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DYNAMICS OF LEAF PHENOLIC CONTENT AND CONIDIAL GERMINATION IN RELATION TO POWDERY MILDEW RESISTANCE IN GARDEN PEA GENOTYPES

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Summary

The role of phenolics in mechanism of resistance to powdery mildew in resistant garden pea genotypes CHPMR-1, CHPMR-2, CHP-1 and CHP-2 was studied during 2002-2004 at HARP, Ranchi. In case of wall bound phenol, the content in genotypes CHPMR-1, CHPMR-2 and NDVP-8 (susceptible), did not differ significantly between pre-flowering stage and 45 days after flowering, whereas a drastic reduction in the content of wall bound phenol was observed during the peak flowering stage. In genotypes CHP-1 and CHP-2, there was a drastic reduction in the content of wall-bound phenol during 45 days after flowering. The line CHPMR-1 had significantly higher content of wall-bound phenols than that in line NDVP-8 at all the three stages of observation during both the years whereas no such trend was observed in case of wax bound phenol. The line CHPMR-2 had significantly higher content of wax bound phenols at pre-flowering and at 45 days after flowering and wall bound phenol only at peak flowering stage than that in line NDVP-8 during both the years. The maximum activity of Phenylalanine ammonia-lyase enzyme was observed at both pre-flowering and peak flowering stages in case of CHPMR-1, whereas in other genotypes, the activity did not differ significantly. Thus, cell wall-bound phenols might be one of the components of the complex defense mechanism operating in plants of garden pea genotype CHPMR-1 against powdery mildew fungus whereas, in the genotypes CHP-1, CHP-2 and CHPMR-2 both wax-bound and wall-bound phenols might play important role.

सारांश

बागवानी एवं कृषि वानिकी शोध कार्यक्रम, राँची से विकसित सब्जी मटर की चूर्णिल असिता प्रतिरोधी किस्में जैसे– सी.एच.पी.एम.आर.–1, सी. एच.पी.एम.आर.–2, सी.एच.पी.–1 एवं सी.एच.पी.–2 की चूर्णिल असिता प्रतिरोधिता के साथ फिनॉल यौगिकों की भूमिका का अध्ययन किया गया। सी.एच.पी.एम.आर.–1 की चूर्णिल असिता प्रतिरोधिता के लिए पौधे की कोशिका भित्त के साथ संलग्न फिनॉल यौगिक की भूमिका ज्यादा महत्वपूर्ण पाई गई। सी.एच.पी.एम.आर.–2, सी.एच.पी.–1 एवं सी.एच.पी.–2 की चूर्णिल असिता प्रतिरोधिता के लिए पौधों में बाहरी आवरण (एपीडर्मल लेयर) स्थित मोम–संयुक्त फिनॉल यौगिक एवं कोशिका भित्त स्थित फिनॉल यौगिक दोनों की भूमिका पाई गई। अध्ययन से यह भी ज्ञात हुआ कि सी. एच.पी.एम.आर.–1 में प्रतिरोधिता गुण सम्बन्धित एनजाइम फिनाइल–एलानिन एमोनिया लाईएज की गतिविधि पौधों में फल आने के पूर्व तथा फूल आने के समय अधिक होती है।

Introduction

Powdery mildew (*Erysiphe pisi*) is the most serious disease of garden pea particularly in mid and late season crop in the subtropical region. It causes around 24 to 27% yield loss of fresh pods of the crop (Singh, 1987) in addition to loss in quality and taste of shelled green peas. Role of phenolics in resistance mechanism to powdery mildew has been studied by number of workers (Parashar and Sindhan, 1986; Bhattacharya and Shukla, 2000). However, information on dynamics of accumulation of different kinds of phenol with respect to powdery mildew resistance in garden pea is limited. Different phenols like wax- and wall- bound phenols have been reported to influence plant resistance mechanism to diseases at different stages of pathogenic invasion (Kumar *et al.*, 1997). It is logical that an alteration in tissue susceptibility to the pathogen correlates with a corresponding change in the factor that governs host resistance. Bhattacharya and Shukla (2000) observed higher phenol levels, accompanied by higher activities of o-diphenolase and catalase, in resistant crops and in healthy plants of the susceptible cultivar, indicating that high activities of o-diphenolase and catalase were associated with the reduction of mildew severity in pea.

Genotypes might differ in their defense mechanism influenced by accumulation of different kinds of phenols and other biochemical factors at different critical stages of crop growth. Concerted breeding efforts at HARP, Ranchi has resulted in development of four resistant and high yielding lines of garden pea viz., CHP-1, CHP-2, CHPMR-1 and CHPMR-2. The present investigation was an attempt to reveal the mode of resistance mechanism in different genetic background of these powdery mildew resistant pea genotypes with respect to the role of phenolics and activity of PAL enzyme.

Materials and methods

The present study was undertaken during 2002 to 2004 at Horticulture and Agro-forestry Research Programmme (HARP), Plandu,, Ranchi-834010 located at 620 m above mean sea level with a longitude of 85° 20′ to 85° 95′ and latitude of 23° 15′ to 23° 18′ North. The experimental material consisted of four powdery mildew resistant lines viz., CHPMR1, CHPMR2, CHP1, CHP2 developed at HARP, Ranchi (Anonymous, 2000) through hybridization followed by selection, and one susceptible line NDVP-8. The experiment was laid out in the field in Randomised Block Design with 6 replications (one plot of 3m x 2m size per replication) with plant spacing of 30 cm x 10 cm. In both the years, the crop was grown during the last week of October to end of February. Recommended package of practices were uniformly followed in all the plots to raise successful crop.

Scoring of the chosen genotypes for resistance to powdery mildew (in 0-5 point scale) was carried out during 2001-02 to 2003-04 on the basis of symptom expression on the top five leaves at 45 days after peak flowering as suggested by Kumar (1995).

For studying the content of acid soluble wax- and wall-bound phenol in the leaf and stipule in the five genotypes in response to powdery mildew incidence, random plant sample from top five leaves were collected at three stages of plant growth viz., pre-flowering (40 days after sowing), peak flowering and 45 days after peak flowering stage. Estimation of wax- and wall- bound phenol content was done following the method suggested by Kumar and Sridhar (1985). For extraction of acid soluble-wax bound phenol, 10 gm of freshly collected leaves were washed in chloroform for removal of wax on the surface. After drying of chloroform, the wax bound phenol was hydrolysed in 1N HCl overnight and partitioned thrice in ethyl acetate. The pooled ethyl acetate fraction was evaporated under vacuum followed by dissolving the residue (phenol) in 5 ml of distilled water. The content of phenol was estimated through spectrophotometer (UV5704SS, ECIL Make) using Folin Ciocalteu reagent (Malik and Singh, 1980). During estimation of acid soluble wall bound phenol, the leaves, after removal of wax bound phenol were ground and washed in acetone for removal of all the chlorophyll pigments followed by washing in hot water for prevention of enzymatic oxidation of phenols. The residue comprising of cell wall material was air dried after washing with diethyl ether. The extraction and estimation of the content of acid soluble wall bound phenol was done using the same procedure as followed in case of wax bound phenol. The data was subjected to analysis of variance.

The activity of Phenylalanine ammonia-lyase (PAL) enzyme of the leaves of the five pea genotypes was measured during 2003-04 at pre-flowering stage and peak flowering stage. The activity of PAL enzyme was measured using the procedure given by Thimmaiah (1999). The enzyme was extracted by grinding 3.0 g of leaf tissue in 2.6 ml of 0.2M sodium borate buffer (pH 8.7) containing 2-mercaptoethanol (0.8 ml/litre) and collecting the supernatant after centrifuging at 7000g. The enzyme activity was assayed by incubating 0.1 ml of enzyme extract in a test tube containing 1ml of 0.05M Tris-HCL buffer, pH 8.8, 0.5 ml of 0.01M L-Phenylalanine and 0.4 ml of water at 30°C for 5 minutes and estimating the amount of cinnamic acid formed by observing the absorbance at 268 nm and drawing a standard curve using standard *t*-cinnamic acid. The data was subjected to analysis of variance.

Results and discussion

Powdery mildew is a troublesome disease under prolonged warm, dry day time conditions and when nights are cool enough for dew formation. A number of works have been carried out around the globe for development of powdery mildew resistant lines of peas. Screening for resistance is done on the basis of natural field infections using a late sowing technique to encourage the disease. From the present investigation, the data on scoring pattern of different genotypes with respect to disease intensity is presented in Table 1. As evident from the table, the powdery mildew scores of the developed lines viz., CHP 1, CHP 2, CHPMR 1 and CHPMR 2 were at par with each other during all the three years of observation and screened as resistant. The disease score value of the line NDVP 8 was significantly higher than the other genotypes and screened as susceptible.

Phenols are products of shikkimate pathway in the plant that play an important role in imparting resistance to different diseases. Lignifications of cell wall may act as a physical barrier by limiting the diffusion of fungal enzymes toxins from the infection site to the healthy tissue. These phenolics esters are not restricted

to lignified cells, but also occur in the cell walls of growing tissue (Harris and Hartley, 1976). The data on dynamics of wax bound phenol and wall bound phenol content in the leaves and stipules of different pea genotypes in the present study (mean value of years 2002-03 and 2003-04) have been depicted in Figures 1 & 2, respectively. As evident from Fig 1, the content of wax-bound phenol declined from pre-flowering stage to 45 days after flowering in all the genotypes. In case of wall bound phenol, the content in genotypes CHPMR-1, CHPMR-2 and NDVP-8, did not differ significantly between pre-flowering stage and 45 DAF, whereas a drastic reduction in the content of wall bound phenol was observed during the peak flowering stage (Fig 2). In genotypes CHP-1 and CHP-2, there was a drastic reduction in the content of wall-bound phenol during 45 DAF. Parashar and Sindhan (1986) reported a decline in total phenol and ortho dihydroxy phenol with increasing age of the plant in both resistant and susceptible genotypes of pea in response to powdery mildew infection.

As evident from the Table 2, the genotype CHP-1 had significantly higher content of wax-bound phenol, at peak flowering and 45 DAF than that in the susceptible line NDVP-8 whereas they were at par during pre-flowering stage in both the years of observation. The line had significantly higher content of wall bound phenol than that in NDVP 8, at peak flowering stage whereas, the content was lower during pre-flowering and 45 DAF. Similar trend was also observed in case of line CHP-2 except that it had significantly higher wall bound phenol content at pre-flowering stage. It must be noted here that both the lines (CHP-1 and CHP-2) have been derived from common parents (HC-17-11 and Arka Ajeet). Similar parentage of the lines might have contributed towards similar types of accumulation of wax and wall bound phenols. Higher accumulation of wax-bound phenol at peak flowering and 45 DAF might have played a major role in the resistance mechanism in these two pea genotypes. The higher accumulation of wall bound phenol in the susceptible line NDVP 8 during the later period may be attributed to the initiation of hypersensitivity reaction in this genotype at later stages of plant growth in response to the disease infection.

The line CHPMR-1 had significantly higher wall bound phenols than that in line NDVP-8 at all the three stages of observation during both the years whereas no such trend was observed in case of wax bound phenol. This

Table 1. Reaction of pea genotypes to powdery mildew

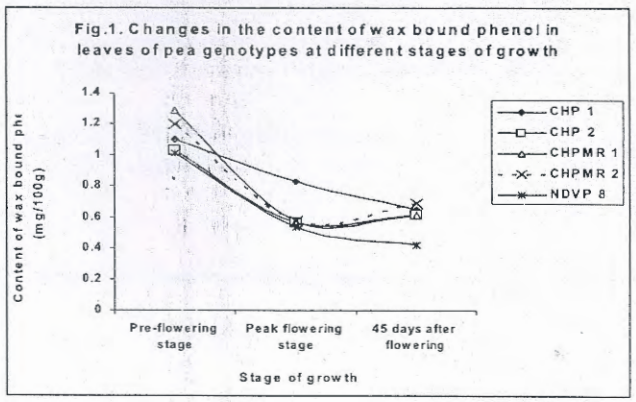
Genotypes	Powdery mildew severity score		
	2001-02	2002-03	2003-04
CHP 1	1.32	1.50	1.40
CHP 2	1.75	1.50	1.50
CHPMR 1	1.38	1.67	1.50
CHPMR 2	1.34	1.50	1.50
NDVP 8	5.00	5.00	5.00

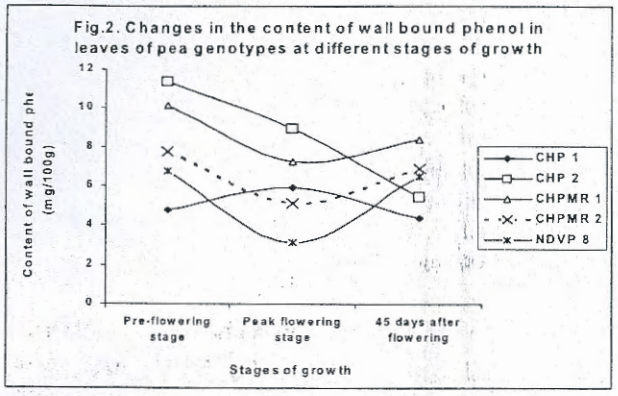
Powdery mildew severity scale: Highly resistant – 0-1.0, Resistant – 1.1-2.0, Moderately resistant- 2.1-3.0, Susceptible-3.1-5.0

indicated the sole role of wall bound phenol in governing resistance to powdery mildew in this genotype. Kumar *et al.* (1997) had reported that resistance offered by cell wall bound phenols to pathogens may not be a universal mechanism, but may be of significance under certain conditions.

The line CHPMR-2 had significantly higher content of wax bound phenols at pre-flowering and at 45 days after flowering and wall bound phenol only at peak flowering stage than that in line NDVP-8 during both the years of observation. This indicated at the decisive role of wax bound phenols in imparting resistance to powdery mildew. Though the content of wax bound phenol was not significantly higher during the peak flowering stage, higher accumulation of pre-formed phenols at pre-flowering stage and higher value at 45 DAF might have contributed towards the resistance in the genotype in both the years.

The Phenylalanine Ammonia-Lyase enzyme (PAL) plays a pivotal role in the biosynthesis of plant phenolics and has been extensively studied for correlating disease resistance in different vegetable crops. However, Guleria *et al.* (2005) hinted that PAL by itself is not the sole limiting factor in the production of phenolics in pea. In the present investigation, at both pre-flowering and peak flowering stages the maximum activity of PAL enzyme was observed in





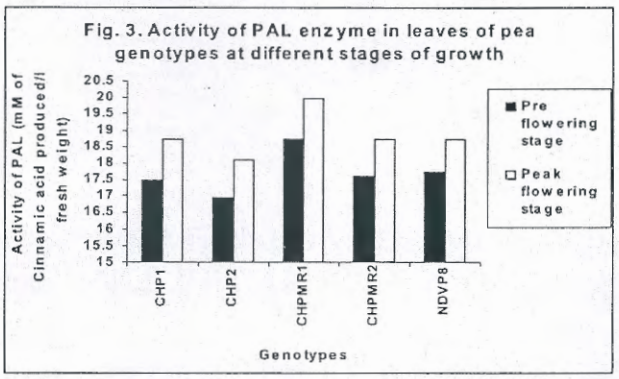


Table 2. Content of wax bound- and wall bound phenol in leaves of pea genotypes at different stages of plant growth

Genotypes	Pre-flowering stage				Peak flowering stage				45 days after flowering			
	Wax bound phenol (mg/100g freshweight)		Wall bound phenol (mg/100g freshweight)		Wax bound phenol (mg/100g freshweight)		Wall bound phenol (mg/100g freshweight)		Wax bound phenol (mg/100g freshweight)		Wall bound phenol (mg/100g freshweight)	
	2002-03	2003-04	2002-03	2003-04	2002-03	2003-04	2002-03	2003-04	2002-03	2003-04	2002-03	2003-04
CHP 1	0.89	1.31	5.28	4.22	0.96	0.70	6.31	5.49	0.74	0.56	5.99	2.71
CHP 2	0.78	1.28	12.36	10.39	0.62	0.51	9.65	8.20	0.68	0.56	8.65	2.25
CHPMR 1	1.32	1.25	9.66	10.59	0.60	0.55	8.25	6.27	0.72	0.50	10.26	6.48
CHPMR 2	1.02	1.37	9.01	6.50	0.58	0.57	6.52	3.74	0.71	0.67	9.35	4.39
NDVP 8	0.77	1.26	6.99	6.45	0.58	0.49	3.61	2.59	0.36	0.48	8.88	4.08
S.E.m.	0.032	0.024	0.461	0.433	0.028	0.029	0.553	0.479	0.026	0.026	0.509	0.427
C.D. at 5%	0.094	0.071	1.356	1.273	0.083	0.086	1.628	1.411	0.079	0.077	1.497	1.315

case of CHPMR-1 whereas in other genotypes, the activity did not differ significantly. The activity of PAL enzyme was found to increase during peak flowering stage (Fig.3). Kalia (1998) also reported increased activity of PAL enzyme in powdery mildew resistant genotypes in response to infection. However, the decline in the content of wax bound- and wall bound phenol in the leaves of different genotypes during peak flowering stage in the present investigation can be attributed to role of PAL on biosynthesis of other products of shikkimate pathway.

Thus, resistance offered by cell wall bound phenols might be one of the components of the complex defense mechanism operating in plants of garden pea genotype CHPMR 1 against powdery mildew fungus whereas, in the genotypes CHP 1, CHP 2 and CHPMR 2 both wax bound and wall-bound phenols might play important role.

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