Int. J. Indust. Entomol. Vol. 9, No. 2, 2004, pp. 255~259

[Short Communication]

International Journal of *Industrial Entomology*

Post-Infectional Biochemical Changes in Mulberry Due to *Xanthomonas campestris* pv. *mori* Induced Bacterial Leaf Spot

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(*Received 5 August 2004; Accepted 12 November 2004*)

Post-infectional biochemical changes due to *Xanthomonas campestris* pv. *mori* (*Xcm*) infection in five elite mulberry varieties *viz.*, S_1, S_{1635}, V_1, RFS_{175} and JRH was studied under inoculated condition. It was revealed that total soluble sugar and protein content was significantly declined in all the varieties due to *X. campestris* infection. Total phenol content was at par prior to inoculation in all varieties, but it was significantly increased in S_1, RFS_{175}, S_{1635} and JRH 7 days after inoculation. The correlation coefficient (r) between total soluble sugar and total phenol content was found positive (r = 0.825) and statistically significant. Similarly, correlation coefficient (r) between total soluble protein and phenol content was found positive (r = 0.897) and statistically significant. The present study indicates that *X. campestris* infected leaves are nutritionally inferior in quality and the duration of phenol production in a mulberry variety play decisive role on disease resistance.

Key words: Bacterial leaf spot, Post infectional biochemical change, Mulberry, *Morus* sp., *Xanthomonas campestris* pv. *mori*.

Introduction

Mulberry (*Morus* sp.), the sole food plant of silkworm (*Bombyx mori* L.) is cultivated in large scale in the Gangetic plains of West Bengal for silkworm rearing since medieval period. Bacterial leaf spot (BLS) caused by *Xanthomonas campestris* pv. *mori* (*X. campestris*) is a major foliar disease of mulberry in West Bengal. The disease appears after on set of monsoon and continued up to the month of October. The symptom of the disease is characterized by appearance of small angular water soaked spots on the lower surface of leaves, which later turn brownish surrounded by yellow halo. The necrotic tissues subsequently fell off and form shot holes. As the pathogens intervenes the foliar tissues during course of disease, leaf productivity and quality of mulberry is adversely affected. The disease severity in the ruling mulberry cultivars in terms of percent disease index (PDI) ranges from 15 – 20% during July - August (Maji *et al.*, 1996). The pathogen is a rod shaped ranging from 1.0 – 2.5 μm × 0.4 – 0.7 μm, gram negative and motile bacteria. The bacteria produce small circular, entire, convex, yellow colonies on nutrient agar. Mucoid growth observed on nutrient agar supplemented with 2% glucose or sucrose (Maji *et al.*, 1998).

Feeding of silkworm with high carbohydrate and protein content leaves is prerequisite for healthy growth of silkworm and good quality and quantity of cocoon. Chanturiya (1968) reported that powdery mildew disease decreased the rate of oxidation process of carbohydrate synthesis. Also, the nitrogenous matter was lowered and its ratio in diseased leaves. This change occurs because of the demands of feeding matter by the pathogen itself from the infected tissues, due to greater intensity of the basic metabolism of the pathogen and also reduced synthesis by the plant due to infection. Sundareswaran *et al.* (1988) reported that there was significant reduction of crude protein, reducing sugar and total sugar in the rust infected leaves of six high yielding varieties. Umesh Kumar (1991) reported that powdery mildew, leaf spot and leaf rust infection increased total soluble sugar but total soluble protein showed both increased and decreased. However, no information is available on the effect of bacterial leaf spot on the metabolic alternations in the mulberry leaf. The present investigation was carried out to study some

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post infectional biochemical changes of some popular high yielding mulberry varieties due to *Xanthomonas campestris* pv. *mori* infection.

Materials and Methods

Five elite mulberry varieties namely S_1, S_{1635}, V_1, RFS_{175} and JRH were raised in earthen pots and kept in green house. Plants were inoculated with 24 hrs old bacterial culture suspension (1×10^8 colony forming unit/ml) of *X. campestris* 20 days after pruning. To measure the post-infectional changes of total soluble sugar, total soluble protein and total phenol content, leaves were collected 5th leaves from first glossy leaves prior to inoculation and 7, 14, 21 and 28 days after inoculation (DAI). Total soluble sugar was measured by Anthrone method (Yeem and Willis, 1954), total soluble protein by Lowry *et al.* (1951) method and total phenols by Swain and Hills (1959).

BLS disease severity of each varieties was assessed from randomly selected five branches from each pot using a 0 – 5 visual rating scale 28 days after inoculation (Maji *et al.*, 2000). In this scale, 0 - healthy leaf, 1 = 1 – 5% leaf area infected, 2 = 6 – 10% leaf area infected, 3 = 11 – 25% leaf area infected, 4 = 26 – 50% leaf area infected, 5 = 51% and above leaf area infected. Percent of disease index (PDI) was calculated by the following formula.

$$\text{Percent disease index} = \frac{\text{Sum of all numerical rating}}{\text{Total no. of leaves counted} \times \text{Maximum grade (5)}} \times 100$$

Data were subjected to analysis of variance to determine significant differences between varieties using F-ratio test. To compare the treatment means, critical differences were calculated by Fishers least significant differences test at α = 5%. Simple correlation coefficient and regression analysis was done for determining relationship between total soluble sugar and total phenol, total soluble protein and total phenol and total phenol and disease severity.

Results

Total soluble sugar

Analysis of variance revealed that among the five mulberry genotypes, highest sugar content was observed in S_{1635} followed by JRH, V_1, RFS_{175} and S_1. There was no differences in the Total soluble sugar (TSS) content in the varieties like RFS_{175}, S_{1635}, and V_1 up to 14 DAI but a significant reduction in the TSS content was noticed in all the varieties on 21 DAI. was at par up to 14 DAI in RFS_{175}, S_{1635}, and V_1 but significantly declined 21 DAI in all varieties (Table 1).

Total soluble protein

The highest protein content was observed in S_{1635} followed by JRH. A progressive reduction of total soluble protein contents in diseased leaves was observed in all the tst varieties due to *Xcm* infection (Table 2).

Total phenol

Analysis of variance revealed that the pre-infectional phenol content was at par in all the test varieties. The phenol content increased significantly in S_1, S_{1635}, JRH and RFS_{175} except V_1. Total phenol content was significantly higher in S_{1635} and JRH throughout study period whereas in S_1 and RFS_{175} phenol content gradually declined. In V_1, phenol content was at par both pre and post infection period (Table 3).

Table 1. Changes in total soluble sugar content in mulberry leaves due to *X. campestris* infection

Variety	Total soluble sugar content (mg/g fr. wt)					Mean
	Days after inoculation					
	0	7	14	21	28	
S_1	41.43	40.96	37.89	30.89	28.72	35.98
RFS_{175}	44.15	43.90	42.46	31.57	26.79	37.77
S_{1635}	44.88	45.61	43.45	39.12	31.92	41.00
V_1	44.58	44.61	40.58	31.12	29.63	38.10
JRH	43.14	44.06	41.45	38.21	30.33	39.44
Mean	43.63	43.83	41.17	34.18	29.48	
CD at 5% variety			1.31			
Days			1.31			
Variety × days			2.93			

Table 2. Changes in total soluble protein content in mulberry leaves due to *X. campestris* infection

Variety	Total soluble protein content (mg/g fr. wt)					Mean
	Days after inoculation					
	0	7	14	21	28	
S_1	18.96	18.00	16.01	12.30	11.82	15.42
RFS_{175}	20.89	20.68	19.62	12.11	11.04	16.87
S_{1635}	24.86	24.99	20.12	15.07	14.66	19.94
V_1	21.25	21.47	19.64	10.94	10.35	16.73
JRH	22.50	22.85	17.55	14.45	16.20	18.71
Mean	21.69	21.60	18.59	12.97		
CD at 5% variety			0.53			
Days			0.53			
Variety × days			1.19			

Table 3. Changes in total phenol content in mulberry leaves due to *X. campestris* infection

Variety	Total phenol (mg/g fr. wt)					Mean
	Days after inoculation					
	0	7	14	21	28	
S_1	10.10	13.00	12.16	10.02	8.48	10.75
RFS_{175}	9.20	14.58	13.64	12.90	13.84	12.83
S_{1635}	10.48	15.24	17.47	16.31	16.73	15.25
V_1	10.47	9.95	12.31	11.03	9.56	10.67
JRH	10.78	16.66	16.88	17.15	17.15	15.72
Mean	10.21	13.89	14.49	13.15	13.15	
CD at 5% variety			1.03			
Days			1.03			
Variety × days			2.31			

BLS disease severity

BLS disease severity was found 15.80 PDI in RFS $_{175}$, followed by 8.36 PDI in S_1, 4.31 PDI in JRH, 2.50 PDI in S_{1635} and 2.43 PDI in V_1. Analysis of variance revealed that BLS disease severity was significantly higher in RFS_{175} and S_1 but in other three varieties disease severity was at par.

Correlation and regression studies

The correlation study between total soluble sugar and total phenol content revealed a significant positive correlation ($r = 0.825$) between the two factors. In regression analysis, it was observed that coefficient determination ($R^2 = 0.68$) was significant at 5% level. Analysis of inter-relationship between total soluble sugar and total phenol content showed that a unit increase of total soluble sugar content resulted increase of 1.057 (mg/g fr. wt) of total phenol content. Similarly, a strong positive correlation ($r = 0.897$) between total soluble protein and phenol content was established and found statistically significant. In regression analysis, the coefficient determination ($R^2 = 0.80$) was significant at 5% level.

Discussion

A healthy and nutritious leaf is a prerequisite for healthy growth of silkworm (*Bombyx mori* L.) and good cocoon harvest (Chowdhury, 1992). Apart from effect of foliar fungal diseases (Chanturiya, 1968; Sundareswaran *et al.*, 1988; Umesh Kumar, 1991), little information is available on the effect of *X. campestris* on mulberry leaves. Results presented in the present investigation provide first time information on the effect of *X. campestris* infection on total soluble sugar, total soluble protein, total phenol content and correlation between total soluble sugar and total phenol, total protein and total phenol, total phenol and disease severity on different high yielding mulberry varieties. The results indicate that decrease of sugar content with advancement of *X. campestris*. The decrease of sugar con-

tent with advancement of disease may be due to i) decrease of photosynthetic assimilative surface due to formation of water soaked and brown necrotic spot, yellow halo, and shot holes in the leaves, ii) disruption of chloroplast structure, iii) utilization of soluble sugar by the pathogen, iv) wasteful host respiration due to pathogenesis and v) utilization of soluble sugar for host defense reactions such as synthesis of polyphenols and phytoalexin (Asahi *et al.*, 1980).

The reduction of protein contents in diseased leaves has been reported several workers in different crops (Samborski *et al.*, 1958; Wang *et al.*, 1958; Agarwal *et al.*, 1982; Lathura *et al.*, 1988). Nayudu and Walker (1961) reported that decrease of protein content occurred in the infected leaves due to utilization of protein by pathogen, reduction in protein synthesis or due to increase of activity of proteolytic enzymes. Howell and Krusberg (1966) opined that reduction of protein content occurred in the diseased leaves due to break down of protein by proteolytic enzymes secreted by the pathogen.

Accumulation of phenolics occurs in many plants after infection. Similar observations were also recorded in *Xanthomonas campestris* pv. *malvacearum* infected cotton leaves (Borkar and Verma, 1991) and *X. campestris* pv. *cyamopsidis* infected cluster bean leaves (Lodha *et al.*, 1993). The post infectional increase of phenol content could be due to a number of factors, including enhancement of synthesis, translocation of phenolics to the site of infection and hydrolysis of phenolic glycoside (Sharma *et al.*, 1983).

Variety wise disease severity was significantly low in those varieties where phenol content was high. The phenolic compounds have long been correlated with the resistance of plants to infectious agents (Link, 1933; Walker and Link, 1935; Sokolova *et al.*, 1958; Farkas and Kiraly, 1962; Couture *et al.*, 1971; Luthra *et al.*, 1988). Several workers reported that phenol concentration is usually higher in resistant than the susceptible ones (Lily and Ramadasan, 1979; Arora and Wagle, 1983; Luthra *et al.*, 1988). Vidhyasekaran (1974) and Bilgrami and Dubey (1982) opined that the speed of phenol production plays a decisive role in disease resistance. Regarding mechanism of phenolics on disease resistance, Vidhyasekaran (1997) opined that phenolics may alter the porosity of pathogens and inhibit certain enzymes of pathogens or DNA transcription. Phenolics may also inhibit production of toxins pectic enzymes by pathogens.

The present studies suggest that rate and duration of phenol synthesis in mulberry has a direct bearing with disease resistance. The results also corroborate the findings of Saini *et al.* (1988) that sugar along with phenols play a role in expressing resistance. Phenolic compounds and carbohydrate have been correlated with disease resistance mechanisms in different crop plants (Mandokhot *et al.*, 1979; Chand and Verma, 1980; Gupta *et al.*, 1984). Asahi (1980) opined that soluble sugars are precursors of phytoalexins and polyphenols, thus the variety having high soluble sugars are efficient producer of phenolic substances. Walker (1975) reported that co-existence of free sugars and phenols results in glycosylation of phenols by sugars forming phenolic glycosides, which are more soluble in cell sap and thus are involved more efficiently in the expression of disease resistance. Singh (1984) opined that proteins and enzymes in large quantities also contribute to post-infectional resistant reaction in plants.

The present study indicates that i) *Xcm* infected leaves are nutritionally inferior in quality. ii) The duration of phenol production in a mulberry variety play a decisive role on disease resistance.

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