over different locations (Schroeder and Providenti 1965; Tiwari et al. 1997a). Based on these studies, we may infer that the accessions showed resistant disease reaction for three consecutive years over two locations and to four isolates in the laboratory may probably carrying gene *er-1* while others that showed instability in the disease reaction may have gene *er*-2. However, further confirmation is needed by developing mapping populations with specific gene or gene combinations.

Among four isolates, the *kangra* isolate was found most virulent as it infected 43 accessions as compared to *rungway, trilokinath* and *stingri*, which infected 19, 24 and 26 accessions, respectively. We did not find any relationship between the place of origin of accessions with resistance or susceptible reactions as out of 701 accessions from 60 countries only 14 accessions from 10 countries showed stable reaction/complete resistant to all isolates. It is further mentioned that these accessions could form a new set of accessions resistant to powdery mildew as none of them have tested earlier against the same isolates in this region.

Natural sources of resistance with variable disease reaction to powdery mildew in pea have been identified in the germplasm from difference places of the world regardless of their origins (Heringa et al. 1969; Kumar and Singh 1981; Tiwari et al. 1999; Ahmad et al. 2001; Liu et al. 2003; Fondevilla et al. 2006).

We believe that plant breeders invariably have resistant germplasm in their breeding stocks but majority of them have poor agronomic background carrying several undesirable gene. The dominant nature of many undesirable genes and associated linkage drag makes gene transfer more cumbersome. Therefore, it is good to obtain additional information on the extent of genetic diversity and agronomic performance of resistant germplasm accessions (Ghafoor et al. 2005; Smýkal et al. 2008; Ceyhan et al. 2008; Singh et al. 2011). The germplasm accessions, which have high levels of resistance and agronomic superiority may reduce the time taken to eliminate the undesirable gens through repeated back crossing by plant breeders.

Germplasm evaluated in the present experiment showed high level of variance coupled with high heritability and genetic advance for primary branches/plant, pods clusters/plant, pods/plant, 100-seed weight and seed yield/plant. This suggest that these traits may be under the influence of additive gene interactions and use of simple selection methods would be sufficient for further improvement (Cockerham 1961; Rana and Gupta 1993a, 1994; Kalia and Sharma 1988; Mehrani 2002; Javid et al. 2002; Kumar 2008; Ahmad et al. 2010). However, traits like days to flowering, pod length and seeds/pod, which have narrow range of variance and low genetic advance may have non-additive gene interactions, thus needed to be improved by hybridization (Gritton 1980; Duke 1981; Khan and Malik 1989; Amurrio et al. 1993; Vaid and Tyagi 1997). The influence of additive and non-additive gene actions on seed yield in pea has been frequently reported in the literature (Ceyhan 2003; Singh and Singh 1990; Kumar 2008). The correlation coefficients among traits may help plant breeders to select products with improved agronomic performance not merely on the basis of yield performance but also through associated traits (Mehrani 2002; Mehmet and Ceyham 2006; Singh et al. 2011).

Similarity indices and pattern of relationships obtained through genetic diversity and PCA are useful to evaluate potential breeding value of germplasm through traits loaded on PC1 and PC2 (Keneni et al. 2005). The minimum inter-cluster distance (17.15) occurred between clusters II and III and maximum (32.84) between clusters I and III indicating genetic distance and closeness among accessions due to different genetic constitutions. The occurrence of genotype of same geographical region in different clusters and vice versa is likely due to the free exchange of seed materials among different regions and character constellations that might be associated with particular region in nature but lose their individuality under human interference (Saxena et al. 1975; Singh and Tripathi 1980; Tiwari et al. 2004; Kumar 2008; Ahmad et al. 2010).

The clustering could not fully separated vegetable, pulse and field pea into different clusters. Smýkal et al. (2008) while grouping 164 different pea types using RBIP and SSR markers found that molecular data not fully separated fodder pea types from other pea types, and suggested that no global genomic differences exist between the two pea types. Previous PCA analysis based on SSAP transposon polymorphisms have pointed out similar close relationships in different pea types (Maxted and Ambrose 2001; Vershinin et al. 2003; Yadav et al. 2007; Kosterin and Bogdanova 2008; Jing et al. 2010).

It is concluded, that germplasm evaluated in the present experiment has high level of resistance to powdery mildew along with sufficient amount genetic diversity and agronomic superiority, which can be used for breeding pea varieties with resistance to powdery mildew and high yield. The phenotypic selection for agronomic performance within pea accessions applied in this work has resulted into a group of good pea breeding lines for future use.

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