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Changes in crude protein, phenols and lignin in different mustard leaves infected with powdery mildew disease in both naturally infected and fungicide treated plants

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ABSTRACT

The experiment was conducted with five different cultivars, the results of these study showed that phenols play important roles in disease resistance in both fungicide treated and untreated plants. The phenol content decreased in diseased plants with the advancement of diseases from S_1 to S_3 stage. Change in lignin content was significantly higher in control plants as compared with the diseased plants in all mustard cultivar. Among the different cultivars, lignin content was higher at pre infectional stage (S_1) than the infectional stage (S_2) in diseased plants. In control plants it showed increasing pattern except in single cultivars.similar trend also observed for crude protein.

INTRODUCTION

Powdery mildew caused by *Erysiphe polygoni*. DC is one of the major diseases among the leaf diseases. Powdery mildew of mustard (*Erysiphe polygon*i. DC) is an obligate parasite. Pathologists reported that damage to mustard crop may be very severe (@ 17.4%) when disease appears in early stages of plant growth[4]. The disease is successfully controlled by synthetic chemicals, however indiscriminate uses of such chemicals have created resistance in pathogen and environmental pollution becomes potential threat to human and animal health. To obviate these losses it is imperative to study the host parasite interaction at the biochemical level that leads to the better understanding of the biochemical and molecular mechanism of disease resistance in plants and possibly to the solution of even more important task of inducing resistant in the susceptible plants. All natural resistance is governed by genes and which are expressed through biochemical products such as phytoalexins, phenolics, lignins, callose formation, cellwall degrading enzymes, epicuticular wax composition and pathogen related protein. Hence, efforts are being made to find out alternative, which may be safe and eco-friendly so, always need to develop a resistant cultivar through breeding method or through genetically modified plant. Therefore the studies made to identified maximum change in cultivar for phenols, protein and lignin.

MATERIALS AND METHODS

Leaves of diseased scored cultivars viz two medium susceptible (3 & 3.5, SKM-9801, Skm- 9804) one susceptible(4.5: GM-1) and two highly susceptible (5 :Varuna and Skm-9818) were harvested at 75 DAS,

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TABLE 1 : Changes in crude protein content (mg.g^{-1}.dry.wt) in leaves of mustard cultivars at different stages of infection

Cultivar	Treatment	(S_1) Pre infectional stage	(S_2) infectional stage	S_3 Post infectional stage	Mean (VxT)
V_1 (SKM-9804)	Diseased	94.75	99.00	92.13	95.29
	Control	73.875	70.187	69.56	71.21
	(Mean)	84.315	84.595	80.84	
V_2 (SKM-9801)	Diseased	88.94	96.56	92.56	92.687
	Control	69.25	71.56	65.18	68.663
	(Mean)	79.095	84.06	78.87	
V_3 (VARUNA)	Diseased	94.625	94.375	89.93	92.978
	Control	73.375	72.5	70.63	72.167
	(Mean)	84.0	84.438	80.28	
V_4 (SKM-9818)	Diseased	90.125	90.56	80.63	87.112
	Control	77.315	76.875	66.875	73.71
	(Mean)	83.755	83.718	73.76	
V_5 (GM-1)	Diseased	101.62	89.937	85.625	92.392
	Control	78.75	78.25	75.625	77.542
	(Mean)	90.185	84.09	80.625	
Mean (VxS)		84.27	83.98	78.98	
	S.Em	CD at5 %		S.Em	CD at5 %
S	0.22	0.636	TxV	0.402	0.519
V	0.284	0.821	VxS	0.492	1.42
T	0.179	1.42	VxTxS	0.697	2.01

TABLE 2 : Changes in lignin content (mg.g^{-1}.fr.wt) in leaves of mustard cultivars at different stages of infection

Cultivar	Treatment	(S_1) Pre infectional stage	(S_2) infectional stage	S_3 Post infectional stage	Mean (VxT)
V_1 (SKM-9804)	Diseased	0.18	0.10	0.44	0.24
	Control	0.26	0.18	0.58	0.34
	(Mean)	0.22	0.14	0.51	
V_2 (SKM-9801)	Diseased	0.21	0.20	0.61	0.34
	Control	0.22	0.23	0.65	0.367
	(Mean)	0.215	0.215	0.63	
V_3 (VARUNA)	Diseased	0.22	0.18	0.53	0.310
	Control	0.26	0.29	0.63	0.393
	(Mean)	0.24	0.235	0.58	
V_4 (SKM-9818)	Diseased	0.19	0.14	0.50	0.277
	Control	0.22	0.34	0.68	0.413
	(Mean)	0.205	0.24	0.58	
V_5 (GM-1)	Diseased	0.13	0.25	0.56	0.313
	Control	0.24	0.27	0.64	0.383
	(Mean)	0.185	0.26	0.60	
Mean (VxS)		0.213	0.218	0.582	
	S.Em	CD at5 %		S.Em	CD at5 %
S	0.003	0.01	TxV	0.009	0.025
V	0.005	0.01	VxS	0.005	0.016
T	0.028	0.08	VxTxS	0.012	0.035

when there were no visual symptoms of disease infection and leaves were green and healthy (S_1). Subsequently leaves were harvested at 85 DAS, when the disease covered with 60-70% powdery mass (infection process S_2) and also at 100 days when plants were in advanced stages of powdery mildew infection (S_3). For estimating total phenols, protein and lignin.

For the estimation of crude protein content the standard micro-kjeldahl procedure (A.O.A.C., 1965) was followed. The protein content was determined by the method of Lowry et al.[10]. Estimation of total phenol content was carried out by following method of Bray and Thorpe[9].

RESULTS AND DISCUSSIONS

Total protein

The total protein content in leaves of fungicide treated plants i.e. control and naturally infected plants of five Brassica cultivars at different stages of disease development are presented in TABLE 1 and figure 1.

At the pre infectional stage (S_1), the total protein content in leaf obtained from control plant significantly varied from cultivar to cultivar and varied between 78.75 to 69.25mg.g^{-1}.dry.wt. Incase of leaves from diseased plants, protein content varied from 88.94-101.62mg.g^{-1}.dry.wt. In general, it was seen that leaves obtained from control (Treated) plants had significantly lower level of total protein as compared with the diseased plant.

The total protein content in leaves from control plants at infectional stage (S_2) varied from 70.19 to 96.56 (mg.g^{-1}. dry wt). The total protein content was significantly decreased from S_1 to S_2 stage, except cultivar V_2 where it little increased. Incase of diseased leaves obtained from infectional stage (S_2), resulted significantly higher amount of the total protein as compared to the value recorded at the pre infectional stage except in cultivar V_3 and V_5 where it decreased by 0.26% and 11.5% respectively.

The control plants at post infectional stage (S_3) showed significantly decline in the total protein content as compared with the pre infectional stage (S_1) and infectional stage (S_2). Among the cultivars V_5 (75.6mg.g^{-1}. dry wt) had significantly higher value of protein con-

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TABLE 3 : Changes in total phenol content (mg.g^{-1}.fr.wt) in leaves of mustard cultivars at different stages of infection

Cultivar	Treatment	(S_1) Pre infectional stage	(S_2) infectional stage	S_3 Post infectional stage	Mean (VxT)
V_1 (SKM-9804)	Diseased	2.77	2.649	2.48	2.633
	Control	3.253	3.105	3.588	3.315
	(Mean)	3.011	2.877	3.034	
V_2 (SKM-9801)	Diseased	2.649	2.637	2.484	2.59
	Control	2.977	3.026	3.407	3.137
	(Mean)	2.813	2.832	2.946	
V_3 (VARUNA)	Diseased	1.759	1.904	1.863	1.842
	Control	2.70	2.787	3.399	2.962
	(Mean)	2.229	2.346	2.631	
V_4 (SKM-9818)	Diseased	1.975	1.915	1.898	1.929
	Control	2.407	2.468	2.772	2.549
	(Mean)	2.191	2.191	2.335	
V_5 (GM-1)	Diseased	1.865	1.734	1.639	1.746
	Control	2.321	2.416	2.923	2.553
	(Mean)	2.093	2.075	2.281	
Mean (VxS)		2.467	2.464	2.645	
	S.Em	CD at5 %		S.Em	CD at5 %
S	0.014	0.03	TxV	0.025	0.032
V	0.018	0.05	VxS	0.02	0.056
T	0.0115	0.0325	VxTxS	0.044	0.125

tent. Diseased leaves had also showed similar trend as observed for control plants through the magnitude of reduction was different. These observations are supported by Singh (2000) who evaluated total protein content in *Brassica* species against downey mildew and white rust of mustard. They revealed that the susceptible cultivars contained higher amount of total protein than the resistant and moderately resistant cultivars at all growth stages.

Overall it was observed that treated plants (control) resulted greater changes in cultivar V_3 and V_4 with the advancement of stages as compared with the others cultivars. Incase of diseased plant cultivar V_2 and V_5 showed greater change in the total protein.

Total phenol

Total phenol content in fungicide treated plants *i.e.* control and naturally infected with powdery mildew fungus in disease leaves of all five *Brassica cvs* at different stages of disease development have been depicted in TABLE 2 and figure 2.

At pre infectional stage (S_1), the leaf tissue obtained from control plant showed significantly higher phenol

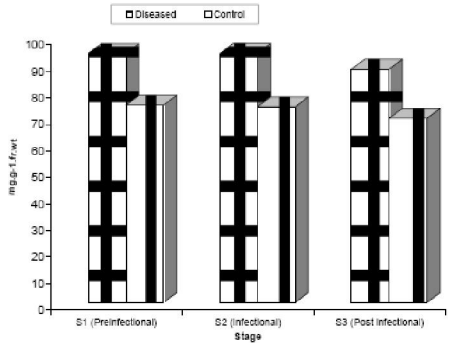

Figure 1 : Changes in mean value of (TxS) crude protein content (mg.g^{-1}. dry.wt) in leaves of mustard cultivars at different stages of infection. (S.Em, 0.311 and CD at 5%, NS)

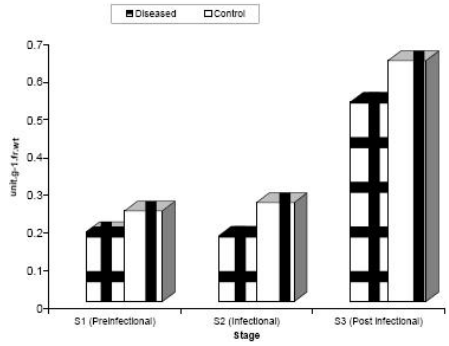

Figure 2 : Changes in mean value of (TxS) lignin content (mg.g^{-1}.fr.wt) in leaves of mustard cultivars at different stages of infection. (S.Em, 0.64 and CD at 5%, 1.86)

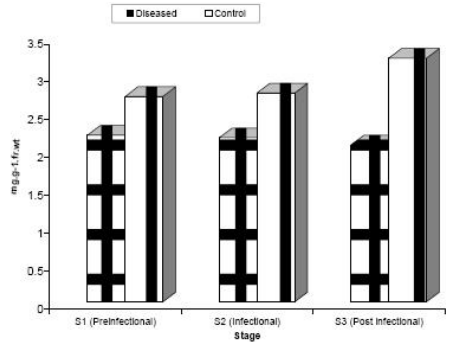

Figure 3 : Changes in mean value of (TxS) total phenol content (mg.g^{-1}. fr.wt) in leaves of mustard cultivars at different stages of infection. (S.Em, 0.02 and CD at 5%, 0.056)

content with cultivar V_1 (3.25mg.g^{-1} . fr.wt) and minimum with V_5 (2.32mg.g^{-1} .fr.wt) and it varied from cultivar to cultivar similarly the phenol content in leaf tissue of diseased plants varied from 1.76 to 2.77 (mg.g^{-1}.fr.wt). Overall, leaves obtained from control plants had significantly higher level of phenol content (19.33%) as compared with the diseased plants. Total phenol content in leaf tissue of treated plants at infectional stage (S_2) varied from 2.42 to 3.11mg.g^{-1}.fr.wt and the content was significantly increased in all the cultivars from S_1 to S_2 stage, except in cultivar V_1 where it was de-

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creased by 4.31%. Incase of diseased leaf tissue obtained at infectional stage (S_2), resulted significantly decreased in the phenol content as compared to the value recorded at pre infectional stage (S_1), except in cultivar V_2 where no change observed.

Thus at infectional stage (S_2) there was decrease in phenol content of leaf tissue obtained from diseased plants as compared with the pre infectional stage (S_1).

The control plants at post infectional stage (S_3) showed significant rise in phenol content as compared with both infectional (S_2) and pre infectional stage (S_1).

Among all the cultivars, it was observed that treated plants resulted less changes in cultivars V_3 and V_5 with the advancement of stages. Incase of diseased plants, all cultivars showed significant reduction in phenol content from S_1 to S_3 stage and cultivar V_5 showed greater percent change from S_1 to S_3.

Above results are in agreement with Parashar and Sindhan[5], who observed that resistant variety had higher level of total phenol and also orthodihydroxy phenols than susceptible varieties at both 80 and 90 days of pea plant in relation to powdery mildew.

Gupta et al.[2-4] indicated that an initial increase in the level of total phenols followed by continuous decrease with the age of plant in all *brassica spp*. due to alternaria blight disease. Tolerant species of brassica registered considerable higher amount of total phenols compared to susceptible ones at all stages of plant development. They also reported that phenol content declined after infection in all the species of *brassica* depending upon the severity of disease.

lignin content

Lignin content in fungicide treated plants i.e. control and naturally infected plants of five Brassica cultivars at different stages of disease development are presented in TABLE 3.

At the pre infectional stage (S_1), the lignin content in leaf tissue from control plants significantly varied from cultivar to cultivar. Higher levels of lignin content were recorded with the cultivars V_1 and V_3 (0.26 unit g^{-1} fr.wt). The lignin content of diseased plants varied from 0.13-0.22 unit/g fresh wt. Thus at pre infectional stage (S_1), it was seen that leaves obtained from control plants had significantly higher level of lignin content (13%) as compared with the diseased plants.

Lignin content in leaf of control plants at infectional stage (S_2) varied from 0.18 to 0.34 unit.g^{-1} fr.wt. All the cultivars showed that the lignin content was significantly increased from S_1 to S_2 stage. The percent increase was varied from 6.69-58.30 % except in cultivar V_1 where it decreased. Incase of diseased plants at infectional stage (S_2), the content significantly decreased as compared to the value recorded at the pre infectional stage except for the cultivar V_5 where the lignin content remarkably increased.

The treated plants, at post infectional stage (S_3) showed almost three times higher level of lignin content in all the cultivars as compared with the infectional stage (S_2). Diseased leaves at post infectional stage (S_3) had higher lignin content than the values recorded at infectional stage (S_2).

Among the cultivars, it was observed that treated plants resulted less changes in cultivar V_2 and V_5 with the advancement of stages. Incase of diseased plants all the cultivars showed significant rise in the lignin content from S_1 to S_3 stage. The cultivar V_5 showed greater percent change from S_1 to S_3 stages. These results are in agreement with Hegazai et al.[1]. They observed significant positive correlation between the level of lignin and the susceptibility of rice cultivars to brown spot disease. The level of lignin was also found higher in healthy while the infection caused a reduction in lignin content in both the cultivars.

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