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Changes in pectic substances and calcium content in leaves of *Piper betle* infected by *Fusarium scirpi*

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It was found that pectic substances increased considerably in leaf tissues of all five cultivars (i.e. Kali, Mitha-Bangla. Dhal. Sanchi, Mitha) of *Piper betle* due to infection by *Fusarium scirpi.* The maximum increase was found in Mitha-Bangla cultivar (8.85 mg/g dry wt.) and minimum in cultivar Mitha (6.60 mg/g dry wt.).

The calcium content of all the cultivars of *P. betle* due to infection by *F. Scirpi* was also found to increase. The maximum increase was found in Mitha-Bangla cultiver (5.58 mg/g dry wt.) and minimum in cultivar Kali (4.54 mg/g dry wt.).

Both the pectic substances and calcium content were found to increase upto 7 days after inoculation and then they started to decline.

Key words : Pectic substances, calcim content, *Piper betle, Fusarium scirpi*

INTRODUCTION

Betelvine has been grown for centuries in some areas but uptil now there is no generalised plant protection management system which can protect the crop from several diseases. According to maiti and Sen (1979), the fungal diseases of betelvine is the most important so far the lose is concerned. Of these, *Phytophthora* wilt, *Sclerotium* wilt, and leaf spots diseases have been reported by several workers (Saxsena, 1977 ; Chile *et al.,* 1984 ; Chattopadhyay and Sengupta, 1956 ; Mehrotra and Tiwari, 1967 ; Singh and Joshi, 1972 ; Raut and Sukla, 1973 ; Amhed and Pathak, 1974 ; Maiti and Sen, 1977 ; Maji *et al.*, (1984).

Maji *et al.* (1984) first reported the leaf spot disease of betelvine caused by *Fusarium scirpi.* Uptil now on works have so far been done on this organism in association with *Piper betle.* As such in the present investigation attempts have been made to find out the host parasite interactions of this organism and the observations are presented in the following pages.

MATERIALS AND METHODS

The seeds (setts i.e. five nodes vines) of *Piper betle* (Cultivars *Mitha, Sanchi, Dhal-Bangla, Kali-Bangla* and *Mitha-Bangla*) were collected from Tamluk, Midnapur, West Bengal and were cultivated for experiment at Tamluk, Midnapur, West Bengal.

At 4 months of plant growth, healthy plants were inoculated with the conidia (8×10^6 / ml) of virulent strains of *Fusarium scirpi* in separate plants in the following way.

First the leaves of the plants were rubbed by sterilized sand particles and then the formerly prepared conidial suspension was sprayed over the rubbed areas of the leaves by automizer (sprayer) and then the leaves were covered with moist polythene bags. Simultaneously some rubbed leaves sprayed with distilled water and covered with moist

polythene bags were also kept as control just like inoculated ones. Different sets of plants both normal and inoculated were harvested after 2 days, 5 days, 7 days, 10 days and 15 days after inoculation. Each type of harvested leaves were then washed with distilled water and were kept in different polythene bags and were stored at 4°C for biochemical studies.

Method for estimation of calcium

An amount of 5 g of fresh materials (leaves) was crushed, diluted with distilled water and volume was made upto 50 ml with distilled water. This prepared solution was used for calcium estimation following the method of Vogel (1961).

Method for estimation of pectic substances

Fresh healthy and infected leaves were dried in a hot air oven at 60°C for 24 hr and then the dried plant materials were crushed for producing powder in a clean morter with pestle. Then this powder was stored in a stoppered bottle in a desiccator and pectic substances were estimated following the method of McComb and McCready (1952) and McCready and McComb (1952).

RESULTS AND DISCUSSION

Changes in Pectic substances

Necessary experiments were done to eveluate the change in pectic substances in cell walls of healthy and inoculated leaves of *P. betle* and the results obtained are presented in Table 1.

The data in Table 1 showed that due to infection by *F. scirpi*, the pectic substances increased to 8.32, 8.86 and 9.36 (µg/g) upto 2, 5 and 7 days after inoculation respectively in Kali cultivar then it declined to 8.70 and 7.86 (µg/g) at 10 and 15 days after inoculation. In Mitha-Bangla, it increased to 9.42, 9.70 and 10.26 (µg/g) after 2, 5, and 7 days after inoculation respectively. It then decreased to 9.26 and 7.93 (µg/g) at 10 and 15 days after inoculation respectively. In Dhal cultivar the pectic substances content (mg/g) increased to 8.20, 8.74 and 9.40 at 2, 5 and 7 days after inoculation and then declined to 8.54 and 7.90 at 10 and 15 days

Table 1 : Data (mean) showing the amount[a] of pectic substances (mg/g dry wt) in cell wall of healthy and infected leaves of *Piper betle* inoculated by *Fusarium scirpi.*

Cultivars	Healthy	Pectic substances (mg/g dry wt.)				
		Days after inoculation				
		2	5	7	10	15
Kali	7.70 ± 0.20	8.32 ± 0.20	8.86 ± 0.03	9.36 ± 0.04	8.70 ± 0.02	7.86 ± 0.20
Mitha Bangla	8.85 ± 0.15	9.42 ± 0.07	9.70 ± 0.05	10.26 ± 0.08	9.26 ± 0.05	7.93 ± 0.80
Dhal	7.70 ± 0.21	8.20 ± 0.08	8.74 ± 0.06	9.40 ± 0.08	8.54 ± 0.06	7.90 ± 0.85
Sanchi	6.60 ± 0.20	7.12 ± 0.20	8.00 ± 0.05	9.00 ± 0.20	8.20 ± 0.04	7.85 ± 0.20
Mitha	6.40 ± 0.25	7.00 ± 0.05	7.70 ± 0.08	8.80 ± 0.02	8.00 ± 0.20	7.70 ± 1.08

[a]Data have been expressed in mg per g dry cell wall materials. Each value represents an average of three separate determinations from different prepartion ± S.E.M.

Table 2 : Data (mean) showing the concentration[a] of calcium in cell wall of healthy and infected leaves of *piper betle* inoculated by *Fusarium scirpi.*

Cultivars	Healthy	Calcium (mg/g dry wt)				
		2	5	7	10	15
Kali	3.84± 0.06	3.96 ± 0.06	4.20 ± 0.08	4.54 ± 0.10	4.02 ± 0.10	3.64 ± 0.12
Mitha Bangla	4.48 ± 0.14	4.68 ± 0.10	4.96 ± 0.04	5.58 ± 0.06	5.40 ± 0.08	4.02 ± 0.10
Dhal	3.94 ± 0.10	4.36 ± 0.04	4.78 ± 0.06	4.98 ± 0.10	4.26 ± 0.06	4.06 ± 0.06
Sanchi	4.00 ± 0.16	4.22 ± 0.06	4.52 ± 0.06	4.84 ± 0.06	4.66 ± 0.06	4.40 ± 0.10
Mitha	3.88 ± 0.10	4.12 ± 0.08	4.40 ± 0.10	4.76 ± 0.08	4.38 ± 0.08	4.00 ± 0.06

[a]Data have been expressed in mg per g dry cell wall materials. Each value represents an average of three separate determinations from different prepartion ± S.E.M.

after inoculation respectively. In Sanchi cultiver it increased to 7.12, 8.00 and 9.00 (µg/g) at 2, 5 and 7 days after inoculation and decreased thereafter to 8.20 and 7.85 (µg/g) at 10 and 15 days after inoculation respectively. In Mitha cultivar it increased to 7.00, 7.70 and 8.80 (µg/g) at 2, 5, and 7 days after inoculation and thereafter decreased to 8.00 and 7.70 (µg/g) at 10 and 15 days after inoculation respectively.

Changes in Calcium content in cell wall

The data in Table 2 showed the changes in calcium content of five cultivars of *P. betle* due to infection by *F. scirpi.*

In Kali the calcium content was 3.84 (healthy) which gradually increased to 3.96, 4.20, 4.54 (mg/g dry wt.) at 2, 5 and 7 days after inoculation respectively. Then it declined to 4.02 and 3.64 (mg/g dry wt.) at 10 and 15 days respectively.

In Mitha Bangla, the calcium content in healthy leaves was 4.48 (mg/g) which gradually increased to 4.68, 4.96 and 5.58 at 2, 5, and 7 days after inoculation respectively. Then it declined to 5.40 and 4.02 (mg/g dry wt.) at 10 and 15 days respectively.

In Dhal the calcium content (healthy) was 3.94 which gradually increased to 4.36, 4.78 and 4.98 (mg/g dry wt.) at 2, 5, and 7 days after inoculation respectively. Then it declined to 4.26 and 4.06 (mg/g dry wt.) at 10 and 15 days respectively.

In Sanchi the calcium content in healthy tissue was 4.00 (mg/g dry wt.) which gradually increased to 4.22, 4.52 and 4.84 (mg/g dry wt) at 2, 5 and 7 days after inoculation. Then it decreased to 4.66 and 4.40 (mg/g dry wt.) at 10 and 15 days respectively.

In Mitha the calcium content was 3.88 (mg/g dry wt.) in healthy leaves which increased to 4.12, 4.40 and 4.76 (mg/g dry wt.) at 2, 5, and 7days after inoculation. Then it declined to 4.38 and 4.00 at 10 and 15 days after inoculation.

The data in Table 1 showed that there was a considerable increase in pecti substances in leaf tissue of all the five cultivars of *P. betle* due to infection by *F. scirpi.* The pectic substances (mg/g dry wt.) was maximum in tissues of Mitha-Bangla (8.85) among the five cultivars which was followed by Kali (7.70), Dhal (7.70), Sanchi (6.60) and Mitha (6.40). The pectic substances increased gradually with the increase of infection time upto 7 days (i.e. 7 days after inoculation) by *F. scirpi.*

The calcium content of all the cultivars of *P. betle* was also found to increase upto 7 days after inoculation and then it started declining. The calcium content was maximum in cv. Mitha-Bangla (4.48 mg/g dry wt.) in healthy leaves tissues and it increased to 5.58 (mg/g dry wt.) and was minimum in cv. Kali (3.84 mg/g dry wt.) and it incrreased to 4.54 (mg/g dry wt.).

Such changes in the concentrations of pectic substances and calcium in the respective leaf tissues of different cultivars of *P. betle* indicate the involvement of pectin degrading enzymes (PME and Endo-PG) of Pathogenic origin in disease symptom development.

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