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Inheritance of *er1*-Based Broad-Spectrum Powdery Mildew Resistance in Pea (*Pisum sativum* L.)

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Abstract

The knowledge about the nature and number of gene(s) controlling resistance is the pre-requisite for the success of powdery mildew resistance breeding program in pea. Seven biparental cross combinations involving three highly resistant (It-96, No. 267 and JI 2302) and two highly susceptible (Climax and PF-400) pea genotypes were evaluated for their response to powdery mildew disease. The quantitative microscopic scale of disease assessment coupled with detached leaf assay was employed for the evaluation of disease response of the crosses and their generations (F_1, F_2, BC_s, and BC_r) against two highly virulent conidial isolates of *Erysiphe pisi*. The disease response of 677 F_2 plants has revealed a typical monohybrid Mendelian 3 (susceptible): 1 (resistant) segregation, moreover, the evaluation of 254 BC_r plants gave a perfect 1 (susceptible): 1(resistant) segregation. No complementation was observed among all the F1 plants of three complementation crosses, suggesting that the same allele (*er-1*) conditions complete and broad-spectrum resistance in all the powdery mildew resistant pea genotypes in homozygous recessive form.

Keywords: biotrophs, *Erysiphe pisi*, legume, genetic analysis

Introduction

Pea is nutritionally an important legume crop. In Pakistan, the area under pea crop is consistently increasing but on the contrary there is stagnation in average yield (Anonymous, 2010-2011). Diseases are the major limiting factor to yield and quality of pea harvest. Pea is attacked by a number of different fungal, bacterial, viral and nematode diseases; fungi are the most common cause of pea diseases (Falloon and Viljanen-Rollinson, 2001).

Powdery mildew caused by an obligate biotrophic fungus, *Erysiphe pisi* Syd. is the most troublesome disease of pea, wherever pea is cultivated in the world. The powdery mildew fungus of pea causes severe decline in yield and deteriorate the quality of pea harvest (Agrios, 1988; Rubiales *et al*., 2009). Under favorable conditions for disease development the magnitude of yield losses can increase many times as plants fail to reach reproductive phase (Uppal *et al*., 1953).

In Pakistan powdery mildew disease appears in epidemic form almost every year when the plants are in the pod formation stage (January-February). During the transitional period between the end of winter and the onset of spring season, the weather becomes favorable for the epidemic emergence of powdery mildew disease on pea crop (Thompson and Kelly, 1982). Different sulphur based synthetic fungicides are used to reduce the yield losses caused by the onset of powdery mildew disease.

The excessive use of fungicide has developed resistance in *E. pisi* against the high doses of chemical sprays used during previous years. It is an established fact that any disease is the result of an interaction among the pathogen (virulent), environmental conditions (suitable for pathogenicity and virulence) and susceptible host. It is difficult to manage the first two components of disease triangle; the only component that can be effectively managed is to replace the resistant host with the susceptible one, the disease will not develop ultimately. But, unfortunately no powdery mildew resistant and high yielding pea cultivar is commercially available for cultivation in Pakistan.

The resistance against powdery mildew in pea is under stable genetic control, but there are contradicting reports regarding the nature and number of gene(s) controlling resistance against powdery mildew. Harland (1948) used Peruvian accessions to demonstrate that resistance against powdery mildew in pea is controlled by a single recessive gene (*er*), Pierce (1948) also found similar results using Stratagem cultivar. As a result of an extensive research undertaken by Heringa *et al*. (1969) it was found that the powdery mildew resistance in local pea genotypes was due to a single recessive gene (*er1*) while another gene (*er2*) confers resistance to Peruvian accessions in recessive form.

The resistance conditioned by *er2* gene was later on reported to break down under field conditions (Schroeder and Providenti, 1965). There are also reports that duplicate recessive genes (*er1* and *er2*) are required for field resistance (Kumar and Singh, 1981). The evidence for polygenic nature of powdery mildew resistance was provided by Gupta *et al*. (1995). Most recently it was demonstrated

that there is another different gene (*Er3*) which independently confers resistance against powdery in dominant form (Fondevilla *et al*., 2010).

To date different research groups have conducted independent experiments, some advocating for *er1* gene (Cousin, 1965; Mishra and Shukla, 1984; Kalia and Sharma, 1988; Vaid and Tyagi, 1997) and some for *er2* gene as the source of resistance against powdery mildew in pea (Sokhi *et al.*, 1979; Ram, 1992; Tiwari *et al*., 1997).

All the previous efforts to elucidate the inheritance of powdery mildew resistance in pea were undertaken under field conditions with few exceptions of the experiments which were conducted in glasshouse under controlled conditions of temperature and humidity (Heringa *et al*., 1969). In principal, in all the classical experiments the qualitative scale of visual assessment (naked eye or microscopic) of disease, based on the percentage of leaf area affected with powdery mildew was used for the classification of PMR (Powdery mildew resistant) and PMS (Powdery mildew susceptible) plants.

The visual assessment of the percentage of leaf area affected with powdery mildew may involve human error and results can be misleading. In order to incorporate powdery mildew resistance in high yielding pea cultivars it is a prerequisite to have the source of gene(s) and have the knowledge about the number and nature of gene(s) governing the trait. There also exists significant pathogenic variation in *E. pisi* isolates (Azmat *et al*., 2012a).

Therefore, keeping in view the dietary importance of pea, magnitude of yield losses incited by powdery mildew and the issue of pathogenic variation, the objective of this study was to undertake the genetic analysis of disease resistance to reveal the nature and number of gene(s) controlling powdery mildew resistance in local pea genotypes grown in Pakistan. For having more reliable and reproducible inferences the quantitative microscopic scale of disease assessment using detached leaf assay was used for making all the observations

Materials

Plant material and isolates of E. pisi

Four pea genotypes including two highly susceptible commercial cultivars (Climax and PF-400) and two highly resistant local genotypes (It-96 and No. 267) were selected on the basis of their consistent response to powdery mildew disease (Azmat *et al*., 2012a; Azmat *et al*., 2012b). One pea genotype JI 2302 with known source of *er1* gene (Heringa *et al.* 1969) was also included. Seven different crosses were made among these genotypes; four crosses involved PMR and PMS genotypes while three crosses (complementation cross) were made between PMR genotypes.

The F_1 plants of four crosses (Climax × It-96; Climax × No.267; PF-400 × It-96 and PF-400 × No.267) were used to develop BC_r (back-cross with resistant parent), BC_s (back-cross with susceptible parent) and F_2 seeds for each cross (Fig. 1). Two highly virulent conidial isolates (MUZ-1 and MUZ-2) of *E. pisi* were selected amongst 23 isolates of *E. pisi*, collected from different agro-ecological zones of Pakistan (Azmat *et al*., 2012a). The selected isolates were collected from district Muzaffarabad (34.21°N 73.28°E) of Azad Jamu and Kashmir; these isolates have shown disease symptoms even on the genotypes that were previously considered as resistant to powdery mildew (Azmat *et al*., 2012a).

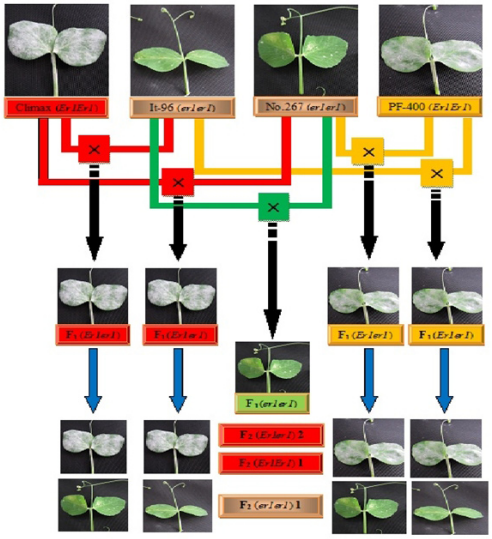

Fig. 1. Five different crosses along with their F_1 and F_2 generations involving two powdery mildew susceptible and resistant genotypes each

The isolates of *E. pisi* were propagated and maintained separately on a susceptible cultivar ('Meteor'-Faisalabad) in a growth chamber under ideal conditions required for the rapid multiplication of powdery mildew inoculumn.

Preparation of powdery mildew inoculumn

The fresh inoculumn of both isolates was prepared using the powdery mildew affected leaves, which were fully covered with powdery mass. The leaves containing inoculumn were homogenized in 0.1% water–agar and 0.0025% Tween-20 solution (Reeser *et al*., 1983).

Sowing of plants and powdery mildew inoculation

All the six generations (P_1, P_2, F_1, F_2, BC_s and BC_r) of the four crosses were sown in a field. The soil was a well-drained silt loam soil with a pH of 7.4. The field was well prepared.

Seed was sown on 75 cm wide beds with a distance of 10 cm between plants. A composite NPK fertilizer was added at the rate of 40-40-25 kg·a^{-1}. Irrigation was applied to the experimental material at 7 to 10 days intervals and manual weed control was applied to all plots.

At six to eight node stage, 10 disease free leaves from all the plants of each generation were excised and placed in Petri plates containing 1% agar, 6 ml of 5% sucrose solution and 150 mgl^{-1} benzimidazole (Warkentin *et al*., 1995; Sillero *et al*., 2006). The leaves were individually inoculated using a hand-held inoculator maintaining a conidial density of 20–50 spores/mm^2 (Azmat *et al*., 2012a, b). The inoculated leaves were placed adaxial surface up in sealed Petri plates. Control Petri plates were maintained to check cross infectivity. The Petri plates were placed in a growth chamber at 22 °C with a 14:10 h light: dark photoperiod with light intensity of 400 μmol m^{-2} s^{-1}.

Microscopic assessment of powdery mildew response of different generations

48 hours after inoculation the inoculated leaves were placed in de-staining solution (1 lactic acid: 2 glycerol: 1 d_2H_2O) for 48 hour and then stained with coomassie blue. The stained samples were observed under dissecting microscope (400X). The slides were prepared by placing the adaxial surface of stained leaves upward in mounting medium (50% glycerin) on microscopic slides. The cover slip was placed over the leaves after adding few drops of mounting medium.

The disease reaction of inoculated leaves of all generations was microscopically quantified using following susceptibility percentage (% S) based 0-5 scale: 0 (*Immune*) = zero susceptibility, 1 (*Highly resistant*) = <1-5%, 2 (*resistant*) = 6-10%, 3 (*Moderately susceptible*) = 11-40%, 4 (*Susceptible*) = 41-70%, 5 (*Highly susceptible*) = 71-100%. The score 0-2 was considered as resistant while score 3-5 represented susceptible disease response. The susceptibility percentage was calculated using following formula:

$$\%\,S = \frac{Conidia\ with\ mycelial\ growth}{Totla\ number\ of\ germinated\ conidia} \times 100$$

The average of susceptibility percentage (based on 10 observations) was used to determine the disease response score of individual plant from each generation.

Chi-square

The segregation ratios in F_2 generations of four crosses were tested for their fitness to mono-genic, two types of di-genic and tri-genic ratios using Chi-square test (Harris, 1912). The segregation ratios of BC_r of all the crosses were also tested for goodness of fit.

Results

All the F_1 plants of four PMR×PMS crosses were susceptible to the both isolates of powdery mildew (*E. pisi*), suggesting that susceptibility to powdery mildew is a dominant trait and the resistance to powdery mildew is governed by recessive gene(s) (Table 1).

The pea plants in the F_2 generation of all PMR×PMS crosses were segregated in perfect 3 (Susceptible): 1 (Resistant) ratio, suggesting that resistance is conferred by a single recessive gene in homozygous form.

A total of 677 F_2 plants were subjected to detached leaf assay coupled with microscopic disease quantification, which resulted in 517 susceptible and 160 powdery mildew resistant plants suggesting Mendelian monohybrid

Table 1. Chi-square values and probabilities of goodness of fit of segregation ratios of F_2 and back-cross-generations of four PMR×PMS crosses

		Expected no. of Plants		Observed no. of Plants				
Cross	Generation	S	R	S	R	Ratio	χ2 Value	P-Value
Climax × It-96	F1	30	0	30	0			
	F2	132.75	44.25	138	39	3:1	0.831	0.1-0.3
	BCs	65	0	65	0			
	BCr	35	35	34	36	1:1	0.057	0.4-0.8
PF-400 × It-96	F1	30	0	30	0			
	F2	121.5	40.5	115	47	3:1	1.391	0.1-0.23
	BCs	57	0	57	0			
	BCr	32	32	29	35	1:1	0.560	0.1-0.45
Climax × No. 267	F1	30	0	30	0			
	F2	118.5	39.5	123	35	3:1	0.684	0.1-0.4
	BCs	55	0	55	0			
	BCr	31	31	35	27	1:1	1.032	0.1-0.3
PF-400 × It-96	F1	30	0	30	0			
	F2	135	45	141	39	3:1	1.067	0.1-0.3
	BCs	68	0	68	0			
		29	29	32	26	1:1	0.621	0.1-0.4

ratio (χ^2= 0.674, Prob. 0.1-0.4). The same disease response data of F_2 plants were subjected to other possible digenic and trigenic segregation ratios using chi-square analysis; only the monogenic segregation ratio has shown goodness of fit.

The segregation ratios of back cross generations (BC_s and BC_r) of all cross combinations has validated the above mentioned results; all the tested BC_s plants have shown susceptibility to powdery mildew while the plants in BC_r generations were perfectly segregated in 1(Susceptible): 1(Resistant) ratio. In total 254 BC_r plants were included in this study which segregated in 130 powdery mildew susceptible and 124 resistant plants (1S:1R; χ^2 = 0.142, Prob. 0.1-0.7).

The F_1 plants of the crosses involving PMR genotypes (complementation cross) were also tested for susceptibility reaction to powdery mildew using microscopic quantification of powdery mildew. All the F_1 plants were resistant to the most virulent isolates of *E. pisi* (MUZ-1 and MUZ-2). Since no complementation was observed in all crosses therefore, it may be suggested that the resistance against powdery mildew in local pea genotypes (It-96 and No.267) is governed by the same gene (*er1*) that confers resistance in JI 2302 (Fig. 2).

Table 2. Chi-square values and probabilities of monogenic, two types of digenic and trigenic segregation ratios of F_2 generations of four crosses

		Ratio 3:1 (monogenic)		Ratio 9:7 (digenic)		Ratio 15:1 (digenic)		Ratio 63:1 (trigenic)	
Cross	F_2 plants	χ^2 Value	P-Value	χ^2 Value	P-Value	χ^2 Value	P-Value	χ^2 Value	P-Value
Climax × It-96	177	0.831	0.1-0.35	33.91	≤0.01	75.28	≤0.01	482.26	≤0.01
PF-400 × It-96	162	1.391	0.1-0.23	14.33	≤0.01	143.35	≤0.01	793.62	≤0.01
Climax × No. 267	158	0.684	0.1-0.4	29.94	≤0.01	67.89	≤0.01	435.47	≤0.01
PF-400 × No.267	180	1.067	0.1-0.3	35.67	≤0.01	73.01	≤0.01	473	≤0.01
Total of four crosses	677	0.674	0.1-0.4	111.34	≤0.01	348.91	≤0.01	2144.16	≤0.01

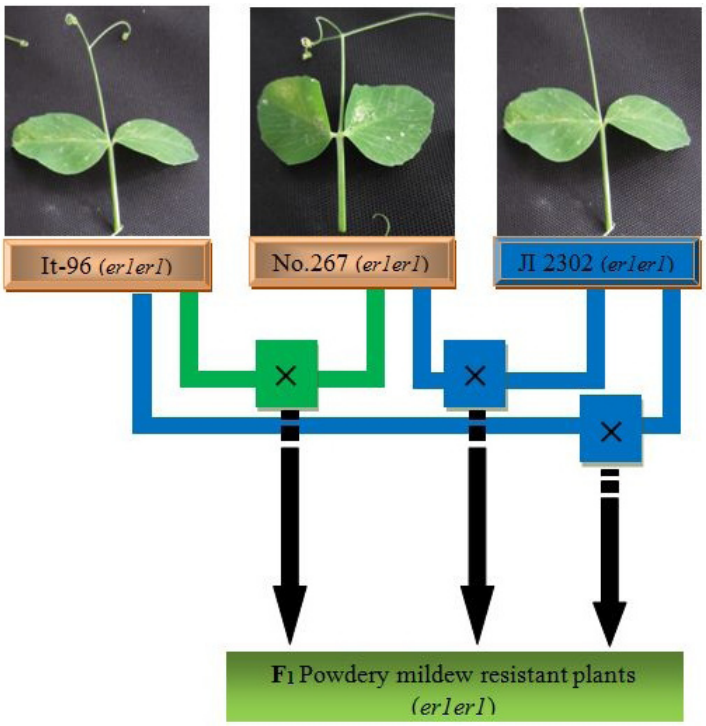

Fig. 2. Crossing scheme with their corresponding F_1 among three powdery mildew resistant genotypes. Two genotypes (It-96 and No.267) are Pakistani in origin

Discussion

The development of powdery mildew resistant pea cultivars is the most appropriate and cost effective means of reducing yield losses. In the present study, for the first time the detached leaf assay coupled microscopic quantification of disease under controlled environment was used to study the nature and number of gene(s) controlling powdery mildew resistance in pea.

It was found that resistance to powdery mildew in pea is controlled by a homozygous recessive gene at a single locus. Since none of the F_1 of crosses involving PMR genotypes showed susceptible disease response therefore, it is concluded that the same gene (*er1*) confers resistance in local pea genotypes (It-96 and No.267) that was previously found in pea genotype JI 2302.

These results have confirmed the findings of other studies independently conducted in different locations and environmental conditions (Pierce, 1948; Kalia and Sharma, 1988; Janila *et al*., 2001; Janila and Sharma, 2004; Vaid and Tyagi, 1997). Contradicting results to the recessive and monogenically governed powdery mildew resistance (Heringa *et al*., 1969; Sokhi *et al*., 1979; Kumar and Singh, 1981; Fondevilla *et al*., 2010) may be due to the erroneous scoring of powdery mildew susceptible plants as being powdery mildew resistant due to escape mechanism. The difference in the resistance mechanism against powdery mildew among different pea genotypes may be due to the real genetic differences among them. The results presented in this study are based on precise and reproducible microscopic quantification of disease. The microscopic examination of powdery resistant and susceptible individual plants (leaves) had shown that in powdery mildew resistant plants the pathogenesis was terminated after the germination of conidia while notable mycelial growth was observed on the leaves of powdery mildew susceptible plants.

There are mainly two types of disease resistance; race-specific resistance (vertical resistance) and non-race-specific resistance (horizontal resistance). The non-race-specific resistance is generally preferred by plant breeders as it confers broad-spectrum resistance, which enables plants to pose resistance to different virulent isolates of the pathogen (Jørgensen and Wolfe, 1994). The PMR pea genotypes used in this study have shown complete resistance to different geographically distributed virulent isolates of *E. pisi* (Azmat *et al*., 2012a) suggesting their efficient use in powdery mildew resistance breeding programs. The disease response data of PMR genotypes (It-96 and No.267) both under field and controlled conditions have validated that unlike *er2*-based partial powdery mildew resistance (Heringa *et al*., 1969), *er1*-based resistance is complete, stable and durable (Azmat *et al*., 2012a; Azmat *et al*., 2012b). Different breeding strategies viz., back cross technique can be used to incorporate powdery mildew resistance in high yielding pea cultivars and/or pedigree selection can also be employed to create new genetic combinations with resistant back-ground after crossing powdery mildew resistant and high yielding pea genotypes. Using different morphological and molecular markers, Sarala (1993) and Timmerman *et al*. (1994) have demonstrated that *er1* gene is present on the chromosome number six of pea genome. The *er1*- linked or *er1* gene-specific molecular markers can be used to increase the efficiency and pace of powdery mildew resistance breeding program.

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