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BIOCHEMICAL CHANGES IN CARTHAMUS TINCTORIUS L. CULTIVARS UNDER THE PATHOGENESIS OF *ALTERNARIA CARTHAMI*

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ABSTRACT

In the present investigation, changes in biochemicals like carbohydrates, chlorophylls, soluble protein and total free amino acids of healthy and infected leaves were analyzed in four cultivars of Carthamus tinctorius L. under pathogenesis of Alternaria carthami. The biochemicals were decreased in infected leaves as compared to healthy leaves. Carbohydrates and protein content was significantly declined in Nari-6 whereas chlorophylls and total free amino acids were greatly reduced in the infected leaves of Nari-38 and Nari-NH-1 respectively over healthy leaves as compared to other cultivars. The present investigation shows a decrease in carbohydrates, chlorophylls, protein and total free amino acids and also decrease in resistance in all cultivars under the pathogenesis of Alternaria carthami. The decrease in disease resistance was confirmed on the basis of morphological observations of the cultivars where they showed increased susceptibility to the pathogen in comparison to healthy cultivars.

KEYWORDS: *Carthamus, Alternaria, carbohydrates, protein, chlorophylls, amino acids.*

INTRODUCTION

Safflower (*Carthamus tinctorius*, L.) is one of the important oil seed crops and it is susceptible to number of fungal diseases i.e. *Alternaria* leaf spot (*Alternaria carthami*), *Cercospora* leaf spot (*Cercospora carthami*), Fusarium wilt (*Fusarium oxysporumf.sp. carthami*), Ramularia leaf spot (*Ramularia* spp.), Rust (Puccinia carthami) etc. Leaf blight caused by *Alternaria carthami* Chowdhury is one of the major diseases of safflower (Deokar et al. 1991). Foliar diseases are most destructive in India which reduces the yield about 90 percent.

The earlier studies carried by Chung (2012) showed that infection caused by *Alternaria* species produces toxin which leads to lipid peroxidation, generation of hydrogen peroxide and followed by cell death. During the coarse of infection, the fungal toxin produced by the fungus drastically alters the plant photosynthetic network along with physiological and biochemical status (Prakash, 2004; Mohsan *et al.*, 2011; Lin *et al.*, 2011). The role of sugars in plant pathogen interaction has been studied by the earlier workers and it was observed that ketohexose variation during infection is directly related to the degree of pathogen infection and plant resistance. The studies carried out by Srivastava & Pandey (2012) showed that the total carbohydrate decreased due to fungal infection. The fungal infection usually leads to the chlorosis and necrosis of the leaves which decreases the photosynthetic products (Ciuffetti *et al.*, 2010). Similar study was carried out by Chen *et al.*, (2005) showing the destructive effect of the pathogen on the host metabolites and on chloroplasts. There is breakdown of the proteins to amino-acids in the host cells during infection, which leads to decrease in protein content was shown by Odebode and Sanusi (1996). There is not enough evidence available on the comparative studies among the biochemical changes of healthy and infected safflower leaves. Hence it was felt necessary to investigation the biochemical changes occurring in Carthamus tinctorius L. cultivars under the pathogenesis of Alternaria carthami Chowdhury.

MATERIAL AND METHODS

The seeds of four cultivars of *Carthamus tinctorious* L. like- Nari-38 (spiny variety), Nari-6 (non-spiny variety), Nari-H-23 (spiny hybrid) and Nari-NH-1 (non-spiny hybrid) were collected from Nimkar Agricultural Research Institute, Phaltan, Dist. Satara (MS) and shown in RBD manner in 4 replications during Rabi season of the year 2014 and 2015. The plant protection measures were applied to each replication except one which was treated as controlled (untreated) one (No plant protection measures applied). Healthy and leaves infected with *Alternaria carthami* (leaf blight) were collected respectively from the replication with plant protection measures applied and the controlled replication of each cultivar. Carbohydrates were determined by the method of Nelson (1944), soluble protein by the method of Lowry et al., (1951), chlorophylls and total free amino acids were analyzed by using the method suggested by Sadasivam and Manickam (1996).

RESULT AND DISCUSSION

The observation Table 1. shows that, reducing sugar and non-reducing, total sugars, starch and total carbohydrates were drastically declined in infected leaves of all cultivars as compared healthy leaves. Nari-H-23 shows least decrease of reducing sugar (34.92%) followed by Nari-38 (51.71%), Nari-NH-1(55.39%) and Nari-6 (71.53%). The cultivar Nari-6 exhibited highest decreases of reducing sugar. The non reducing sugar of infected leaves was declined in all cultivars except Nari-38 which reveals increase in Nonreducing sugar by 20.37% over healthy leaves. Total sugar was significantly declined in Nari-6 (71.3%) followed by Nari-H 23 (38.86%) and Nari-NH-1(31.64%) whereas it was found least decreases in Nari-38 (29.32%). Nari-H-23 showed least decrease in starch (13.43%) followed by Nari-6 (47.73%), Nari-38 (50.07%) and Nari-NH-1(71.01%). The least decrease of total carbohydrate was found in Nari-H-23 (24.07%) followed by Nari-38 (42.1%), Nari-NH-1(53.23%) and Nari-6 (58.68%). Similar declining pattern of carbohydrates were recorded in greengram by Kulkarni et al., (2009) and Sindhan et al. (1999) infected with *Colletotrichum truncatum* (Schw.) Andrus and Moore and *Cercospora* leaf spot respectively. The reduction in carbohydrate contents after infection was due to rapid hydrolysis of sugars during pathogenesis through enzymes secreted by pathogens and subsequent utilization by pathogens for their development (Jaypal and Mahadevan, 1968).

The amount of chlorophyll ‘a’, ‘b’ and total chlorophyll of infected leaves was significantly declined in all cultivars over healthy leaves (Table 2). The least decrease in chlorophyll ‘a’, ‘b’ and total chlorophyll was found in Nari-6 about 56.01%, 49.76% and 53.44% respectively. Similarly the least decrease in chlorophyll ‘a’, ‘b’ and total chlorophyll was found in Nari-H-23 was about 58.36%, 60.2% and 59.7 % respectively. The highest decrease of chlorophyll ‘a’ was observed in Nari-NH-1 (74.16%) whereas chlorophyll ‘b’ and total chlorophyll about 96.88% and 83.08% respectively in Nari-38.

Similar findings were observed by Mesta (2006), while working on *Alternaria* blight of sunflower and Kulkarni *et al.,* (2009) in greengram infected with *Colletotrichum truncatum* (Schw.) Andrus and Moore. Alqarawi *et al*., (2013) showed the significant alterations of chlorophyll ‘a’ and chlorophyll ‘b’ in mangrove infected with *Alternaria alternata* (fr.) keissler compared to control plants. Gabara *et al*., (2012) suggested that decrease of chlorophyll in infected plants may be due to inhibition of photophosphorylation by fungal toxins.

Soluble proteins as well as total free amino acids were also decreased in infected leaves in all cultivars as compared to healthy leaves (Table 3). The least decrease of soluble protein was found in Nari-NH-1 (11.26%) while Nari-6 shows least decrease of total free amino acids (7.01%). The content of soluble protein and total free amino acids were significantly reduced in Nari-6 (31.75%) and Nari-NH-1(65.78%) respectively as compared to other cultivars. Similar findings were reported in sunflower seed infected with *Sclerotinia* head rot by Kumar et al., (1998) and Thomas and Mathew (2014) in *Lawsonia inermis* L. infected with *Asterina lawsoniae* Henn. & nyn. This indicates the inhibition of synthesis of amino acids and protein is mainly due to infection. There seems no degradation of protein to free amino acids. The enzymes involved in amino acids synthesis are activated as a result of infection, which lead to decrease in amino acids content in the infected leaves (Rudolph, 1963). The decreased content of soluble protein in the infected leaves is co-ordination with the increase in protease enzymes in the infected leaves was observed by Thomas and Mathew (2014) in *Lawsonia inermis* L. and Pareek and Varma (2015) in cluster bean.

Table 1. Effect of *Alternaria carthami* infection on carbohydrates in safflower cultivars.

Cultivars	Parameters	Carbohydrates mg g^{-1} fresh weight				
		Reducing sugar	Non-reducing sugar	Total sugar	Starch	Total carbohydrates
Nari-38	Healthy leaves	14.61±0.35	6.58±0.56	21.2±0.22	34.01±0.21	55.21±0.41
	Infected leaves	7.05±0.21 (51.71)*	7.92±1.08 (20.37)*	14.98±0.87 (29.32)*	16.98±1.62 (50.07)*	31.96±0.75 (42.1)*
Nari-6	Healthy leaves	10.15±0.55	8.51±0.47	18.67±0.87	21.50±0.29	40.18±1.16
	Infected leaves	2.89±0.24 (71.53)*	2.46±0.48 (71.03)*	5.35±0.69 (71.3)*	11.24±0.08 (47.73)*	16.6±0.69 (58.68)*
Nari-H-23	Healthy leaves	24.65±5.80	6.04±3.25	30.69±2.66	42.68±0.40	73.37±2.37
	Infected leaves	16.04±0.08 (34.92)*	2.72±0.33 (54.95)*	18.76±0.39 (38.86)*	36.95±1.52 (13.43)*	55.71±1.17 (24.07)*
Nari-NH-1	Healthy leaves	10.2±0.84	11.52±1.01	21.72±0.22	26.39±3.45	48.11±3.61
	Infected leaves	4.55±1.02 (55.39)*	10.3±1.28 (10.62)*	14.85±0.40 (31.64)*	7.65±0.46 (71.01)*	22.50±0.44 (53.23)*

(Values are mean ± SD of triplicates. * Per cent increase or decrease over Healthy leaves)

Table 2. Effect of *Alternaria carthami* infection on chlorophylls in safflower cultivars.

Cultivars	Parameters	chlorophylls mg g^{-1} fresh weight		
		Chlorophyll 'a'	chlorophyll 'b'	Total chlorophyll
Nari-38	Healthy leaves	9.944±0.184	9.216±0.180	19.161±0.241
	Infected leaves	2.954±0.095 (70.28)*	0.287±0.089 (96.88)*	3.242±0.180 (83.08)*
Nari-6	Healthy leaves	9.215±0.105	6.426±0.277	15.641±0.351
	Infected leaves	4.053±0.157 (56.01)*	3.228±0.235 (49.76)*	7.281±0.295 (53.44)*
Nari-H-23	Healthy leaves	9.914±0.072	6.233±0.247	16.148±0.215
	Infected leaves	4.127±0.167 (58.36)*	2.48±0.210 (60.2)*	6.608±0.289 (59.07)*
Nari-NH-1	Healthy leaves	14.037±0.074	9.361±0.169	23.399±0.147
	Infected leaves	3.626±0.104 (74.16)*	2.419±0.196 (74.15)*	6.046±0.293 (74.15)*

(Values are mean ± SD of triplicates. * Per cent increase or decrease over Healthy leaves)

Table 3. Effect of *Alternaria carthami* infection on soluble protein and total free amino acids in safflower cultivars.

Cultivars	Parameters	Soluble protein mg g^{-1} fresh weight	Toatl free amino acids mg g^{-1} fresh weight
Nari-38	Healthy leaves	35.89±0.57	13.70±0.82
	Infected leaves	28.67±0.61 (20.12%)*	7.58±0.41 (44.68%)*
Nari-6	Healthy leaves	33.30±0.32	16.62±0.71
	Infected leaves	22.73±0.59 (31.75%)*	15.45±4.06 (7.01%)*
Nari-H-23	Healthy leaves	35.54±0.13	22.45±0.82
	Infected leaves	30.62±0.65 (13.83%)*	9.04±1.79 (59.74%)*
Nari-NH-1	Healthy leaves	26.76±0.65	22.16±2.29
	Infected leaves	23.75±0.63 (11.26%)*	7.58±0.82 (65.78%)*

(Values are mean ± SD of triplicates. * Per cent increase or decrease over Healthy leaves)

Based on the present findings, it is concluded that the Nari-6 and Nari-H-23 exhibit least decrease of biochemicals in infected leaves making them more resistant whereas Nari-38 and Nari-NH-1 show highest decrease of biochemicals in infected leaves which makes them more susceptible to *Alternaria carthami* infection.

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