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BIOCHEMICAL CHARACTERISTICS IMPARTING RESISTANCE AGAINST *ALTERNARIA* BLIGHT IN CAULIFLOWER GENOTYPES

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ABSTRACT

Alternaria **blight disease has been reported from all the continents of the world, affects most of the cruciferous crops. *Alternaria* blight incited by foliar pathogen, *Alternaria brassicae* has become a major constraint in the successful cultivation of this crop. Development of resistant varieties is the most appropriate approach to control the disease and the concept is now developing to explore the built-in plant defense mechanism in relation to pathogen attack. In the present study, an attempt was made to identify biochemical characteristics imparting resistance against *Alternaria* blight in cauliflower genotypes. The moderately resistant genotypes had higher total chlorophyll, total phenols, total soluble sugars, total proteins, flavonoids and ascorbic acid than the susceptible genotypes. The chlorophyll content was found to be lesser in the infected leaves than the healthy ones. The reduction in total phenols, total soluble sugars, total proteins, flavonoids and ascorbic acid was at a higher rate in susceptible ones than the moderately resistant genotypes. These biochemical characteristics might be responsible for imparting resistance against *Alternaria* blight in cauliflower genotypes.**

Key words: *Alternaria* blight, Biochemical, Cauliflower, Resistance

INTRODUCTION

Considering the importance of vegetables in human diet, agricultural diversification and generation of employment, there is a tremendous scope to increase the vegetable production. But there are several constraints which limit the productivity of the crops *viz.* non- availability of germplasm and its evaluation; poor yield of the existing genotypes, luxuriant vegetative growth, abiotic and biotic stresses (Pandey 1994). *Alternaria* blight incited by *Alternaria brassicae* has become a major constraint in the successful cultivation of cauliflower and may lead to about 80 percent reduction in seed yield of cauliflower (Prasad and Vishunavat 2006). The fungus causes light to dark brown spots on leaves, petiole and stem. On seeds, symptoms include dark lesions immediately after germination that can result in damping-off, or stunted seedlings. The fungus infected curd gets rotten, thus making the crop commercially unmarketable. *Alternaria* blight can be checked by the application of fungicides like Roral, Difolatan, Bavistan etc. but they are not cost-effective; application is cumbersome and cause health hazards (Chattopadhyay and Bagchi 1994). These problems have diverted the attention towards the use of resistant cultivars as the cultivation of resistant genotypes is the effective and cheap method to combat the fungal disease as compared to the chemical control. The identification of a plant trait that is associated with resistance to *Alternaria* could be useful for screening of resistant sources and therefore the identification of genotypes resistant to *Alternaria* blight and the factors involved in imparting resistance are worth studying. The inherent resistance in plants against various pathogens is dependent on several complex and inter-related factors viz. morphological, physiological and biochemical. The aim of the present investigation was to identify the biochemical factors imparting *Alternaria* blight resistance in cauliflower leaves.

MATERIALS AND METHODS

Estimation of biochemical components such as total chlorophyll, total carotenoids, total soluble sugars, total proteins, total phenols, total flavonoids

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and ascorbic acid was carried out in two moderately resistant (Pusa Sharda and N-9102) and two susceptible genotypes (No.2 and 24-2) genotypes of cauliflower. A field experiment was carried out in 2008 and 2009 at Field Area of Department of Vegetable Crops, Punjab Agricultural University, Ludhiana and twenty cauliflower genotypes were raised in randomized block design with three replications following the procedures outlined in Package of Practices. Nursery was raised in August and transplanting was done in September, 2008 and 2009. After the onset of disease, plants were subjected to field screening against *Alternaria* blight on the basis of the key developed by Conn et al (1990). The degree of disease severity was determined by using 0 to 9 scale on the basis of percent leaf area blighted and then percent disease index (PDI) was calculated using the formula of Wheeler (1969). On the basis of PDI, genotypes were categorized as immune (0), resistant (less than 1), moderately resistant (1-10), moderately susceptible (11-25), susceptible (26-50) and highly susceptible (51-100). Out of twenty genotypes, two moderately resistant genotypes with the highest PDI (Pusa Sharda and N-9102) and two susceptible genotypes with the lowest PDI (No.2 and 24-2) were selected for the biochemical estimations. Standard procedures were followed for the estimation of total chlorophyll (Hiscox and Israeltam 1979), carotenoids (Kirk and Allen 1965), total soluble sugars (Dubois *et al* 1956), total proteins (Lowry *et al* 1951), total phenols (Swain and Hills 1959), total flavonoids (Balabaa *et al* 1974) and ascorbic acid (Roe and Oesterling 1943). Samples were collected at two stages of crop development i.e. i) seedling stage and ii) curd initiation stage (after the onset of disease).

RESULTS AND DISCUSSION

Total chlorophyll: The moderately resistant genotypes had higher chlorophyll content than the susceptible genotypes at both the stages (Table 1). At stage I, maximum chlorophyll content was recorded in moderately resistant genotype Pusa Sharda i.e. 1.64 mg g^{-1} FW and minimum was recorded in susceptible genotype No.2 i.e. 1.26 mg g^{-1} FW. At stage II, the chlorophyll content of moderately resistant genotype N-9102 was almost 1.12 times higher than the susceptible genotype No.2. There was a significant difference in the chlorophyll levels of healthy and diseased leaves of

TABLE 1: Variation in total chlorophyll (mg g^{-1} DW) and carotenoids (mg g^{-1} DW) in cauliflower genotypes with different reactions to Alternaria blight at two stages of crop development.

Genotypes	Percent Disease Index	Disease Reaction	Total chlorophyll content				Carotenoid content			
			Seedling stage	Curd Initiation Stage			Seedling stage	Curd Initiation Stage		
				Healthy	Diseased	Decrease over healthy (%)		Healthy	Diseased	Decrease over healthy (%)
Pusa Sharda	9.84	MR	1.64	2.44	1.84	24.59	0.517	0.686	0.582	15.16
N-9102	10.06	MR	1.59	2.41	2.07	14.10	0.499	0.687	0.497	27.65
24-2	29.11	S	1.26	2.18	1.32	39.44	0.380	0.514	0.354	31.12
No.2	32.28	S	1.26	2.18	1.24	43.11	0.363	0.519	0.365	29.67
C.D.(P=0.05)			Genotypes=0.125, Stages=0.06, G×S=0.217				Genotypes=0.012, Stages=0.006, G×S=0.022			

all the genotypes. After pathogenesis, all the genotypes showed a reduction in the chlorophyll content, but the susceptible genotype No.2 recorded maximum reduction (43.11) and moderately resistant genotype N-9102 recorded minimum reduction percent (14.10). Higher chlorophyll content might be associated with host's resistance to blackspot disease of rapeseed mustard caused by *Alternaria brassicae* (Kolte and Awasthi 1999). The reduction in chlorophyll may be attributed to toxic metabolites produced by pathogen which may destroy the chloroplast (Senthil *et al* 2010) or to the inhibition of synthesis of chlorophyll rather than the degradation of pre-existing pigments (Ammajamma and Patil 2008).

Carotenoids: At stage I and II, the moderately resistant genotypes showed higher carotenoid content than the susceptible genotypes (Table 1). Moderately resistant genotypes exhibited carotenoid levels within a range of 0.499-0.517 mg g^{-1} FW, maximum content being possessed by genotype Pusa Sharda. After the onset of disease, there was a decrease of 29.67-31.12 percent in susceptible genotypes. Where as in moderately resistant genotypes, there was a decrease of 15.16-27.65 percent. The fungal pathogens affect the pigment levels by affecting the chloroplast structure. Ghose *et al* (2010) reported a drastic reduction of about 53.24% and 58.04% in total chlorophyll and carotene respectively in blight infected mulberry leaves over the healthy ones which corroborates our results.

Total soluble sugars: Total soluble sugars were significantly higher in moderately resistant genotypes (Table 2). At stage II, moderately resistant genotypes exhibited significantly higher levels of total soluble sugars in both healthy and diseased leaves, maximum being possessed by Pusa Sharda (62.46 and 56.22 mg g^{-1} DW respectively). A significant reduction in the total soluble sugar levels was observed in all the genotypes after the infection, but the percent depletion was at higher rates in susceptible genotypes compared to moderately resistant genotypes. Due to infection, the depletion of sugars was up to 9.99-12.51% in moderately resistant genotypes while 17.65-24.49% in susceptible genotypes. Sunkad and Kulkarni (2006) reported higher levels of sugars in resistant and

TABLE 2: Variation in total soluble sugars (mg g^{-1} DW) and total proteins (mg g^{-1} DW) in cauliflower genotypes with different reactions to *Alternaria* blight at two stages of crop development.

Genotypes	Percent Disease Index	Disease Reaction	Total soluble sugars: Seedling stage	Total soluble sugars: Curd Initiation Stage			Total protein content: Seedling stage	Total protein content: Curd Initiation Stage		
				Healthy	Diseased	Decrease over healthy (%)		Healthy	Diseased	Decrease over healthy (%)
Pusa Sharda	9.84	MR	27.18	62.46	56.22	9.99	9.89	17.30	15.47	10.57
N-9102	10.06	MR	27.32	57.03	49.89	12.51	8.85	16.76	14.32	14.55
24-2	29.11	S	18.60	48.26	36.44	24.49	5.06	11.42	8.43	26.18
No.2	32.28	S	14.43	45.64	37.58	17.65	4.67	11.02	8.66	21.41
C.D.(P=0.05)			Genotypes=1.26, Stages= 0.63, G×S= 0.218				Genotypes= 0.045, Stages= 0.022, G×S= 0.079			

TABLE 3: Variation in total phenols (mg g^{-1} DW), total flavonoids (mg g^{-1} DW) and ascorbic acid (mg g^{-1} DW) in cauliflower genotypes with different reactions to Alternaria blight at two stages of crop development.

Genotypes	Percent Disease Index	Disease Reaction	Total phenols				Total flavonoids				Ascorbic acid			
			Seedling stage	Curd Initiation Stage			Seedling stage	Curd Initiation Stage			Seedling stage	Curd Initiation Stage		
				Healthy	Diseased	Decrease over healthy (%)		Healthy	Diseased	Decrease over healthy (%)		Healthy	Diseased	Decrease over healthy (%)
Pusa Sharda	9.84	MR	11.10	14.91	13.27	10.99	5.85	7.12	6.29	11.65	2.35	3.08	2.57	16.55
N-9102	10.06	MR	10.37	15.24	13.25	13.05	6.00	7.32	6.27	14.34	2.04	3.13	2.51	19.80
24-2	29.11	S	6.26	8.17	6.30	22.88	2.70	4.71	3.48	26.11	1.40	1.95	1.43	26.66
No.2	32.28	S	5.85	7.91	5.96	24.65	2.52	5.07	4.01	20.91	1.16	1.49	1.09	26.84
C.D. (P=0.05)			Genotypes=0.201, Stages= 0.100, G×S= 0.348				Genotypes=0.103, Stages= 0.051, G×S= 0.178				Genotypes=0.061, Stages= 0.031, G×S= 0.105			

moderately resistant groundnut genotypes than susceptible genotypes in response to *Puccinia arachidis* infection. The changes in carbohydrate content in response to infection can be attributed to direct parasite utilization and indirectly to the altered host metabolism. The depletion may be attributed to the secretion of certain metabolites to degrade the carbohydrates and used by the respective pathogen for its growth (Ponmurugan and Baby 2007).

Total proteins: Moderately resistant genotype Pusa Sharda showed maximum and significantly higher protein content (9.89 mg g^{-1} DW) in comparison to susceptible genotypes (4.67-5.06 mg g^{-1} DW) (Table 2). At stage II, similar trend as stage I was followed. Post pathogenesis, all the genotypes showed reduced levels of total proteins. The decline in proteins was more pronounced in susceptible genotypes (21.41 and 26.18 percent). Ponmurugan and Baby (2007) reported higher protein content in tolerant tea cultivars than the susceptible to *Phomopsis theae* infection. Although higher protein in resistant genotypes may serve as index of resistance, its role remains yet to be elucidated (Kiran *et al* 2003). Changes in protein occur when the pathogen penetrates the host cells resulting in disturbances in protein and related metabolisms (Khan *et al* 2001). The reduction in the protein content might be due to blockage of protein synthesis or degradation of proteins in the host plants (Sunkad and Kulkarni 2006).

Total phenols: Maximum and significantly higher levels of total phenols were observed in moderately resistant genotypes (Pusa Sharda and N-9102), whereas the susceptible genotypes (No.2 and 24-2) recorded 2.0 times lower levels of total phenols than moderately resistant genotypes (Table 3). Post-pathogenesis, the healthy leaves of moderately resistant genotypes contained higher total phenol content than the moderately susceptible, susceptible and highly susceptible genotypes. In the diseased leaves, the total phenols decreased in all the genotypes but at a higher rate in susceptible genotype (22.88 and 24.65%), and at lower rate in moderately resistant genotypes (10.99-13.05%). Madhavi *et al* (2005) also reported that species of wild sunflower resistant to *Alternaria helianthi* possessed higher levels of both constitutive phenols as compared to susceptible genotypes. The depletion of phenols after infection can be related to sugar depletion also. The unavailability of sugars as substrates for phenol synthesis can lead to the reduced phenolic levels (Ponmurugan and Baby 2007). It is possible that infected plants are unable to manufacture the phenols or might have used phenols as substrates for toxin production (Siddiqua and Kashem 1993).

Total flavonoids: At seedling stage, the total flavonoid content was significantly higher in moderately resistant genotypes i.e. 5.85 and 6.00 mg g^{-1} DW in Pusa Sharda and N-9102 respectively, which was about 2.5 times higher as compared with susceptible genotypes (Table 3). At curd initiation stage, significantly higher levels of flavonoids were recorded in moderately resistant genotypes, which were almost 1.5 times that of susceptible genotype. After the onset of disease, the flavonoid content was significantly lower in infected leaves than healthy leaves in all the genotypes. The reduction percent was 11.65 and 14.34% for moderately resistance genotypes while 20.91 and 26.11% for susceptible genotypes. Chakrabarty *et al* (2002) recorded higher amounts of flavonols in resistant varieties of diploid cotton against grey mildew as compared to susceptible genotypes. Infected tissues of cocoa (*Theobroma cacoa* L.) due to *Crinipellis perniciosa* had significantly lower levels of total flavonoids as compared to the resistant genotypes (Scarpari *et al* 2005). Flavonoids are fairly well distributed in the plant kingdom and possess insecticidal and antimicrobial activity (Satisha *et al* 2008).

Ascorbic acid: Ascorbic acid provides general resistance and is an antioxidant found throughout the cells and acts as a scavenger of reactive oxygen species. At seedling stage, the moderately resistant genotype Pusa Sharda recorded maximum ascorbic acid content (2.35 mg g^{-1} FW) whereas susceptible genotype No.2 recorded minimum levels of ascorbic acid (1.16 mg g^{-1} FW) (Table 3). At the second stage, i.e. after the onset of disease, the healthy leaves of moderately resistant genotypes had ascorbic acid content which was twice the susceptible genotypes. Moderately resistant genotypes recorded decrease in ascorbic acid which was approximately 16.55 and 19.80% in Pusa Sharda and No.2 respectively. However, about 26 % reduction was observed in the susceptible genotypes. Arora (2004) studied role of phytoanticipins in resistance against red rot of sugarcane and observed that resistant genotypes exhibited high level of ascorbic acid as compared to susceptible genotypes which decreased further after pathogenesis. Guleria *et al* (1997) also observed higher ascorbic acid levels in resistant pea cultivars than the susceptible ones, which exhibited 10.82% reduction following powdery mildew infection. Malhotra (1993) suggested that lower amount of ascorbic acid in the chlorophyll depleted diseased parts is an indication that ascorbic acid biosynthesis appears to be markedly influencing the chlorophyll content. In the present study also, the ascorbic acid content decreases in the diseased leaves and the chlorophyll content also decreases. So ascorbic acid synthesis might be related to chlorosis caused by the pathogen.

The results indicate that moderately resistant genotypes had higher total chlorophyll, carotenoid, total soluble sugars, total proteins, total phenols, flavonoids and ascorbic acid content than the susceptible genotypes and the decrease in various biochemical factors following the infection was at lower rates in moderately resistant genotypes.

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