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Further Studies on Mosaic Disease of Maize (*Zea mays* L.)

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Abstract. *Schizaphis graminum* (RONDANI) is proved to be an additional vector of maize mosaic virus (MMV). The pH range for the infectivity of the virus in extracted juice is found to be from 4·4 to 9·0, the optimum being 5·6 to 7·2. Effect of certain chemicals on the virus *in vitro* has also been studied. Cross protection between MMV and Sugar-cane mosaic virus (SMV) indicated positive results.

It has been concluded on the basis of similar physical properties, tolerance towards certain chemicals, host range, symptomatology, aphid vectors and positive immunological tests, that MMV and SMV are related viruses.

CHONA and SETH (1960) reported the occurrence of mosaic disease of maize from Delhi, which is distinct from all other virus diseases so far described on maize. The diseased plants are characterised by stunting and severe mosaic mottling (Fig. 1). The Maize mosaic virus (MMV) has been shown to be sap transmissible; its physical properties and host range among grasses have also been reported. MMV, however, does not infect sugar-cane. Three species of aphids are established as its vectors. The disease is now known to occur in various parts of the country and has been reported from the Punjab, Himachal Pradesh, Rajasthan, West Bengal and Andhra Pradesh by PALIWAL and RAYCHAUDHURI (1965a), who have also reported the existence of three strains of the virus. BHARGAVA and SHUKLA (1966) reported the disease from Uttar Pradesh and established *Myzus persicae* (SULZ.) as another vector of the virus. PALIWAL and RAYCHAUDHURI (1965b) reported the presence of intracellular viral inclusions in the epidermal strips of the affected maize plants. They have shown that the viral particles are rod shaped (PALIWAL and RAYCHAUDHURI 1966). The influence of MMV on host development under varying levels of potassium fertility, its infectivity to sorghum and loss in yield in maize has been studies by RAYCHAUDHURI et al (1966). SHEPHERD (1965) from California reported a similar juice inoculable virus on corn which does not infect sugarcane and studied its physical properties, vectors, serological behaviour, cross-protection with sugar-cane mosaic virus (SMV), and also purified the virus and found the particles to be rod shaped.

The present studies include additional aphid vector of the virus, *in vitro* effect of certain chemicals and pH variation on viral infection, as also cross-protection tests with SMV in order to establish its relationship with MMV. These studies were carried out with Delhi Strain of the virus reported by

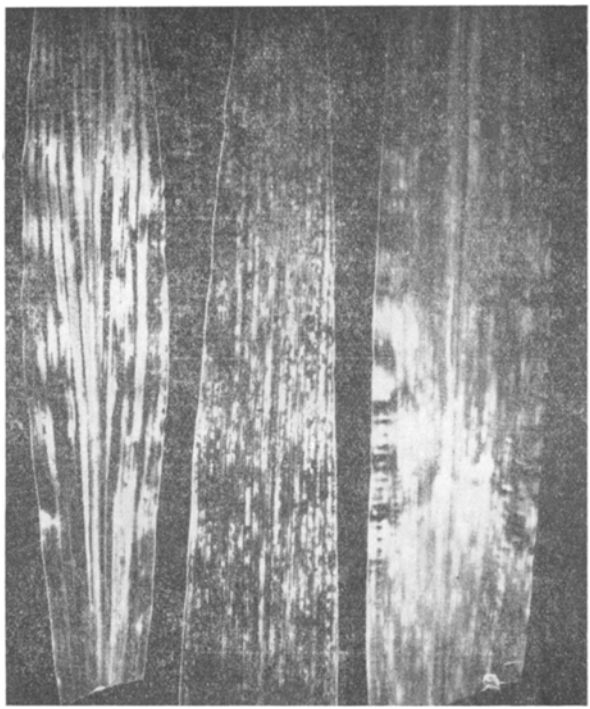

Fig. 1. Maize leaves showing severe mosaic symptoms.

CHONA and SETH (1960) which has a widespread distribution. Its physical properties are: thermal death point 50—55° C, dilution end point 1 : 50 to 1 : 100 and longevity at room temperature (28—32° C) 16 hours.

Material and Methods

For insect transmission tests healthy colonies of *Schizaphis graminum* (RONDANI) were reared on barley plants raised in the insectary. The insects were starved for 3—4 hours before giving an acquisition feeding period of 10—20 minutes on diseased maize plant, after which they were transferred to healthy maize plants.

Effect of pH variation and chemicals on MMV was determined in the extracted sap. For each gram of diseased leaf material $\frac{1}{2}$ cc of sterilized distilled water was used. The juice after extraction was centrifuged for 30 minutes at 3,000 r.p.m. The pH of the aliquots was adjusted to the desired value with the help of Beckman's pH meter by addition of either a few drops of normal HCl or NaOH. For obtaining the desired strength of the chemicals, known volumes of centrifuged juice was mixed with equal volumes of double the required strength of the chemicals. For control, sterile water at different dilutions was used in blank experiments. SMV was extracted from sugarcane variety *Surkha saharanpuri* for cross-protection tests. Healthy sets of the same variety were used to raise the nursery of sugarcane plants for detection of SMV.

In all these tests only, young, actively growing test plants were inoculated and all tests were carried out in the insect-proof glasshouse.

Results

Aphid transmissions: Sixty two maize seedlings were inoculated with *Schizaphis graminum* by transferring 20—30 aphids per plant. After giving the required acquisition feeding period on diseased maize plant, the insects were allowed to feed on test plants for 24 hours and subsequently the plants were sprayed with 0·1% Ekatox to kill the aphids. In all, 14 out of 62 test plants took infection, showing clear mosaic symptoms. This establishes *S. graminum* as fifth aphid vector of MMV besides, *Macrosiphum granarium* KIRBY, *Aphis gossypii* GLOVER, *Rhopalosiphum maidis* (FITCH) and *Myzus persicae* recorded earlier.

Effect of pH: For determining the pH range of the virus *in vitro*, tests were conducted by adjusting the pH of the samples of infectious juice, ranging from 1·7 to 11·4. After the adjustment of the pH of each sample to the desired level, inoculation were made (i) immediately and (ii) after storage for 4 and 24 hours at 7—10° C. The pH of each sample was again determined after 24 hours of storage which revealed that there had been no appreciable change.

The results of inoculations have shown that the virus was rendered innocuous immediately at pH 4·0 and below. However, at pH 4·4 it remained viable even after 24 hours when stored at 7—10° C. Under the same conditions at pH 9·0 the virus remained viable for only 4 hours while beyond pH 9·0 the virus is instantly inactivated. The optimum pH range, that is the pH of the sap at which highest percentage of infection was obtained, lies betweeu pH 5·6 and pH 7·2.

Effect of certain chemicals on infectivity of MMV. The effect of some protein precipitating and denaturing agents as also some organic solvents on the infectivity of the virus was studied by making preparations of desired dilutions of chemicals with the infective plant extract. Inoculations were made employing a set of 6 maize seedlings for each dilution, and control were also maintained. The inoculations were done immediately as also after 4 and 24 hours of storage of the mixture at 7—10° C. Acetone (40%) alcohol

(25%), chloroform (20%) commerical nicotine sulphate (20%) glycerine 5%) lysol (1%) and formalin (0·5%) rendered the virus innocuous within 24 hours of incubation at 7—10° C, while the virus was immediately inactivated by 0·125% copper sulphate and 0·125% potassium permanganate. Similar results were obtained by RAFAY (1935) employing some of these chemicals against SMV.

Cross protection tests: Cross protection tests were conducted in order to determine the relationship between MMV and SMV. In these tests a batch of 10 maize seedlings was inoculated with MMV, while another similar batch was inoculated with SMV. The inoculation of both the batches was done simultaneously under identical conditions. After a week both sets of test plants developed systemic mosaic symptoms. They were then re-inoculated with the challenging virus, that is, the first batch of maize plants was inoculated with SMV and the second batch with MMV. After about 2 weeks healthy maize and sugar-cane plants were inoculated separately with the juice from each batch of infected plants. The inoculum from both the batches produced mosaic symptoms on maize plants, while sugar-cane plants could only be infected with the juice from the second batch. These tests were repeated thrice and the same type of protection was observed, that is, MMV protected maize plants against SMV when the plants are already fully infected with MMV.

In another experiment, a set of maize plants was inoculated with a mixture of equal volumes of MMV and SMV extracts to determine if any protection was afforded when two viruses are introduced simultnaeously in their common host. After about three weeks, back inoculations from these plants had shown that maize as well as sugar-cane plants could be infected with the inoculum from these plants, indicating that the simultaneous introduction of the two viruses in maize did not afford protection to maize plants against SMV and probably both of them multiplied in their common host when they are introduced simultaneously.

In a third set of experiments, interval of 1, 3 and 8 days was given between the inoculations with the immunizing virus and the re-inoculation with the challenging virus. Back inoculations on maize and sugarcane had shown in all these cases that when the maize plants were first immunized with MMV, SMV could not get a chance to multiply. This indicates that complete protection is always afforded when the interval between the introduction of MMV and subsequent inoculation with SMV is 24 hours or more. However, the protection in the reverse order could not be confirmed owing to lack of proper differential host plant.

Discussion

Physical properties, tolerance towards certain chemicals, host range, symptomatology and aphid vectors of both MMV and SMV have much in common, which suggests the relationship between the two viruses. The cross

protection tests have shown an additional evidence of relationship between the two. Hence MMV may perhaps be considered as a strain of SMV.

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Bylo dokázáno, že *Schizaphis graminum* (Rondani) je dalším přenašečem viru mozaiky kukuřice. Virus neztrácí infekčnost při pH 4,4—9,0 pH 5,6—7,2 je pro infekčnost viru ve šťávě optimální. Byl studován účinek některých chemikálií na virus in vitro. Křížové testy, provedené s virem mozaiky kukuřice a virem mozaiky cukrové třtiny, daly positivní výsledky. Podobné fysikální vlastnosti, tolerance k některým chemikáliím, okruh hostitelů, symptomatologie, přenašeči (mšice) a positivní imunologické testy svědčí o tom, že virus mozaiky kukuřice a virus mozaiky cukrové třtiny jsou příbuzné.

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Было доказано, что *Schizaphis graminum* (Rondani) является следующим переносчиком вируса мозаики кукурузы. Вирус не теряет инфекционную способность при pH 4,4—9,0; наибольшая инфекционная способность проявляется при pH 5,6—7,2. Было испытано действие некоторых химических веществ на вирус in vitro. Перекрестные тесты с вирусом мозаики кукурузы и вирусом мозаики сахарного тростника дали положительный результат. Сходство физических свойств, способность переносить воздействие некоторыми химическими веществами общие хозяева, симптомы заражения, переносчики (тля) и положительные результаты иммунологических проб, свидетельствуют о родственных связях между обоими вирусами.