ACCUMULATION OF STARCH IN RICE LEAVES INFECTED WITH *HELMINTHOSPORIUM ORYZAE*

ACCUMULATION of starch around the infection court has been reported to be a common phenomenon in obligate parasites[1-3], but as far as facultative parasites are concerned no precise knowledge is available in this context. In brown spot disease of rice incited by *Helminthosporium oryzae*, accumulation of starch around the diseased areas was observed.

Several factors like increased translocation, alteration in metabolic activity or decrease of β-amylase activity might individually or collectively play an important role. In their studies with enzymatic changes in *Helminthosporium* induced brown spot disease, Shishiyama *et al.*[4] observed increased catalase and decreased β-amylase activity in rice leaves following infection, which might influence accumulation of starch.

TABLE I

Variation of starch accumulation in rice leaves infected with Helminthosporium oryzae

Leaf position	Variety	Concentration of accumulated starch following infection		Diurnal variation of starch accumulation index 120 hours after inoculation		
		72 h	120 h	6 A.M.	10 A.M.	6 P.M.
Topmost leaf	Benibhog	+	+	1·2	1·85	1·6
	CH 13	+	++	1·65	2·5	2·3
Second leaf from top	Benibhog	+	++	1·5	2·4	2·05
	CH 13	++	+++	2·0	3·2	2·8

+ = Concentration.

Two weeks old seedlings of two varieties of rice namely resistant CH13 and susceptible Benibhog were inoculated with the conidial suspension of *H.oryzae* grown on potato dextrose agar at 27 ± 1° C for 10–15 days. Freshly infected leaves were boiled in 80% ethanol for 20 minutes to remove chlorophyll· Achlorophyllous leaves were immersed in iodine-potassium-iodide reagent (1·5 g potassium iodide and 0·3 g crystal iodine in 100 ml distilled water) for 30 minutes. Presence of starch was detected by blue colour reaction. Concentration of starch was measured colorimetrically and 'starch accumulation index', *i.e.*, the ratio of area of starch accumulation to the spotted area was measured with the help of stage and ocular micrometer.

Starch accumulation increased with expansion of lesion and its maturation. Maximum concentration of starch accumulation was observed 120 hours after inoculation. Diurnal variation in 'starch accumulation index' was measured at 120 hours after inoculation. Data presented in Table I showed that starch accumulation index was greater at 10 A.M. in comparison with the value at morning and evening. Concentration of accumulated starch and starch accumulation index were greater in the resistant host in comparison to susceptible host. But starch accumulation was found to be greater in the second leaf than in the topmost leaf of both the varieties, though the topmost leaf is considered to be comparatively resistant,

Dept. of Plant Pathology, Bidhan Chandra Krishi Viswa Vidyalaya, Mohanpur, Nadia, West Bengal, *December* 3, 1976.

S. B. CHATTOPADHYAY.
A. K. BERA.

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TOBACCO WILDFIRE DISEASE IN INDIA

DURING the 1976 growing season, a leaf disease of tobacco (*Nicotiana tabacum* L.) has been the cause of much concern to tobacco growers in Coimbatore District because of its destructiveness. Diseased specimens were brought to this department by tobacco cultivators. The apparently water-soaked margin of the diseased areas, the tissues of which, upon microscopic examination, were found to be teeming with bacteria, suggested that the disease was probably of bacterial origin. It is the present purpose of this report to adequately describe the disease and its causal organism.

The disease was observed to cause large necrotic lesions in the leaf with water-soaked areas along the midrib and veins. The infected leaves were distorted

with dead rotten areas presenting a ragged appearance (Fig. 1-B). Such diseased material was macerated, diluted with sterile distilled water and used as inoculum in the preliminary inoculations. The causal organism was isolated under asceptic conditions in nutrient agar and purified subsequently by dilution plate method. Artificial inoculation with the organism produced water-soaked areas along the midrib and veins (Fig. 1-A) as observed under field infections (if heavy cell

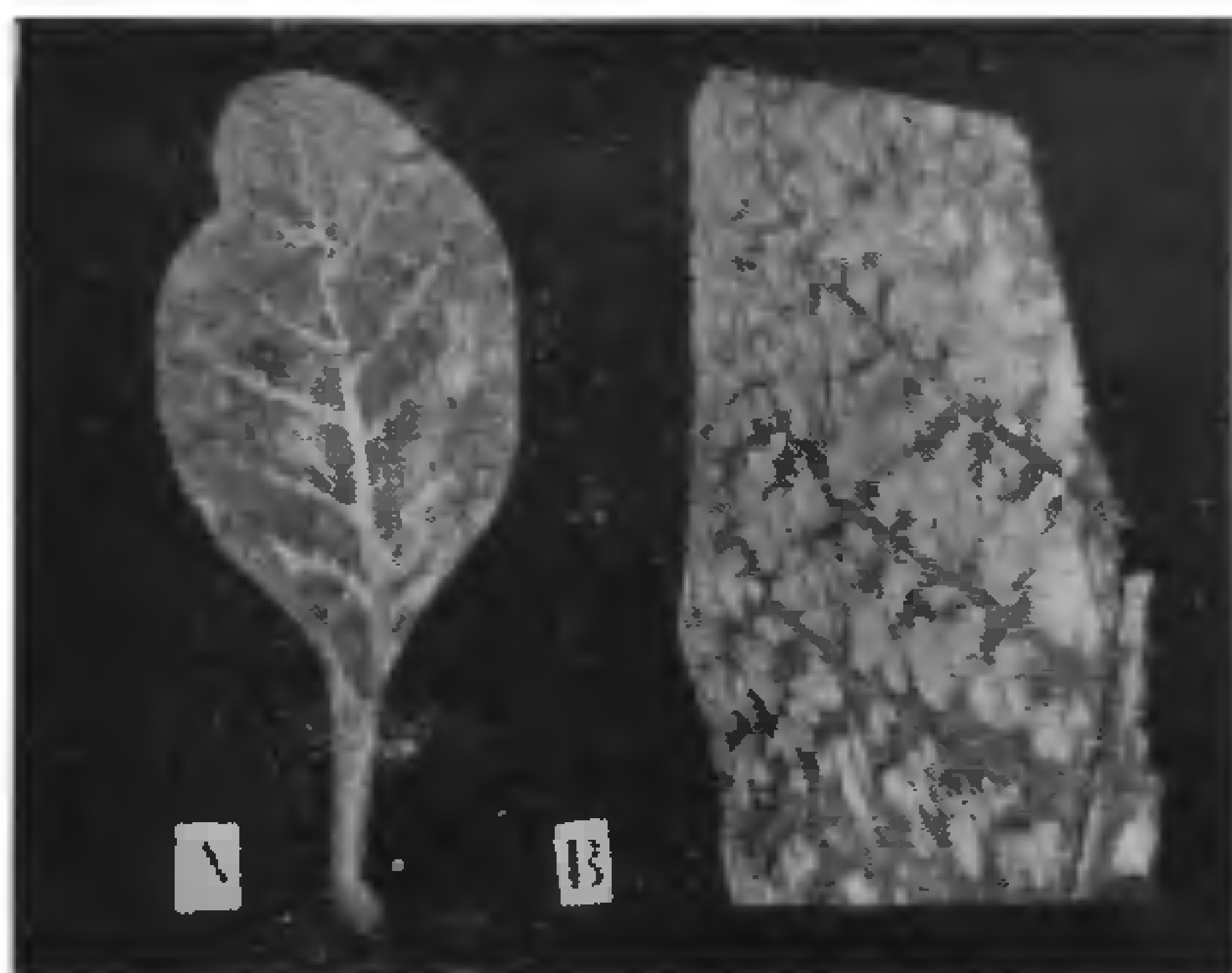

FIG. 1 A-B

concentration was used in inoculum) and also lesions consisting of a small central dead area surrounded by a broad yellow halo[3]. Under artificial conditions, the lesions required about a week to develop fully. In repeated pathogenicity tests the organism produced similar symptoms on tobacco. Inoculation of the pathogen upon *Capsicum annuum*, *Arachis hypogaea*, *Solanum melongena* and *Lycopersicum esculentum* gave negative results.

The pathogen is a rod-shaped bacterium with size of the cells varying from $2 \cdot 0$ to $4 \cdot 5 \times 0 \cdot 9$ to $1 \cdot 6\,\mu$ and average size being $3 \cdot 2 \times 1 \cdot 1\,\mu$. The organism is motile by a single polar flagellum. It is gram negative and, however, is not acid fast. It produces acid but not gas in sugars like glucose, galactose, fructose, xylose, sucrose, glycerol and mannitol; does not produce indole; produces a diffusable green, fluorescent pigment in King's medium B[6]. It is oxidase negative but liquefies gelatin; the organism does not hydrolyse starch but reduces nitrates to nitrites. The process of denitrification is rather difficult to demonstrate in yeast extract-peptone broth but can be readily detected in a synthetic medium[4]. From the above description of this phytopathogenic bacterium and from its capability to initiate disease symptoms on tobacco alone, the organism is identified as *Pseudomonas tabaci* (Wolf and Foster) Stevens[1,2,5], the incitant of tobacco wildfire disease. The authentic culture has been deposited at Type culture collection at Indian Agricultural Research Institute, NewDelhi and in the type culture collection at the Tamil Nadu Agricultural University (item No. 56). This appears to be the first time this pathogen has been found in India.

Department of Plant Pathology,
Tamil Nadu Agric. Univ.,
Coimbatore 641 003,
January 10, 1977.

SAMUEL S. GNANAMANICKAM.
T. K. KANDASWAMY.

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A COMPARATIVE STUDY OF EXTRACELLULAR ENZYMES PRODUCED BY PATHOGENIC FUNGI

SINCE not much is known about the enzyme make up of pathogenic fungi, particularly, the enzymes produced extracellularly, the present investigation was undertaken to study the different kinds of enzymes produced by some of these fungi in relation to their possible role in pathogenicity with the respective host. Extracellular amylase production has been reported from phytopathogenic fungi[1,2]. Cellulase enzymes necessary for the breakdown of cellulose of higher plant cell walls were also known to have been produced extracellularly by a number of phytopathogenic fungi[3-5]. Ribonuclease and deoxyribonuclease, though not related directly to the penetration of the pathogen into the host play an important secondary role of disrupting the protein metabolism of the host and host DNA degradation[6-8]. Little work however has been done so far, to correlate extracellular enzyme production by non-obligate fungi with pathogenicity.

The fungal strains (Table I) obtained from the Indian Agricultural Research Institute, New Delhi, India, were maintained on Czapek-Dox[9] medium and tested for their pathogenic activity prior to enzyme assay. Chemicals used were Na-salt of carboxymethyl cellulose (I.C.I. Ltd.), DNA of calf thymus and Yeast RNA (Nutritional Biochemicals Corporation), Soluble starch (E. Merck), Uranyl acetate (B.D.H.).

The cultures were grown in 100 ml capacity Erlenmeyer flasks containing 25 ml of Peptone-Glucose medium[10] for 10 days at 22° C and 30° C. The supernatant clear culture filtrates obtained after the removal of mycelial debris by