SOME NEW ASPECTS OF MYCOPLASMAL INFECTIONS IN PLANTS

by O. P. BABBAR, *Central Drug Research Institute, Lucknow*

and

U. S. SHUKLA, V. P. AGNIHOTRI *and* KISHAN SINGH,

Indian Institute of Sugarcane Research, Lucknow

Disease symptoms resembling those described to be characteristic of the mycoplasmal infections of plants, appeared in the plants which were infected at the seedling stage, with an avian mycoplasma. As in the natural infections, it was not possible to reisolate the avian mycoplasma from the diseased plants, but the disease symptoms could be transmitted to the healthy seedlings through an aphid vector.

INTRODUCTION

It has been demonstrated that 9-day-old chick embryos (WLH) are ideally suited for the pathogenicity tests of avian mycoplasma (Babbar and Verma 1972). Of the inoculated embryos 25 to 33.4 per cent died, but the remainder exhibited various forms of deformities and marked suppression of growth. Babbar *et al.* (1972) have also shown that two avirulent strains of avian mycoplasma became virulent on transfection with a Ranikhet disease virus (RDV) and also induced symptoms identical to those incited by virulent forms in chick embryos. A number of plant diseases, having stunted growth, have been indicated to be due to mycoplasma. Various kinds of deformities and retarded growth have been reported to be associated with these (Maramorosch 1970). Grassy shoot disease of sugarcane, characterised by premature and excessive tillering and marked stunting, has been reported to be associated with mycoplasma (Corbett *et al.*, 1972), though the pathogen has not been isolated from the infected tissues as yet. Morphological similarity of the reported plant and avian mycoplasmas and the marked suppression of growth in the infected host in both cases, prompted investigations on the effect of the avian mycoplasma on sugarcane and a few other related plant species.

MATERIALS AND METHODS

The biological characters of the DP strain of the avian mycoplasma employed the method of its maintenance and titration as number of colony forming units (CFU/ml) and the procedure to obtain suspensions of washed mycoplasma cells have been detailed earlier (Babbar and Verma 1972 *a, b*; Babbar *et al.* 1972).

Plants were inoculated when 3 to 5-days-old. These were raised from disease free surface sterilised seeds of paddy (I.R. 8), maize (G.II) or one bud setts of sugarcane (Co.S.510) free from GSD or RSD viruses. These seedlings were kept immersed in a known volume and concentration of the suspension of washed cells of DP strain of mycoplasma (3 to 4×10^8 CFU/ml). Each seedling was then pricked at two points

(radicle and plumule) by an entomological pin (no. 20) and incubated at 37°C overnight. For comparison, check was maintained with seedlings similarly treated but with sterile distilled water. Each seedling was then planted in a pot and kept in an insect proof glass-house at a temperature of 25°C to 30°C with relative humidity 40 to 60 per cent.

Four or 5-day-old maize and paddy seedlings, 10-day-old sugarcane plants and *Rhopalosiphum maidis*, the maize aphid, reared up to the 4th generation in the laboratory by the method of Singh and Shukla (1966) were chosen for transmission tests. The aphids were starved for 30 minutes, fed on diseased or healthy plants for 30–40 min and finally transferred to the healthy maize seedlings using 10 to 15 aphids per seedling and 20 seedlings per treatment. Pots, having test seedlings, were maintained in the glass-house and observed for abnormalities up to cob formation in maize and ear formation in paddy and for about a year in case of sugarcane plants. The plants with height less than the minimum confidence limit of the height of the check plants at 5 per cent significance level, were taken as stunted.

Results

Most of the sugarcane plants inoculated with avian mycoplasma had stunted growth and extensive tillering or both (Fig. 1) after 10 weeks. Such symptoms were not observed in the check plants. To evaluate the significance of these symptoms, 4 experiments were conducted, everytime using 50 to 70 germinating buds infected with avian mycoplasma. The deformities in the plants grown were recorded up to 12 months. The results obtained (Table I) showed that 60 per cent of the total number of the inoculated plants exhibited marked stunting as compared to the check. Pre mature and excessive tillering, a patent symptom of grassy shoot disease (Singh *et al.*, 1967), was observed in case of plants inoculated with avian mycoplasma. On an average there were 12.7 tillers per inoculated plant as compared to 7.4 for an uninoculated plant. Highly pronounced stunting of plants, as a result of inoculation was observed in case of maize as well as paddy (Figs. 2 and 3). Changes in colour of foliage too were observed. These symptoms were conspicuous when plants attained the age of 4 to 5 weeks. Experiments with paddy as well as maize were conducted thrice, using 50 seedlings per experiment. Data thus obtained are summarized in Table I. It was observed that 32 to 34 per cent of the plants inoculated with avian mycoplasma were characterised by the symptoms described above. Such disorders were absent from the uninoculated check plants which appeared to grow normally.

From the experiments reported above, it is indicated that the avian mycoplasma can bring about abnormalities in the above three types of plants. However, all efforts to reisolate this organism from the inoculated plants as per procedure adopted by Hampton *et al.* (1969) were unsuccessful. The evidence of the presence of the pathogen in the inoculated plants was, therefore, provided by the transmission of the disease through aphids. Aphids are known to transmit the grassy shoot disease of sugarcane (Chona *et al.* 1960). The results of these experiments (Table II) clearly show that the malady incited by avian mycoplasma in maize plants can be transmitted through aphids to 3-4-day-old maize seedlings in 70 per cent of the plants thus treated.

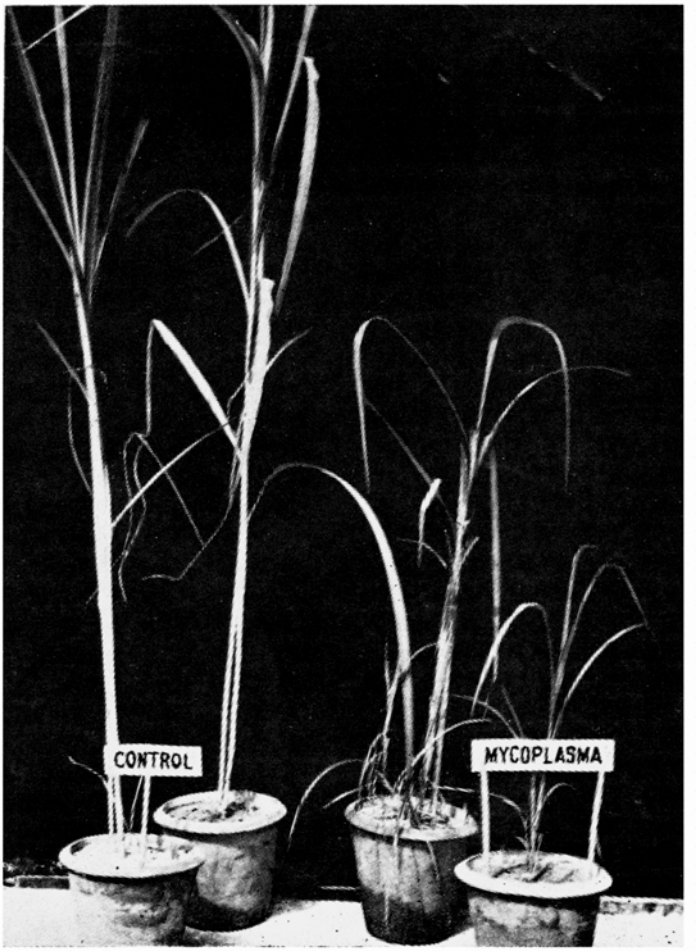

FIG. 1. Stunted growth and excessive tillering in the sugarcane plants infected with avian mycoplasma at the seedling stage.

DISCUSSION

The data presented here clearly indicate that an avian mycoplasma could incite a disease characterised by severe stunting in sugarcane, maize and paddy. Excessive and premature tillering in sugarcane, a patent symptom of grassy shoot disease (Singh and Shukla, 1967) was observed in plants inoculated with avian mycoplasma. This, therefore, raises the question about the nature of the causal organism of the GSD occurring in different parts of the country. It calls for more intensive investigations. Inability to reisolate the avian mycoplasma from the diseased plants suggests that mechanism of the pathogenesis is perhaps similar to that in nature and does not preclude the possibility that the pathogen is not viable in inoculated plants. Transmission of the disease to healthy plants is an indication of the presence of the pathogen

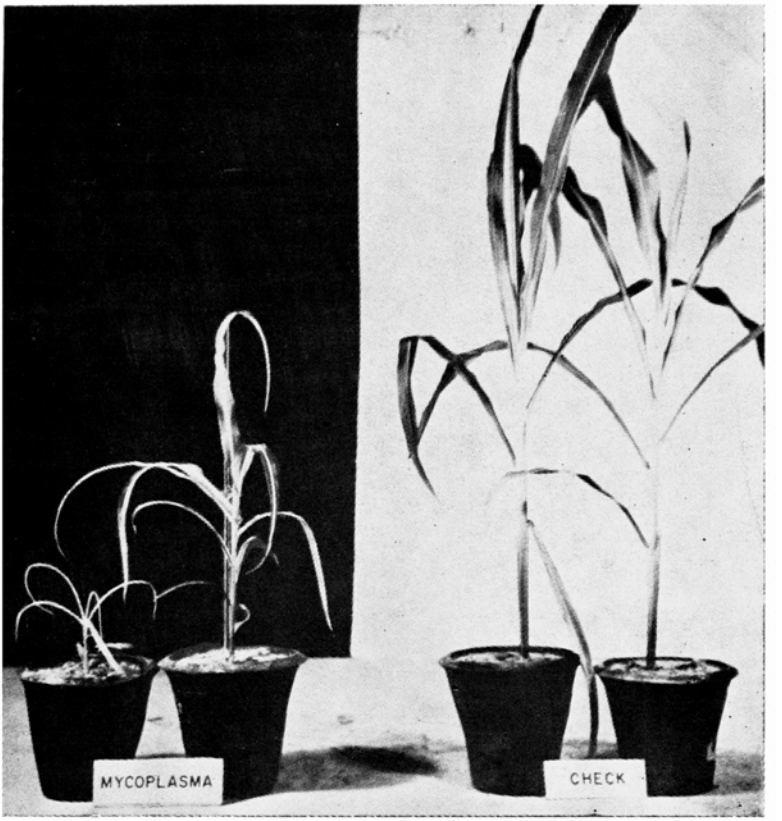

FIG. 2. Stunted growth in maize plants infected with avian mycoplasma at the seedling stage.

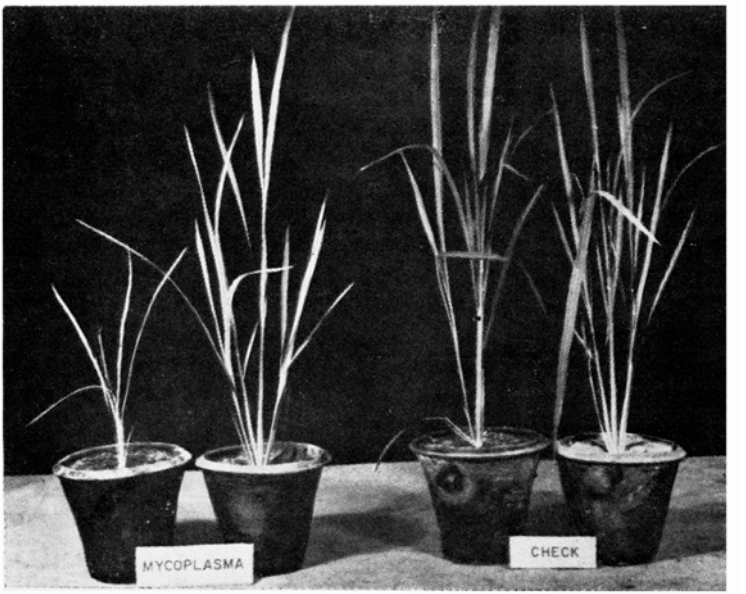

FIG. 3. Stunted growth and other disorders in the paddy plants infected with avian mycoplasma at seedling stage.

TABLE I

Appearance of disease syndrome in plants infected with avian mycoplasma (DP Strain) at the seedling stage

Treatment	% mortality of plants after 4 weeks	Per cent plants showing pathological disorders			
		Stunted growth	Deformities'	Tillering	Foliage disorders
Sugarcane infected with mycoplasma	0	60	3	80/12.1*	0
Control	0	5	0	20/7.4	0
Maize infected with mycoplasma	13.4	34.5	0	0	89**
Control	0	0	0	0	0
Paddy infected with mycoplasma	25.4	32.5	0	10/4	80***
Control	0	0	0	0	0

' Gall like formation or twisted growth

* $\frac{\%\text{ of plants showing tillering}}{\text{Number of tillers/plant}}$

**Yellow colour of the foliage

***Few plants with albinoid leaves and few developed anthocyanin pigment

TABLE II

Transmission of the disease syndrome from the infected maize plants to healthy maize seedlings by Rhopalosiphum maidis

Groups of plants used for vector transmission	Transmission of disease syndrome to healthy maize seedling by *R. maidis*	
	Stunted growth*	Foliage colour**
Diseased plants from seedling infected with avian mycoplasma		
Maize	57.5	90
Paddy	60.0	70
Uninoculated check		
Maize	0.0	0
Paddy	0.0	0

*% of the plants

**% of the plants showing either yellowish colour or leaves with anthocyanin pigment

in the former. It is likely that the procedure of isolation of mycoplasmas from diseased plants is yet not perfect. The role of birds in spreading diseases of similar nature in the three kinds of plants experimented with may have to be looked into.

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