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Biochemical genetics of powdery mildew resistance in pea

P. Kalia and S.K. Sharma*

Department of Vegetable Crops, Himachal Pradesh Agricultural University, Palampur 176 062, India

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Summary. A biochemical study on phenolic (total phenols and orthodihydroxy phenols) content and on the activities of phenol oxidizing enzymes (peroxidase and polyphenol oxidase) in pea cultivars resistant and susceptible to powdery mildew infection revealed that the resistant cultivars contained higher levels of phenolics and phenol-oxidizing enzymes than the susceptible ones. A further study of their F_1s, F_2s and backcross progenies suggested a high heritability for all biochemical traits. The correlation coefficients between the biochemical parameters and the disease index were also high. Both additive ($\hat{d}$) and dominant ($\hat{h}$) components were found to contribute to the inheritance of these constituents.

Key words: *Erysiphe polygoni* – Phenolics – Peroxidase – Polyphenol oxidase – *Pisum sativum*

Introduction

Powdery mildew is caused by *Erysiphe polygoni* DC. and is the most devastating disease of pea (*Pisum sativum* L.). The disease often appears in epidemic form covering all parts of the plant with white floury patches, thereby adversely affecting the photosynthetic activity of the plants. This not only reduces the yield of green pods but also reduces their market value.

In a breeding programme, it is imperative to screen the breeding material by creating artificial epiphytotics. The obligate nature of this parasite makes the possibility of culturing it artifically a distant probability. Breeders have to depend on natural outbreaks of the pathogens, therefore, in order to select resistant plants, and as such, the chances of escapes are enhanced. It would, therefore, be worthwhile to assay the resistance to powdery mildew in terms of biochemical genetic parameters, which are less influenced by the environment. Kirik et al. (1974) observed high peroxidase and polyphenol oxidase activity in resistant pea cultivars. This paper reports on the genetic make-up of the phenolics and phenol oxidizing enzymes in two pea crosses.

Materials and methods

The experimental material comprised parents (P 185 and P 6583 resistant lines, and 'Bonneville', a susceptible cultivar), their F_1s, backcrosses and F_2s. Seeds were sown in a complete randomized block design with three replications in 3 m long rows. In each replication, the rows were spaced 60 cm apart, whereas a 10 cm distance was maintained between plants. Infector rows of 'Bonneville' were interspersed in the experimental field to ensure an ample availability of inoculum for the spread of the disease. Powdery mildew score was recorded as a percentage infection index (McKinney 1923).

For the biochemical analysis, the leaf samples were collected at the time of maximum appearance of the powdery mildew on 'Bonneville'. Composite samples were drawn from parents and F_1s, whereas for the backcrosses and F_2s, samples were taken from all the plants. Total phenols and orthodihydroxy phenols were determined by the methods of Bray and Thorpe (1954) and Arnow (1937), respectively. Peroxidase and polyphenol oxidase activities were assayed following Addy and Goodman (1972) and Mayer et al. (1965) respectively, and expressed as unit/min per g (Farkas and Kirlay 1962). The data were subjected to analysis of variance and a standard test of significance was applied.

The scaling tests of Mather (1949) were performed to detect non-allelic interactions, and gene effects were estimated according to Jinks and Jones (1958).

* Associate Professor (Genetics), Department of Basic Sciences

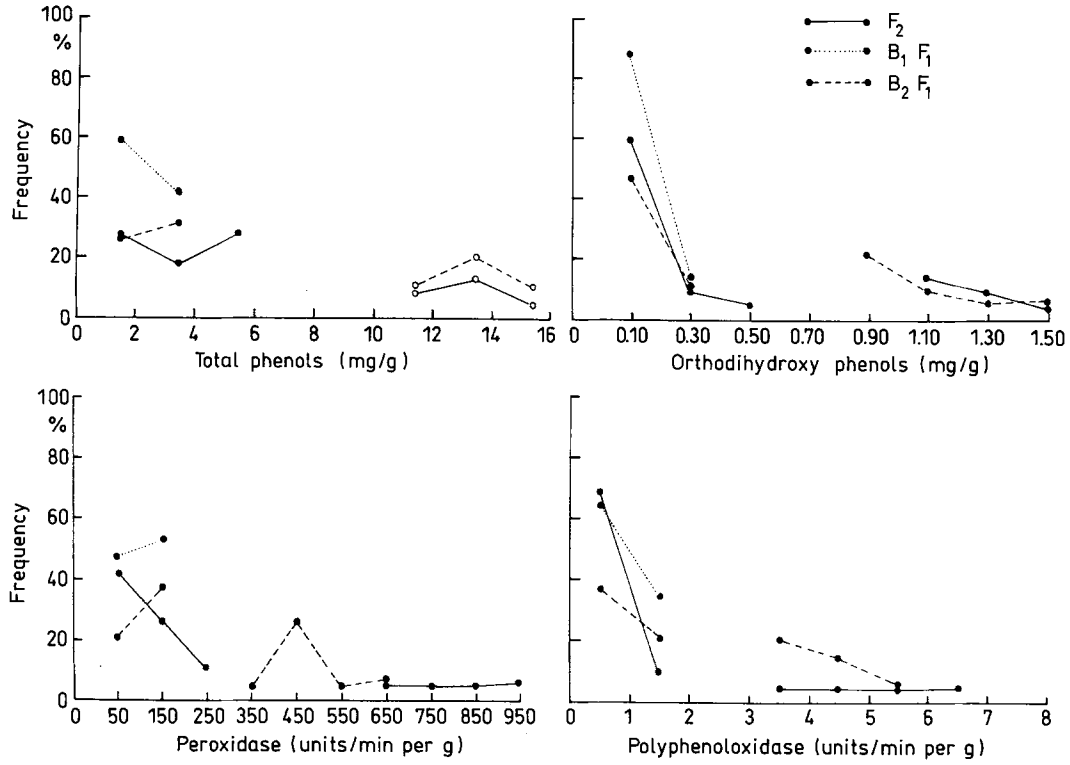

Fig. 1

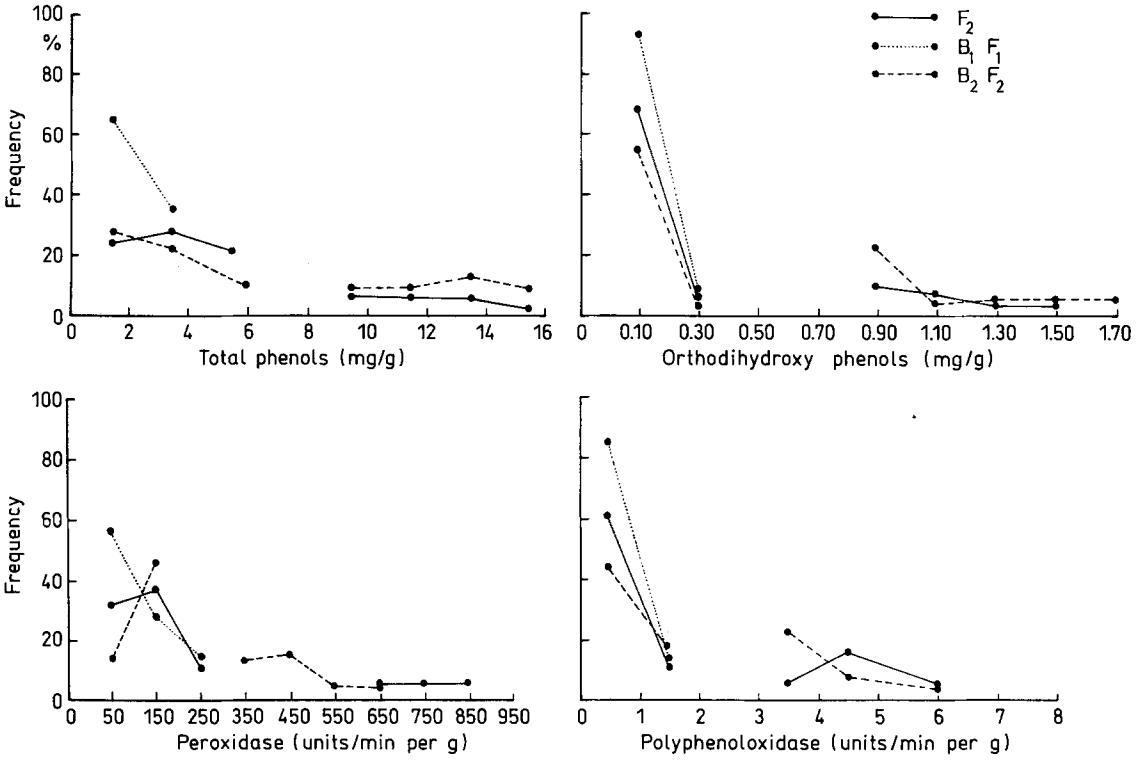

Fig. 2

Figs. 1 and 2. Frequency curves showing distributions of biochemical constituents in segregating generations **1** of the cross 'Bonneville' × P 185, and **2** of the cross 'Bonneville' × P 6583

Table 1. Inheritance of powdery mildew resistance in pea crosses

Cross	Generation	No. of plants		Ratio	χ^2	*P*-Value
		Resistant	Susceptible			
Bonneville × P 185	F_2	61	169	1:3	0.28	0.50–0.70
(Bonneville × P 185) × Bonneville	B_1F_1	0	93	0:1	–	–
(Bonneville × P 185) × P 185	B_2F_1	62	53	1:1	0.70	0.30–0.50
Bonneville × P 6583	F_2	56	152	1:3	0.41	0.50–0.70
(Bonneville × P 6583) × Bonneville	B_1F_1	0	108	0:1	–	–
(Bonneville × P 6583) × P 6583	B_2F_1	83	98	1:1	1.24	0.20–0.30

Table 2. Analysis of variance for biochemical constituents

Character	Source	Mean square
Total phenols	Genotypes	30.240*
	Error	0.740
Orthodihydroxy phenols	Genotypes	0.294*
	Error	0.003
Peroxidase	Genotypes	82861.250*
	Error	2100.650
Polyphenol oxidase	Genotypes	12.560*
	Error	0.060

* Significant at $P < 0.05$

Results and Discussion

The mode of inheritance of resistance to powdery mildew as determined from F_2 and backcross generations in 'Bonneville' × P 185 and 'Bonneville' × P 6583 crosses revealed that resistance is under the control of a single recessive gene (Table 1). In our biochemical studies, the generation means for phenolics and phenol oxidizing enzymes differed significantly (Table 2). The parents were also found to be different for all the traits, indicating their divergence (Table 3). Higher levels of total phenols and orthodihydroxy phenols, and increased activities of peroxidase and polyphenol oxidase were observed in resistant parents (P 185 and P 6583) in comparison to those found in the susceptible cultivar ('Bonneville') and their hybrids. The F_1s and backcrosses with the susceptible parent were comparable to the latter (Table 3), suggesting that the dominant gene for susceptibility hinders the phenolic pathway and the activity of the enzyme.

The frequency distribution graphs of the segregating populations for all the biochemical traits showed a bimodal distribution in the F_2 and the backcross generations of recessive parents of the cross 'Bonneville' × P 185 (Fig. 1) and 'Bonneville' × P 6583 (Fig. 2). This suggests that the synthesis of biochemical constituents is controlled by a major gene. The presence of transgressive segregants in segregating generations indicated the role of modifiers. Thus, there is the possibility that in the future suitable segregants having high levels of phenolics and increased enzyme activity can be isolated which will impart resistance to powdery mildew disease.

A high heritability and genetic advance for all the biochemical traits revealed that disease resistant plants in advance generations can be selected on the basis of high levels of biochemical constituents. This observation further supported by high correlation coefficients between biochemical parameters and the disease index (Table 4).

The scaling tests indicated the inadequacy of the additive-dominance model for the biochemical traits in both crosses (Table 5). Hence, only the epistatic model was appropriate. Both the additive ($\hat{d}$) and dominant ($\hat{h}$) gene effects were important in the inheritance of all biochemical traits in both crosses. As far as epistatic interactions are concerned, dominance × dominance ($\hat{l}$) was positive for total phenols in both crosses, while not one part of the epistatic component was significant for orthodihydroxy phenols in both crosses. However, the magnitude of dominance × dominance ($\hat{l}$) effect was higher than for other interactions. The enzymes peroxidase and polyphenol oxidase had a dominance × dominace ($\hat{l}$) interaction followed by an additive × dominance ($\hat{j}$) effect in each cross. A duplicate type of epistasis was shown by both the crosses for all characters except orthodihydroxy phenols.

The present study indicates that resistant genotypes possess an inherent capacity to produce high phenols and phenol oxidizing enzymes, and this capacity is highly heritable. Although these biochemical constituents may not be directly useful in the breeding of resistant cultivars, they will certainly facilitate the development of varieties which will be relatively stable against environmental influences. The nature of the gene action suggests that early generation isolates should be intermated in segregating populations in order to accumulate favourable additive genes. This system will ensure the full utilization of both additive and non-additive effects and will eventually lead to the fixation of the desired character at the desired level.

Table 3. Range, generation means and genetic parameters for biochemical constituents in pea crosses

Generation	Total phenols (mg/g)	Orthodihydroxy phenols (mg/g)	Peroxidase activity (units/min per g)	Polyphenol-oxidase activity (units/min per g)
Cross I				
Bonneville (P_1)	3.60– 4.41 (3.90)	0.04–0.09 (0.07)	70.04– 89.20 (78.26)	1.30–1.42 (1.36)
P 185 (P_2)	11.16–14.41 (12.54)	0.90–0.98 (0.94)	509.15–677.40 (593.30)	6.32–6.40 (6.37)
Bonneville × P 185 (F_1)	4.42– 6.65 (5.53)	0.08–0.10 (0.09)	20.15–25.43 (23.66)	0.40–0.58 (0.52)
B_1F_1	0.72– 4.41 (2.24)	0.00–0.18 (0.05)	21.66–194.74 (89.18)	0.00–1.83 (0.55)
B_2F_1	0.54–16.29 (6.92)	0.00–1.60 (0.51)	30.94–647.42 (265.15)	0.00–5.06 (1.94)
F_2	0.54–14.93 (5.94)	0.00–1.35 (0.34)	30.94–967.40 (250.02)	0.00–6.37 (1.24)
Cross II				
Bonneville (P_1)	3.60– 4.41 (3.90)	0.04–0.09 (0.07)	70.04– 89.20 (78.26)	1.30–1.42 (1.36)
P 6583 (P_2)	9.82–11.81 (10.81)	0.81–0.86 (0.83)	445.20–504.70 (469.91)	5.38–5.56 (5.46)
Bonneville × P 6583 (F_1)	4.72– 5.30 (5.11)	0.15–0.20 (0.18)	116.80–190.18 (148.99)	0.36–0.61 (0.49)
B_1F_1	0.69– 4.51 (2.25)	0.00–0.20 (0.06)	30.94–218.40 (111.22)	0.00–1.36 (0.30)
B_2F_1	0.71–17.15 (6.75)	0.00–1.98 (0.51)	59.15–624.60 (268.31)	0.00–6.37 (2.16)
F_2	1.53–15.83 (5.60)	0.00–1.62 (0.31)	35.69–915.60 (257.27)	0.00–5.89 (1.45)
Mean	6.14	0.35	232.30	1.98
C.D. at 5% level	1.46	0.09	78.06	0.42
Phenotypic coefficient of variation	52.95	90.35	73.33	103.87
Genotypic coefficient of variation	51.06	88.98	70.63	103.13
Heritability	92.99	97.00	92.76	98.58
Genetic advance	101.39	182.69	140.13	211.28

Values within parentheses are generation means

Table 4. Correlations of biochemical constituents with disease index in pea crosses

Cross	Generation	Total phenols	Orthodihy-droxy phenols	Peroxidase	Polyphenol oxidase
Bonneville × P 185	F_2	−0.99*	−0.96*	−0.97*	−0.96*
(Bonneville × P 185) × Bonneville	B_1F_1	−0.99*	−0.93*	−0.92*	−0.95*
(Bonneville × P 185) × P 185	B_2F_1	−0.97*	−0.97*	−0.95*	−0.97*
Bonneville × P 6583	F_2	−0.94*	−0.91*	−0.91*	−0.97*
(Bonneville × P 6583) Bonneville	B_1F_1	−0.99*	−0.94*	−0.98*	−0.96*
(Bonneville × P 6583) × P 6583	B_2F_1	−0.96*	−0.91*	−0.95*	−0.94*

* Significant at $P < 0.01$

Table 5. Estimation of gene effect on biochemical characters

Cross	m̂	d̂	ĥ	î	ĵ	Î
Total phenols						
Bonneville × P 185	13.66**	−4.32**	−22.75**	−5.44**	−0.72	14.62**
Bonneville × P 6583	11.75**	−3.45**	−17.97**	−4.40**	−2.14*	11.33**
Orthodihydroxy phenols						
Bonneville × P 185	0.74**	−0.43**	−0.96**	−0.24	−0.05	0.31
Bonneville × P 6583	0.55*	−0.38**	−0.59	−0.10	−0.14	0.22
Peroxidase						
Bonneville × P 185	627.20**	−257.52**	−905.18**	−291.42**	163.10**	301.64**
Bonneville × P 6583	544.10**	−195.82**	−752.22**	−270.02*	77.47	357.11*
Polyphenoloxidase						
Bonneville × P 185	3.84**	−2.50**	−7.09**	0.02	2.23**	3.77*
Bonneville × P 6583	4.29**	−2.05**	−7.56**	−0.88	0.40	3.76**

* Significant at $P < 0.05$
** Significant at $P < 0.01$

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