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Changes in total chlorophyll and carbohydrates in different mustard leaves infected with powdery mildew disease in both naturally infected and fungicide treated plants

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ABSTRACT

Changes in total chlorophyll, total soluble sugar, reducing sugar and non reducing sugar were observed in five different cultivars of mustard in response to powdery mildew viz, Skm-9801, skm-9804, GM-1, Varuna and Skm-9818 at different stages of disease infection. In fungicide treated plant ie. Control plants significantly higher value for total soluble sugar, chlorophyll and reducing sugar where as reverse trend obsereved in non reducing sugar content in all cultivars. Among the different cultivars no similar trend was observed in diseased infectional stages.

INTRODUCTION

Mustard, *Brassica Juncea* (Czern. and Coss.) is the most popular one among different species of rapeseed and mustard being grown in winter in India since 2000 BC. These days mustard seed is mainly used to extract edible oil. The seed also serve as an important raw material for the agro based industries and also used as condiments and medicines[15]. The residual cake is valuable by products used as a feed for animals and rich source of organic manure. The crop is affected by several diseases which cause enormous losses in yield. Among the several diseases, alternaria blight ,white rust, downy mildew, powdery mildew and phyllody considered as major diseases, on the basis of their wide distribution and yield losses. 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 and may persist on the host plant of *Brassica spp.* and other weeds may carry the fungal mycelium during off season. Carbohydrates content significantly contributed to disease resistance and could be the most important factor to be considered for improving disease resistance. This investigation made to study effect of hexaconezole on leaf metabolites viz chlorophyll and carbohydrates at various stages of disease development.

MATERIAL 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, 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 chlorophyll, total sugar and reducing sugar.

Chlorophyll estimation was carried by the method of Arnon[1]. 100mg fresh leaves were cut in to small pieces and homogenized with chilled acetone: water

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TABLE 1 : Changes in total chlorophyll 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	0.927	0.860	0.603	0.802
	Control	1.325	0.870	1.848	1.348
	(Mean)	1.126	0.873	1.226	
V_2 (SKM-9801)	Diseased	1.271	0.583	1.051	0.968
	Control	1.325	0.833	1.465	1.208
	(Mean)	1.298	0.708	1.258	
V_3 (VARUNA)	Diseased	1.279	0.583	1.465	1.109
	Control	1.289	0.887	1.845	1.34
	(Mean)	1.284	0.735	1.655	
V_4 (SKM-9818)	Diseased	1.284	0.762	1.096	1.047
	Control	1.289	1.104	1.378	1.257
	(Mean)	1.287	0.933	1.237	
V_5 (GM-1)	Diseased	0.833	0.843	0.63	0.769
	Control	1.296	1.166	1.244	1.235
	(Mean)	1.065	1.005	0.937	
Mean (VxS)		1.212	0.851	1.263	
	S.Em	CD at5 %		S.Em	CD at5 %
S	0.015	0.03	TxV	0.024	0.075
V	0.014	0.04	VxS	0.025	0.079
T	0.09	0.02	VxTxS	0.036	0.105

TABLE 2 : Changes in total soluble sugar 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	10.26	8.68	8.417	9.119
	Control	10.42	17.038	18.14	15.199
	(Mean)	10.34	12.859	13.279	
V_2 (SKM-9801)	Diseased	9.785	8.02	8.68	8.82
	Control	10.68	15.615	17.295	14.533
	(Mean)	10.236	11.818	12.988	
V_3 (VARUNA)	Diseased	8.713	8.008	8.73	8.303
	Control	9.28	14.92	19.03	14.41
	(Mean)	8.726	11.416	13.88	
V_4 (SKM-9818)	Diseased	7.29	7.943	9.05	8.049
	Control	9.29	16.83	18.95	15.023
	(Mean)	8.29	12.386	14.00	
V_5 (GM-1)	Diseased	7.22	9.138	9.23	8.529
	Control	9.48	16.74	19.14	15.12
	(Mean)	8.35	12.939	14.185	
Mean (VxS)		9.189	12.29	13.666	
	S.Em	CD at5 %		S.Em	CD at5 %
S	0.0587	0.128	TxV	0.083	0.104
V	0.045	0.165	VxS	0.101	0.286
T	0.037	0.104	VxTxS	0.143	0.404

(80:20 V/V) using a mortal and pestle. The extract was filtered through whatman No.1 filter paper, filtrate was collected and volume made upto 10ml with 80% Acetone. Absorbance was measured at 645 and 663nm for determination of chlorophyll. The total chlorophyll was calculated as:

$$\textbf{Total chlodrophyl} = \frac{20.2A_{645} + 8.02A_{663}}{a \times 1000 \times W} \times V$$

Where,

a = Length of light path in the cell (usually 1cm)

V = Vol. of the extract in ml

W = Fresh weight of the sample in gram

A_{645} = Optical density measured at 645 nm

A_{663} = Optical density measured at 663 nm

Total soluble sugar content was estimated by following the method of Dubois et al.[4] with some modifications. And from the same extract was used for the analysis of the reducing sugar content. It was estimated by the method of Nelson (1994).

Total chlorophyll content

At pre infectional stage (S_1), the total chlorophyll in leaf from control plant significantly varied from cultivar to cultivar and it was ranged from 1.289 to 1.325mg.g^{-1} fr.wt (TABLE 4). Similarly the chlorophyll content in leaves of diseased plants varied from 0.833-1.284 (mg. g^{-1}. fr.wt). Overall it was seen that leaves from treated plants had significantly higher chlorophyll content (26.52 %) as compared with the diseased plants at pre infectional stage *i.e.*.S_1 (Figure 1).

Total chlorophyll content in leaves of treated plants (control) at infectional stage (S_2) varied from 0.833 to 1.166mg.g^{-1}.fr.wt. The total chlorophyll content was significantly decreased from S_1 to S_2 stage and percent reduction was varied from-7.26 to -37.1 %. Incase of diseased leaves obtained from naturally infected plants (S_2), resulted significantly less amount of total chlorophyll as compared to the value recorded at the pre infectional stage (S_1). Except in cultivar V_5 where it increased.

At post infectional stage (S_3), the treated plants showed significantly higher total chlorophyll content as compared with the pre infectional stage (S_1) and infectional stage (S_2). Cultivar, V_1 (1.848mg.g^{-1}. fr.wt) had significantly higher value than the cultivar V_2 (1.465mg.g^{-1}.fr.wt), while cultivar V_3, V_4, and V_5 were

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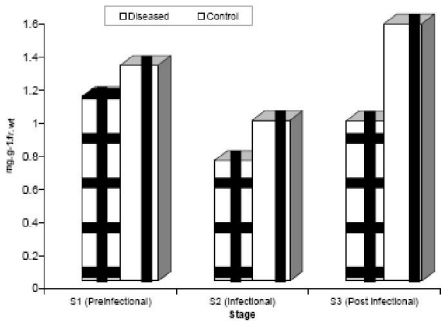

Figure 1 : Changes in mean value of (TxS) total chlorophyll content (mg.g^{-1}.fr.wt) in leaves of mustard cultivars at different stages of infection. (S.Em, 0.01 and CD at 5%, 0.04)

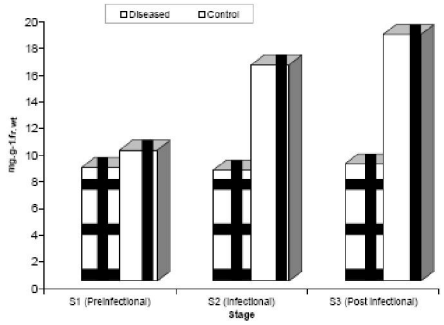

Figure 2 : Changes in total soluble sugar content (mg.g^{-1}.fr.wt) in leaves of mustard cultivars at different stages of infection. (S.Em, 0.064 and CD at 5%, 0.181)

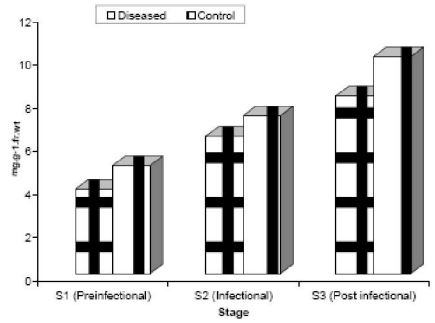

Figure 3 : Changes in mean value of (TxS) reducing sugars content (mg.g^{-1}.fr.wt) in leaves of mustard cultivars at different stages of infection. (S.Em, 0.015 and CD at 5%, 0.043)

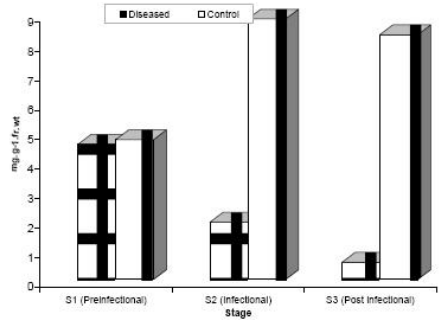

Figure 4 : Changes in non reducing sugar content (mg.g^{-1}.fr.wt) in leaves of mustard cultivars at different stages of infection. (S.Em, 0.014 and CD at 5%, 0.042)

at par. Diseased leaves had little higher value than the value recorded at infectional stage (S_2) except for the cultivar V_1 and V_5. These results are in agreement with Guleri et al.[5] who reported that chlorophyll content decreased at post infectional stage in powdery mildew infected leaves of pea compared to healthy leaves.

The reduction in the chlorophyll content may be due to inhibition of its production by fungus[8] or may be due to enhanced activity of chlorophyllase[3,9].

At the pre infectional stage (S_1), the total soluble sugars content in leaf from control plant significantly varied from cultivar to cultivar and cultivar V_5 had significantly higher value (10.68mg.g^{-1}.fr.wt) and minimum was recorded with V_2 (9.25mg.g^{-1}.fr.wt). Similarly the sugars content in leaves of diseased plants were varied from 7.22-10.26mg.g^{-1}.fr.wt. Overall it was seen that leaves obtained from control plants had significantly higher level of sugars (13 %) as compared with the diseased plants at pre infectional stage (S_1).

Total soluble sugar content in leaves from control plants at infectional stage (S_2) varied from 14.92 to 17.04mg.g^{-1}.fr.wt. The sugar value was significantly increased from S_1 to S_2 stage and the per cent increase was varied between 45.8-81.26%. Incase of diseased leaves obtained from naturally infected plants (S_2), resulted significantly less amount of total soluble sugars (7.94-9.14mg.g^{-1}.fr.wt) as compared to the value recorded at the pre infectional stage(S_1) except cultivars V_4 and V_5 where it increased by 8.9% and 26.9% respectively.

At post infectional stage (S_3), the treated plant showed significantly higher total soluble sugar as compared with the pre infectional stage (S_1) and infectional stage (S_2) cultivar V_1 (18.14mg.g^{-1}.fr.wt) had significantly higher value than the cultivar V_2 (17.29mg.g^{-1}.fr.wt), while cultivar V_3 , V_4, and V_5 were at par. Diseased leaves had little higher value than the value recorded at infectional stage (S_2) except for the cultivar V_1. Singh et al.[13,14] revealed that the *Brassica* cultivar showing differential reaction to downey mildew (*Pernospora parasitica*) incase of sugar content of both resistant and susceptible cultivars. Their findings indicated higher amount of total and reducing sugars in resistant cultivars than the susceptible cultivars at all the growth stages.

These results are in agreement with the result obtained by Gupta et al.[6]. They reported that sugar con-

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TABLE 3 : Changes in reducing sugar 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	4.323	7.671	8.16	6.718
	Control	6.272	9.06	10.048	8.416
	(Mean)	5.297	8.365	9.104	
V_2 (SKM-9801)	Diseased	5.117	5.90	8.01	6.342
	Control	7.377	8.85	9.928	8.718
	(Mean)	6.247	7.375	8.699	
V_3 (VARUNA)	Diseased	3.655	5.745	8.29	5.897
	Control	4.292	5.89	10.085	6.756
	(Mean)	3.973	5.818	9.188	
V_4 (SKM-9818)	Diseased	3.63	5.752	8.29	5.897
	Control	4.286	5.885	10.253	6.756
	(Mean)	3.959	5.819	9.406	
V_5 (GM-1)	Diseased	2.924	6.859	8.56	6.117
	Control	3.229	6.965	9.763	6.653
	(Mean)	3.076	6.914	9.166	
Mean (VxS)		4.5	6.858	9.167	
	S.Em	CD at5 %		S.Em	CD at5 %
S	0.019	0.0306	TxV	0.019	0.025
V	0.014	0.0396	VxS	0.034	0.097
T	0.008	0.025	VxTxS	0.034	0.097

TABLE 4 : Changes in non reducing sugar 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	0.594	1.053	0.31	2.434
	Control	4.15	7.98	7.838	6.656
	(Mean)	5.045	4.516	4.047	4.545
V_2 (SKM-9801)	Diseased	4.67	2.06	0.67	2.467
	Control	3.39	6.77	7.35	5.837
	(Mean)	4.03	4.415	4.01	4.152
V_3 (VARUNA)	Diseased	4.52	2.113	0.555	2.396
	Control	4.98	8.83	8.938	7.583
	(Mean)	4.75	5.471	4.746	4.989
V_4 (SKM-9818)	Diseased	3.55	2.22	0.76	2.177
	Control	5.003	10.94	8.45	8.131
	(Mean)	4.276	6.58	4.605	5.154
V_5 (GM-1)	Diseased	4.29	2.3	0.68	2.423
	Control	6.25	9.76	9.38	8.463
	(Mean)	5.27	6.03	5.03	
Mean (VxS)		4.674	5.403	4.493	
	S.Em	CD at5 %		S.Em	CD at5 %
S	0.010	0.029	TxV	0.019	0.024
V	0.134	0.038	VxS	0.020	0.065
T	0.008	0.024	VxTxS	0.033	0.093

tent was higher, when mustard leaves infected with Alternaria leaf blight, in first and second stage of disease development. Whereas resistant plants showed little higher sugar content than the susceptible varieties. Similar results also were recorded in cotton infected with grey mildew[2].

The higher level of total soluble sugars in fungicide treated plants may be due to mechanism to compensate for increase need of the normal plant growth. In diseased plants the total soluble sugars are used by both the plants and the fungus and the level of total soluble sugars possibly low in infected plants.

Reducing sugars

Reducing sugar 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 3 and figure 3.

At pre infectional stage (S_1), the reducing sugar content in leaf obtained from control plant significantly varied from cultivar to cultivar. Significantly higher reducing sugar content was recorded with the cultivar V_2 (7.38mg.g^{-1}.fr.wt) while minimum with the cultivar V_5 (3.23mg.g^{-1}.fr.wt). Similarly the reducing sugar content in leaves of diseased plants varied between 2.93-5.12mg.g^{-1} fr.wt. Overall it was seen that leaves from control plants had significantly higher level of reducing sugar (22.76%) as compared with the diseased plant at pre infectional stage (S_1).

Reducing sugars content in leaves of treated plants at infectional stage (S_2) varied from 5.89 to 9.06mg.g^{-1} fr.wt. The content significantly increased from S_1 to S_2 stage and percent change was varied from 19.45-116.37%. Incase of diseased leaves obtained from naturally infected plants (S_2), resulted significantly higher amount of reducing sugar (5.72-7.65mg.g^{-1} fr.wt) as compared to the value recorded at the pre infectional stage (S_1). Thus at infectional stage (S_2), there was increased in reducing sugar content in the leaves of all the cultivar obtained from diseased plants as compared with the pre infectional stage (S_1).

At post infectional stage (S_3), the treated plant showed significantly higher reducing sugar as compared with the pre infectional stage (S_1) and infectional stage (S_2). Cultivar V_4 (10.5mg.g^{-1}.fr.Wt) had significantly higher value than all other cultivar. Diseased leaves had

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little higher value than the value recorded at infectional stage (S_2) in all the cultivars. Results obtained from this study are in agreement with Parashar and Sindhan[7,10], Who observed that leaf of resistant variety (*Erysiphe polygoni*) had higher total soluble sugar and reducing sugar content than susceptible varieties after both 80 and 90 days of plant growth. They also showed that sugar content varied with age and in response to inoculation. Singh et al.[13,14] revealed that higher amount of reducing sugar in resistant brassica cultivar to downey mildew than the susceptible cultivar to at all growth stages.

In general, it was observed that treated plant (control) resulted less changes in cultivar V_1 and V_2 with the advancement of stages as compared with the others cultivars. Incase of diseased plant these two i.e. V_1 and V_2 cultivars showed similar trend though the magnitude was different for the reducing sugar while cultivar V_3, V_4 and V_5 showed greater change for reducing sugars. Similar findings were recorded by Yadav et al.[6,16] and reported higher content of reducing sugars in mustard genotype resistant to white rust. Singh et al.[13,14] indicated that reducing sugar content significantly contributed to disease resistance and could be the most important factor to be considered for improving resistance to *A. blight*.

Non reducing sugar

Non reducing sugar 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 depicted in TABLE 4 and figure 4.

At the pre infectional stage (S_1), the non reducing sugar content in leaf obtained from control plant significantly varied from cultivar to cultivar. Significantly higher value was recorded with the cultivar V_5 (6.25mg.g^{-1}. fr.wt) and minimum with V_2 (3.39mg.g^{-1}. fr.wt). Non reducing sugar content in leaves of diseased plants varied from 0.59 to 4.67mg. g^{-1}.fr.wt. Irrespective of cultivars, it was seen that leaves obtained from control had significantly higher level of non reducing sugar (3.38%) as compared with the diseased plant at pre infectional stage (S_1).

Non reducing sugar content in leaves of control plants at infectional stage (S_2) varied from 6.77 to 10.94mg.g^{-1}.fr.wt. The content was significantly increased from S_1 to S_2 stage and percent change was varied from 56.16-118.8%. Incase of diseased leaves obtained from naturally infected plants (S_2), showed significant reduction in non reducing sugar as compared to the value recorded at the pre infectional stage.

The findings are in agreement with Guleri et. al.[5] who noticed the higher percentage of rise in non reducing sugar content in resistant and susceptible cultivars and indicated that non reducing sugar may be involved in disease resistance. Sindhan and Parashar[7,10] stated that there were lower levels of non-reducing sugars in groundnut resistant cultivars to early and late leaf spot as compared to susceptible.

At post infectional stage (S_3), the treated plant showed significantly higher non reducing sugar as compared with the pre infectional stage (S_1). Diseased leaves resulted lower level of non reducing sugar content than the value recorded at infectional stage (S_2) for all the cultivars.

Among the cultivars, non reducing sugar in treated plants resulted less changes in cultivar V_1 and V_5 with the advancement of stages. Incase of diseased plants of all cultivar showed drastic reduction in the content from S_1 to S_3 stages of disease development.

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