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Histological and biochemical changes in *Mycoplasma* infected leaves of eggplant

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Cell length and nuclear diameter were reduced in *Mycoplasma* infected leaves of eggplant. Microspectrophotometric study indicated slight increase of DNA, but not of RNA contents of cells of infected plants as compared to healthy controls. Total chlorophyll and chlorophyll a and b contents of the infected leaves decreased. Photosynthetic efficiency of leaves as measured by Hill reaction rate also decreased in infected plants. Total carbohydrates, proteins and phenol contents of the infected plants decreased significantly. Both RNA and DNA contents of the infected leaves decreased. Gel-electrophoretically separated proteins of infected leaves indicated quantitative and qualitative changes in protein profile as compared to non-infected controls.

Key words : Eggplant, *Mycoplasma*-infection, histological and biochemical changes

INTRODUCTION

Little leaf of eggplant is caused by mycoplasma like organism (Varma *et al.*, 1969). The key symptom of the disease is stunting, profuse branching and extreme reduction of the leaf lamina (Maramorosch, 1976). Although the striking morphological changes caused by MLO infection of eggplant are well documented, the physiological changes in infected plants are not well understood. Generalized stunting, significant reduction of leaf lamina and partial chlorosis of leaves suggest disturbance in relation to metabolism and growth involving photosynthesis, protein synthesis and nucleic acid synthesis in the infected plants.

The objectives of this investigation were : i) to determine the alteration of call and nuclear size and changes in nucleic acid content per cell of the infected tissues, ii) to examine the physiological changes involving carbohydrate, protein, phenolics and nucleic acid contents of the infected tissues, and iii) to compare the gel electrophoretically separated protein profiles of the healthy and infected leaf tissues.

MATERIALS AND METHODS

Healthy and infected leaves of eggplant were collected from cultivators' fields and used immediately. Young leaf tips of healthy and infected plants were fixed in acetic acid; ethanol (1:3) for 1 h, heated with aceto-orcin : 1N HCl (9:1) for few minutes and stored

for overnight. Leaf tips were then squashed on glass slide. The cell or nuclear sizes of cells in squashed preparations were determined by a micrometer.

For microspectrophotometric measurement of nuclear DNA and RNA contents the method of Sau *et al.* (1980) was followed. The leaf tips of healthy and infected plants were fixed with aceto-alcohol (1:3), hydrolysed with 1N HCI at 60°C for 8 minutes and stained with Schiff's reagent for DNA or with pyroninemethyl green for RNA. DNA and RNA contents were estimated with a Carl Zeiss MFV 4002 microspectrophotometer by using respective standards for DNA and RNA.

Chlorophyll from leaves of health and infected plants was extracted with cold 80% acetone and the amount of total chlorophyll, chlorophyll a and chlorophyll b was determined by the method of Arnon (1949). For Hill activity study, chloroplasts from healthy and infected leaves were isolated by the method of Saha and Good (1970). The reaction mixture for Hill reaction contained 0.2 ml chloroplast suspension, 4 ml of suspending medium and 2 ml of aqueous solution of 0.1 mM 2,6-dichlorophenol-indophenol. The initial absorbance was measured at 620 nm after the addition of the dye. The mixture was illuminated with two 500 W lamps for 5 min and the final absorbance was measured using a Bausch and Lomb spectronic colorimeter. Results are expressed as A620/mg chlorophy11/5 min, following the method of Naidu *et al.* (1984).

Carbohydrates from healthy and infected tissues were extracted with boiling 80% ethanol. The tissue samples were homogenized and filtered. The filtrate was evoporated to dryness with a flash evaporator at 37°C and the residue was dissolved in a small volume of distilled water. Carbohydrate content of the aliquots of the preparation was determined by the anthrone method of Morris (1948). Phenol from healthy and infected leaf tissues was extracted with hot 80% methanol following the method of Seevers and Daly (1970). The phenol content of the samples were estimated by Folin-Ciocalteu reagent (Spies, 1955). Sodium phosphate buffer soluble proteins from healthy and infected leaf tissues were extracted. The protein contents of the aliquots were estimated following the method of Lowry *et al.* (1951).

RNA from tissues was extracted by the method of Loening (1967). RNA content of the tissue extracts was estimated by using orcinol reagent. DNA from tissues was extracted by the method of Lyttleton and Peterson (1964). DNA content of the tissue extracts was estimated using diphenylamine reagent of Burton (1968).

Soluble proteins from healthy and infected leaves were extracted by using an extraction medium containing 0.1M tris-HCl buffer, pH, 8.0; 0.1% ascorbic acid (Sigma, USA); 0.1% cysteine (E. Merck, Germany). The homogenate was centrifuged for 40 min. at 15000 g in a refrigerated centrifuge, The supernant served as protein preparation for gel electrophoresis. Polyacrylamide gel tubes (7.5% polyacrylamide) were prepared following the method of Davis (1964) with minor modifications. Gel tubes after preparation were fitted to the electrophoresis chamber containing Tris-glycine buffer (Tris, 0.6 g; glycine, 2.88 g; H_2O, 1 liter; pH, 8.3) and charged with the test protein solutions. Electrophoresis was carried out at 20°C applying 4 mA current per tube at 300 volts using systronics power pack till the marker dye (bromophenol blue, E. Merck, Germany) reached near the lower end of the gel tube. The gel sticks were then removed and stained with amido black (1 g/1 in 7% aqueous acetic acid) for 1 h and destained with 7% acetic acid for 2-3 days. Protein

patterns of the samples were evaluated on the basis of the number and position of the bands. The Rm values of the stained protein bands were calculated with respect to the marker dye band.

RESULTS

Histology and histochemistry of healthy and infected leaves

Histological and microspectrophotometric studies of leaves showing little leaf symptoms were made to determine the nature of the dwarfing at the cellular level. Cell length, diameter of the nucleus and DNA and RNA content per nucleus were determined. Results (Table 1) showed that cell length and nuclear diameter of infected leaves of eggplant were significantly reduced. The DNA content per nucleus increased, whereas the RNA content per nucleus remained unchanged in cells of infected plants.

Table 1. Comparison of cell length, nuclear diameter, DNA/nucleus and RNA/nucleus of healthy and infected leaf tissues of eggplant

Cytological parameters	Tissue type		
	Healthy	Infected	% change
Cell length (μ)[a]	126.7	60.9	-52
Nuclear diameter (μ)	34.4	15.3	-56
DNA/nucleus[b]	56.2	64.9	+15.5
RNA/nucleus[c]	48.2	50.0	+3.7

[a]Cell length and nuclear diameter were measured with a micrometer and the mean value of at least 400 cells is presented.

[b]DNA content per nucleus was measured by microspectrophotometric techniques. Data presented are based on at least 100 interphase nuclei.

[c]RNA content per cell was determine by microspectrophotometry and data presented are based on at least 100 cells in interphase state.

Chlorophyll content and Hill reaction potential of healthy and infected leaves

The chlorophyll contents and Hill reaction activities of the infected leaves as compared to healthy controls were examined in this experiment. The relative concentrations of a, b, and total chlorophyll and the Hill reaction activity per mg chlorophyll was determined. Results (Table 2) showed that in infected leaves the contents of a, b and total chlorophyll reduced significantly as compared to healthy controls. Hill reaction activity of the chlorophylls extracted from infected leaves was also significantly reduced.

Table 2. Comparison of chlorophyll content and Hill activity of healthy and infacted tissues of eggplant

Tissue type	Chlorophyll content (mg. g^{-1})			Hill reaction activity (OD_{620} mg^{-1} chlorophyl per min.)
	Chl a	Chl b	Total	
Healthy	19.2	23.5	42.7	4.8
Infected	10.4	13.6	24.2	1.4
	(45.3)[a]	(42.1)	(43.3)	

[a]Figures in the parenthesis indicate percentage reduction from control tissue.

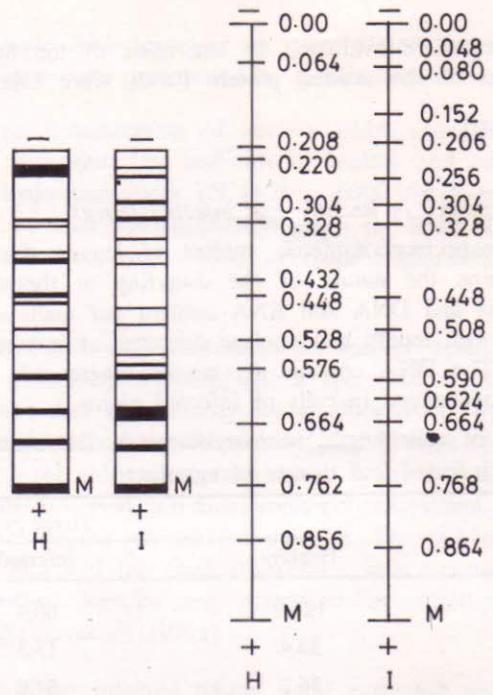

Fig.1, Disc electrophoretic patterns of soluble protein extracts from healthy (H) and infected (I) eggplant leaf tissues on 7.5% polyacrylamide gels. The diagram represents the average value of Rm of five gels from three extracts and three electrophoretic runs. The amount of protein in each gel was about 150 mg.

Comparison of carbohydrate, protein and phenol contents of healthy and infected leaves

Total contents of anthrone positive carbohydrates, buffer soluble proteins and methanol extractable free phenols of healthy and infected leaf tissues were determined. Results (Table 3) showed that carbohydrate, proteins and phenol contents of infected tissues decreased significantly as compared to healthy leaves

Table 3. Comparison of carbohydrate, protein and phenol contents of healthy and infected leaves of eggplant

Tissue type	Carbohydrate[a] (mg. g^{-1})	Protein[b] ($mg.g^{-1}$)	Phenols[c] ($mg.g^{-1}$)
Healthy	12.3	2.5	3.0
Infected	9.5	1.2	1.5
	(22.8)[d]	(52.0)	(50.0)

[a]Anthrone positive carbohydrates were determined.

[b]Buffer soluble proteins were extracted and determined.

[c]Methanol extractable phenolics were determined

d Figures in the parenthesis indicate percentage induction from controls.