EFFECT OF INFECTION BY *COLLETOTRICHUM CAPSICI* AND TOXIN TREATMENT ON THE PERMEABILITY CHANGES OF TURMERIC LEAVES

M. CHANDRASEKHARAN NAIR*

(Department of Plant Pathology, College of Agriculture, Vellayani, Kerala)

AND

K. RAMAKRISHNAN, F.A.SC.

(University of Agricultural Sciences, Bangalore)

MS received 4 May 1974

ABSTRACT

A toxin produced by *Colletotrichum capsici*, pathogen of turmeric leaf spot disease has been found to alter the cell permeability of turmeric leaf tissue even at very low concentrations. This led to leaching of large quantities of water soluble constituents. The possibility of the involvement of a toxin in the pathogenesis is discussed.

INTRODUCTION

IN an earlier communication (Nair and Ramakrishnan 1973) we have reported that the toxin isolated from the culture filtrate of *Colletotrichum capsici* (Syd.) Butl. and Bisby produces pathogen free spots on turmeric leaves clearly resembling the leaf spots induced by the pathogen in morphology. We report here the permeability changes induced by the toxin in turmeric leaves.

MATERIALS AND METHODS

A highly pathogenic isolate of *Colletotrichum capsici* isolated from turmeric leaves was used in the present study. The fungus was cultured over a shallow layer of Fries' medium No. 3 for 15 days and the toxin isolated as per the methods outlined earlier (Nair and Ramakrishnan 1973). 2000 μg of the toxin dissolved in one ml of sterile distilled water served as the stock solution. At this concentration when bioassayed against turmeric leaves at the rate of 0·05 ml per drop, the toxin was capable of inducing 20 mm diameter necrotic spots with an yellow halo, in 24 hr. The stock

* This paper forms part of the Ph.D. thesis of M. C. Nair, submitted to the Tamilnadu Agricultural University.

solution was serially diluted to give 200, 20, 2, and 0·2 μg/ml of the toxin and the effect of these samples in changing the permeability of the host tissue was studied. Turmeric leaves of uniform age were collected from plants grown in a glass house. Leaves were cut into uniform pieces and were permitted to take up the toxin solution by keeping them immersed in the toxic solutions of appropriate concentration for 4 hr. The leaf pieces were then transferred to 30 ml of deionised water in conical flasks to facilitate leaching of water soluble constituents. The electrical conductivity of the leachate was measured periodically using a conductivity bridge. Ten grams of leaf material were used for each test. Leaf pieces of turmeric of the same age artificially infected by *Colletotrichum capsici* were also similarly leached. Healthy leaf pieces treated with deionised water served as control. All estimations were triplicated. The leachate sample from leaf pieces treated for 12 hr with 2000 μg/ml of the toxin was analysed for sodium, potassium, calcium, total carbohydrates, total nitrogen and total phosphorus in comparison with that from healthy control. Potassium, calcium and sodium were estimated using a Perkin-Elmer flame photometer as described by Jackson (1962). Estimation of total nitrogen was done by the Kjeldhal's method (Jackson 1962), total carbohydrates by the anthrone reaction method according to the procedure of Barton (1961) and total phosphorus by the method outlined by Ward and Johnston (1962).

Results

Changes in Permeability

Changes in electrical conductivity of the deionised water in which the toxin treated and infected turmeric leaves were leached for different periods of time are presented in table 1.

It is evident from the data presented in table 1 that the cell permeability of toxin treated and infected tissue was drastically altered leading to the leaking out of water soluble constituents. This alteration was a function of concentration of toxin applied and duration of leaching. In the healthy leaf also some leaching had taken place but this was far less than in toxin treated and infected leaves. Permeability changes seem to take place even within 4 hr of toxin treatment even with the lowest concentration of toxin used.

Leakage of soluble constituents from toxin treated leaf pieces

The analysis of the leachate collected during different periods of leaching for the presence of various soluble constituents is presented in figure 1,

Table 1. *Changes in electrical conductivity of leachate from toxin treated and infected turmeric leaves (as μ mho/cm for 10 g fresh weight of leaves)*

Sl. No.	Hours leached	Healthy control	Pathogen infected tissue	Toxin dose (μg/ml) 2000	200	20	2	0·2
1	0	100·0	100·0	100·0	100·0	100·0	100·0	100·0
2	4	113·3	171·6	300·0	250·0	173·3	146·6	136·6
			(+ 51·4)	(+ 164·7)	(+ 120·6)	(+ 34·6)	(+ 29·3)	(+ 20·4)
3	8	124·3	253·3	411·6	303·3	346·6	221·6	213·3
			(+ 103·8)	(+ 231·1)	(+ 144·0)	(+ 178·4)	(+ 78·0)	(+ 71·6)
4	12	187·0	270·0	914·6	601·6	440·5	411·6	401·6
			(+ 49·7)	(+ 388·7)	(+ 221·7)	(+ 135·5)	(+ 120·1)	(+ 114·8)

Values in parentheses represent percentage increase over healthy control.

Toxin treatment C.D. = 67, S.E. = 23·4.

Infection C.D. = 4·12, S.E. = 1·414.

The toxin treated tissues lost large amounts of potassium, phosphorus, nitrogen and soluble carbohydrates while the loss of sodium and calcium was negligible. When compared with healthy controls the loss in potassium and nitrogen was 500 per cent higher and those of carbohydrates and phosphorus about 250 and 150 per cent respectively.

DISCUSSION

The data presented herein clearly show that infection and toxin treatment were capable of bringing about pronounced changes in cell permeability of the host and this happened at all concentrations of the toxin tried. In this respect the toxin resembles victorin as reported by Wheeler and Black (1963). Altered permeability has been suggested by Wheeler and Hanchy (1968) as the initiative phase of triggering host physiology in plant infections. Bollard and Matthews (1966) have also stated that the first effect of the pathogen on the host tissue appeared to be an increase in permeability, which made nutrients available to the parasite. The present finding that the *Colletotrichum capsici* toxin altered the host cell wall permeability indicates that the toxin plays an important role in pathogenesis. Bollard and Matthews (1966) pointed out that many fungal parasites on entry into the host tissue

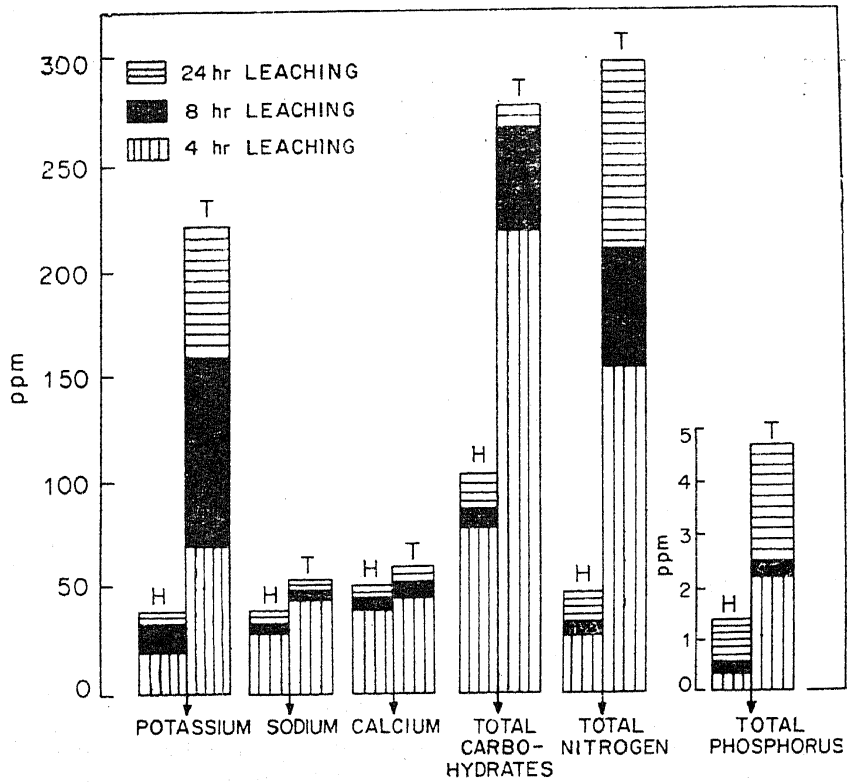

FIGURE 1. Analysis of leachate collected from turmeric leaves treated with toxin during different intervals. H = Healthy; T = Toxin (2000 μg/ml).

apparently secrete some substance or substances that increase permeability. The toxins secreted by the pathogen seem to act in advance of the pathogen in impairing the semi-permeability of the host plasma membrane thereby opening the 'door of the larder' to the advancing pathogen.

REFERENCES

Barton, L. V., Biochemical studies of dormancy and after ripening of seeds. II. Changes in oligobasic organic acids and carbohydrates. *Contrib. Boyce. Thompson Inst.* **21** 147–162 (1961).

Bollard, E. G. and Matthews, R. E. F., *The Physiology of Parasitic Disease in Plant Physiology* (Ed.) Steward, F. E., **4** 417–550 (1966).

Jackson, M. L., *Soil Chemical Analysis* (Prentice Hall of India Ltd., New Delhi) pp 498 (1962).

Nair, M. C. and Ramakrishnan, K., Production of toxic metabolites by *Colletotrichum capsici* (Syd.) Butl. & Bisby and its role in leaf spot disease of turmeric. *Curr. Sci.* **42** 362–363 (1973).

Ward, G. M. and Johnston, F. B., *Chemical Methods of Plant Analysis, Canada Dept. Agric.* Ottawa pp 52 (1962).

Wheeler, H. and Black, H. S., Effect of *Helminthosporium* and victorin upon permeability, *Am. J. Bot.* **50** 686–693 (1962).

Wheeler, H. and Hanchy, P., Permeability phenomenon in plant diseases. *Ann. Rev. Phytopathol.* **6** 331–350 (1968).