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Extent of variation of Citrus Canker symptoms, virulence of the pathogens and post infectional biochemical changes associated with three species of *Citrus* in the plains of West Bengal

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Occurrence of Citrus Canker caused by *Xanthomonas axonopodis* pv. *citri* in the plains of subtropical region of West Bengal has become a matter of concern in recent years. Symptoms of Citrus Canker greatly varied in three species of *Citrus* namely *Citrus aurantifolia, Citrus grandis,* and *Citrus limon.* Symptoms varied from tiny, watery, circular, raised lesion, encircled by yellow halo in acid lime to necrotic, dark brown and very rough, with very insignificant yellow halo surrounding the canker lesion in Assam lemon to usually circular, with distinct orange-yellow halos and sparsely distributed lesions on foliage of pummelo. The causal bacterium, isolated from acid lime from different locations of West Bengal, showed variation in respect to virulence, post infectional biochemical changes (percentage of total sugar and phenol content) in three *Citrus* species.

Key words: Citrus Canker, variation, pathogenic variability, symptoms, sugar and phenol content

INTRODUCTION

In India, in terms of area under cultivation, citrus is the third largest fruit industry after banana and mango. The area and production under citrus cultivation in India are 846.0 thousand hectare and 7464.0 thousand metric tons respectively, with an average productivity of 8.8 tons per hectare (Indian Horticulture Database, 2011). In West Bengal citrus occupies an area of 7.52 thousand hectare with a production of 64.77 thousand metric tons (Economic Review, 2011-12). Among the *Citrus* species, acid limes are popularly cultivated in plains of West Bengal. Incidence of Citrus Canker disease is common and little information is available with regards to the disease in the subtropical plains of West Bengal. The present studies are aimed to record the existence of variability in symptoms of the disease, virulence of the pathogen (*Xanthomonas axonopodis* pv. *citri*) and post infectional biochemical changes in three species of *Citrus.*

MATERIALS AND METHODS

The experimental programmes were carried out through field survey and recording of detail symptoms of Citrus Canker disease in three species of *Citrus* namely acid lime (*Citrus aurantifolia*), Assam lemon (*Citrus limon*) and Pummelo (*Citrus grandis*) in plains of West Bengal. The causal bacterium was isolated following standard procedure described by Sujatha and Gopal (2010) and selected isolates were named as stated in Table 1.Pathogenicity test for the bacterial isolates obtained from different diseased samples was done following leaf injection-infiltration technique (Klement, 1963 and Bhattacharya *et al.*, 2012). In this method bacte-

rial suspension (2.4 x 10^9 cfu/ml) were injected in between the veins in disease free tender leaves of citrus plants after washing them with clean water. Upon injection, the leaf tissue adjacent to the vein becomes water soaked. The size of the water soaked area varied from 6-8 mm in diameter depending upon the amount of suspension pushed. Observations on development of symptom were recorded at 24 h interval up to 10^{th} day.

For testing virulence of selected 10 isolates of *Xanthomonas axonopodis* pv. *citri* (*Xac*) from *Citrus aurantifolia* were inoculated in three species of *Citrus* (*Citrus aurantifolia, Citrus grandis,* and *Citrus limon*). Four branches of each plant were selected from four directions (north, south, east and west). In each selected branch, four healthy leaves were inoculated by injecting bacterial (*Xac*) suspension. In each selected leaves the bacterium (*Xac*) was injected at three different points whereas in control sterile distilled water was injected. The extent of area affected due to development of symptom (average diameter) was recorded on 10^{th} day after inoculation.

Phenol content was estimated following method described by Sadasivam and Manickam (2008) and total sugars by method described by Hedge and Hofreiter (1962).

Correlation analysis was calculated based on independent variable as lesion size and total sugar and phenol content as dependent variables using Software SPSS version 16.00

RESULTS AND DISCUSSION

Symptoms of Citrus Canker on three Citrus spp. *under natural condition*

(i) Acid lime (Citrus aurantifolia)

In natural infection, the initial symptom appeared as tiny, watery, translucent circular lesion on the leaves. The lesion first appeared on lower surface of the leaf and then become prominent on the upper surface of the leaf. With the progress of the disease raised lesions became surrounded by water soaked margins and later on the entire lesion was usually surrounded by faint chlorotic margin or yellow halo. The chlorotic halo became intensified after some days. Most lesions were round in shape, occasionally oval or angular. Lesion gradually converted into raised, rough, corky grayish brown structure with or without crater like appearance. In severe infection numerous small round corky raised lesions were found on a leaf. The lesion size was around 1.0 mm in diameter. Lesions were also found on mid-veins and petioles. Canker lesions were observed on twigs. Cankers on the twigs were more prominent and normally 2 to 3 mm in size, brown to grayish brown in colour. In many cases lesions were elongated and may sometime coalesce to girdle the branches. Such branches above the point of infection became dead producing die back symptom at the end of the rainy season. Severe infection and girdling of the branches were common. Infection on the petiole caused early defoliation. Symptom on the fruit was similar to that produced on leaf but yellow halo was absent. Young fruit drop occurred due to infection at the stalk-end. In leaf miner infected leaf many canker lesions were formed in damaged area close to each other without yellow halo. Symptoms described by earlier authors in other states were more or less similar (Broadbent *et al.*, 1992; Aslam and Abid, 2007; Sahi *et al.*, 2007; Sujatha and Gopal, 2010 and Beheshti *et al.*, 2011).

(ii) Assam lemon (Citrus limon)

The symptom appeared as small, raised necrotic, dark brown lesions on both sides of leaf surface. The lesions were very rough in appearance. Water-soaked area surrounded the canker lesions mostly on the under surface of the leaf but hardly noticed on the upper surface of the leaf. Unlike acid lime and pummelo where yellow halo was intense and prominent, the yellow halo was less prominent (very insignificant) in Assam lemon. The cankers were seen even on the petiole and midveins. The lesion had also been observed in the serrated margins of the leaf. The lesions on both sides of the leaves have prominently raised margins and gave crater-like appearance. Crater like appearance here it was very prominent; compared to Acid lime or Assam lemon, and they were found in majority of the lesions. Lesion size ranged from 0.5 mm to 5 mm diameter but in severe cases lesion measured 7 mm in diameter. Such lesions also tend to coalesce to form larger lesion on twigs, cankerous lesions were also observed. They are usually linear in shape in the older branches. Dieback symptom was also observed in severe case. Kalita *et al.* (1997) recorded similar symptoms on this host from Assam.

Table 1 : Extent of variation of disease severity on three species of *Citrus* and post infectional biochemical changes

Isolates	Acid lime			Assam lemon			Pummelo		
	Lesion size (Sq. mm)	Phenol (mg/g)	Total sugar (%)	Lesion size (Sq. mm)	Phenol (mg/g)	Total sugar (%)	Lesion size (Sq. mm)	Phenol (mg/g)	Total sugar (%)
LN 1	25.11*	3.60*	14.20*	27.00*	7.61*	11.56*	28.00*	3.59*	13.64*
LN 2	32.22	3.65	14.21	26.11	7.63	11.44	32.17	3.47	13.70
LN3	21.78	4.09	13.59	23.22	7.92	11.17	45.34	3.06	14.92
LN4	16.67	4.85	12.83	21.11	8.20	10.82	16.33	4.26	12.93
LN 5	19.33	4.25	13.26	32.56	7.54	11.90	36.00	3.45	13.46
LH 1	23.78	3.64	14.70	20.89	8.24	10.51	19.66	3.51	13.96
LHo 1	30.11	3.56	13.46	20.44	8.22	10.62	26.67	5.06	11.65
BHo 2	23.78	3.55	14.24	43.89	7.23	11.25	11.83	4.33	12.61
LM 1	33.33	3.53	14.38	25.44	8.09	10.99	21.33	4.91	11.84
LNP 1	34.00	3.13	15.72	17.11	9.66	9.80	13.00	4.85	10.90
Control	0.00	2.69	17.12	0.00	6	13.16	0.00	3.02	15.21
Mean	26.01	-	-	25.78	-	-	25.03	-	-

*=Mean of three replications, L=leaf, B=Branch, N=Nadia, H=Howrah, Ho=Hooghly, M=Purba Medinipur, NP=North 24 Parganas

(iii) Pummelo (Citrus grandis)

The plant was relatively resistant to citrus canker as compared to acid lime and Assam lemon, with respect to number of lesions, prominent appearance of the canker lesion and the lesions size. However, cankers were sparsely noticed on the foliage. Water soaked symptoms was not visible or absent in natural infection. Initial infection starts as a small brown slightly raised spot, mostly circular, with a prominent and distinct yellow halo on the upper surface and yellow to orange on the lower surface of the leaf. Very rarely the cankers were observed on the twigs, branches or fruits. In advance stage of the leaf infection the lesion became deep brown, rough, corky, slightly raised and they were more prominent and numerous on the upper surface of leaf. On the corresponding lower surface, dark brown or tan colored spot were visible. In some instances only raised necrotic lesions developed on the upper leaf surface without the brown spot on the corresponding under surface of leaf and number of such raised protuberance was very less as compared to Assam lemon and acid lime. The lesion size may vary from a mere less than 1 mm to about 3.5 mm. diameter. Average lesion size measure 1-2 mm. in diameter. Those necrotic lesions which are greater than 2 mm in diameter tend to develop a rough, corky and crater like symptoms with slightly raised, deep brown margins. The fruits remained unaffected, as no diseased symptoms or infection has been detected so far. In one instance, leaf miner infection considerably increased the size and the number of canker lesion leading to severe curling and distortion of the leaves.

***Pathogenic variability of different isolates of Xanthomonas axonopodis pv. citri on three Citrus* spp.**

The experiment was carried out with 10 isolates of *Xanthomonas axonopodis* pv. *citri* (Table 1) on three *Citrus* species namely acid lime (*C. aurantifolia*), Assam lemon (*C. limon*) and pummelo (*C. grandis*) under field condition during April-May, 2012 to determine the pathogenic fitness or pathogenic aggressiveness. The artificial inoculation on tender leaves with the suspension of *Xac* isolates

Table 2: Correlation study of lesion size against total sugar and phenol on different citrus hosts

Crop	Parameters		Correlation coefficient
Acid lime	Lesion size	*vs* total sugar	0.690*
		vs phenol	-0.835**
Assam lemon	Lesion size	*vs* total sugar	0.642*
		vs phenol	-0.763*
Pummelo	Lesion size	*vs* total sugar	0.664*
		vs phenol	-0.660*

*Correlation is significant at the 0.05 level (2tailed).
**Correlation is significant at the 0.01 level (2tailed).

were made following the method mentioned earlier and disease severity (area of individual lesion) was recorded on 10th day after inoculation.

None of the citrus species tested were free from Citrus Canker disease after artificial inoculation. But they differed in their tolerance/susceptibility against different isolates of *Xac*. In other words, the different *Xac* isolates showed varying degrees of virulence or aggressiveness towards different citrus hosts.

All the *Xac* isolates exhibited their aggressiveness/ virulence on acid lime with varied range of lesion size or area (16.67 to 34.0 mm^2), and among them isolates LNP1 produced highest lesion size or area of 34.0 mm^2 (Table 1). The isolates were considered to be highly virulent with respect to pathogenic fitness with citrus canker disease system on acid lime group of citrus.

On Assam lemon citrus group *Xac* isolates produced disease lesion within a range of 17.11 to 43.89 mm^2 and one isolate BHo2 appeared to be highly virulent inducing 43.89 mm^2 lesion area.

Over all mean disease lesion area was comparatively low (25.03 mm^2) on pummelo group and observed that there was not much distinct pathogenic variability among the *Xac* isolates. Different isolates exhibited only a range of 11.83 to 45.34 mm^2 diseased lesion area.

Accordingly, the overall mean disease severity (26.01 mm^2) was maximum on acid lime group and considered as most susceptible group followed by Assam lemon group (25.78 mm^2) as moderately susceptible group and Pummelo group (25.03 mm^2) as moderately resistant group. Kishun and Chand (1987) reported that canker infestation is relatively more on acid lime and less common on mandarin and sweet orange. Muhammad-Atiq *et al.* (2007) screened fifteen cultivars for the source of resistance against citrus canker and found that the variety *Citrus limonia* (L.) Osbeck cv. Meyer lemon showed highly susceptible reaction, whereas Pummelo white, Shamber grapefruit and Rough lemon were found susceptible (Muhammad-Burhan *et al.*, 2007).

Post infectional biochemical changes on three different Citrus species through artificial inoculation with Xac isolates

The ten isolates of *Xac* isolated from acid limes were inoculated separately on the acid lime, Assam lemon and pummelo leaves following the standard methods. During the course of pathogenesis the biochemical changes in the hosts were studied and compared with the control inoculated with sterile distilled water.

Estimation of phenol from artificially Xac inoculated citrus leaves

The highest phenolic content was exhibited by LN4 (4.85 mg/g) in Acid lime while it was highest by LNP1 (9.66 mg/g) in Assam lemon. Isolate LHo1 showed maximum (5.06 mg/g) phenol content in inoculated leaves of pummelo during this post inoculation pathogenesis process. It was revealed from Table 1 that there was slight increase in phenol content in all the *Xac* inoculated tissues (mg/g fresh leaves) in the initial stages of infection in comparison to their respective controls 3.02 mg/g in acid lime, 6.0 mg/g in Assam lemon and 2.69 mg/g in pummelo respectively. The above findings were in conformity with the works made earlier by Manonmani *et al.* (2009). Rommelt *et al.* (1999) also explained that the accumulation of phenolics was ten times higher in apple leaves with slight infection of *Erwinia amylovora* (Burrill, 1882; Winslow *et al.*, 1920), than in leaves with pronounced disease symptom.

Estimation of total sugar from artificially Xac inoculated citrus leaves

There were slight reductions in post infectional to-

tal sugar content in all the three citrus species over their respective controls. It was found that, maximum sugar content recorded in isolate LNP1 (15.72%) in acid limes while it was minimum (12.83 %) with isolate LN4 in contrast to 17.12% in control. In Assam lemon maximum and minimum sugar content were recorded in LN5 (11.90%) and LNP1 (9.80%) respectively and (13.16%) in control. In pummelo maximum (14.92%) and minimum (10.90%) total sugar content were estimated in LN3 and LNP1 respectively and 15.21% in control. The experimental results indicated that there was a trend of slight decline in the percentage of total sugar in the inoculated leaves as compared to controls. Our results are supported by findings of Manonmani *et al.* (2009).

Relationship of disease severity with post infectional biochemical changes in phenol and total sugar content

Correlation study among the variables *viz.* lesion size, total sugar and phenol reveals that the isolates of *Xac* obtained from acid lime showed to be highly significant in mother host; acid lime, in comparison to different but related hosts *viz.* Assam lemon and pummelo as shown/revealed in correlation matrix in Table 2. These findings rather proofed the fact that all the isolates of acid lime showed higher compatibility and pathogenic fitness towards acid lime with regards to host-pathogen interaction (*i.e.* detrimental interaction and dependency of increment of lesion size with the total sugar content of the plant and phenolic production in the plant system). Also a significantly negative correlation (-0.835**) obtained between lesion size and phenols are in agreement with the fact that higher disease severity or lesion correlates to reduced phenol production or its defense activity and vice versa as noticed in case of acid lime (we know that, phenol is associated with the disease resistance mechanism of a plant due to its high oxidative ability). Similarly, positive correlation (0.690*) was established with disease severity and total sugar content in the plant (acid lime). However, in case of non-host i.e. Assam lemon and pummelo the interaction of the variables lesion size with total sugar content and phenols were weakly correlated Table 2. This may be attributed to the fact that, Assam lemon and pummelo hosts rendered slight incompatibility factor due to different host henceforth highly significant relationship could not be established among the variables.

Thus we are directed to draw a conclusion that there are significant and remarkable variation in Citrus Canker symptoms and that acid lime is most compatible or susceptible for the establishment of citrus bacterial canker disease. Additionally, the present experimental findings revealed that the pathogen causing citrus bacterial canker, *Xanthomonas axonopodis* pv. *citri* can also successfully infect and reach a highly damaging proportion even in related hosts as well. The biochemical changes induced in the plant due to the infection is well established from the above experiment so it is highly imperative to seek for strategy involving induction of phenolic compound in plants either through the use of biotic or abiotic elicitors in disease management programme which will ultimately help in mitigating the disease.

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