Neth. J. Pl. Path. 78 (1972) 110–112 SHORT COMMUNICATION

M II–16, an artificial symptomless mutant of tobacco mosaic virus for seedling inoculation of tomato crops

A. TH. B. RAST

Institute of Phytopathological Research (IPO), Wageningen[1]

Accepted 17November 1971

Broadbent (1964) suggested deliberate inoculation of tomato seedlings as a cultural method to minimize losses in yield and fruit quality by tobacco mosaic virus (TMV). Satisfactory experimental results were obtained with common tomato strains of TMV as inoculum, particularly when combined with an advance in sowing date (Fletcher, 1968; Rast, 1969). A further improvement of the method has been achieved by the use of attenuated strains isolated from infected plants after prolonged periods of heat treatment (Paludan, 1968; Komochi et al., 1966).

The present author tried to obtain mild strains by nitrous acid treatment of undiluted sap from leaves containing a common strain of TMV (cf. Mundry and Gierer, 1958). Samples of treated sap were then inoculated to *Nicotiana glutinosa* leaves and the resulting lesions transferred singly for multiplication to one tomato seedling and one young tobacco plant of the variety 'Samsun nn.'. One of the isolates (code number M-II-16) obtained after a treatment of 45 minutes caused no visible leaf symptoms on both host plants, which were proved to contain TMV by essay on 'Xanthi nc.'. Since the original TMV strain caused normal mosaic symptoms it seemed logical to assume that the isolate represented a mutant.

In cross-protection tests the symptomless mutant protected tomato plants against infection by the distorting and yellow strains of TMV as well as the parent strain, provided these were inoculated at least ten days later. A challenge inoculation with the yellow or aucuba strain was afterwards profitably used to assess the infection rate of mass-inoculation techniques.

The mutant was first introduced into commercial practice in the fall of 1969. Observations on fruit set were made periodically on 54 holdings with early winter crops where a total of 64 blocks of two plots each had been laid out. Plants inoculated with the mutant were grown in one plot and compared with plants in another plot that were not deliberately inoculated and served as controls. Higher numbers of set fruits were counted on the inoculated plants in 38 blocks where the control plants suffered badly from a natural infection with a common strain of TMV. In 8 blocks adverse weather conditions in spring caused the inoculated plants in particular to shed flowers excessively resulting in a lower number of fruits as compared to the control plants. Again in

[1]Stationed at the Glasshouse Crops Research and Experiment Station, Naaldwijk, the Netherlands.

the last 18 blocks a lower number of fruits was counted on the inoculated plants which only lagged behind in fruit set but were in no other way inferior to the control plants. These remained practically symptomless and did not show the detrimental effects of an infection by a common strain of TMV elsewhere in the same glasshouse but further away from the inoculated plants. These observations suggested that the mutant had been spread to the control plants during cultural operations. It should be noted that inoculation of seedlings with the mutant causes a check of growth especially in the winter months. Also that the inoculated plants sooner or later after planting out develop mild mosaic symptoms distinct from the normal mosaic caused by common strains of TMV. The occurrence of an occasional plant with normal mosaic symptoms was not considered a matter of much concern since fruit set was hardly affected.

In 1971 yield experiments were carried out on 3 holdings with early winter crops. In three out of five experiments seedling infection with the mutant gave the highest yields as compared to infection with a common strain which occurred soon after planting out. In the fourth experiment there were no significant differences in yield and in the fifth the highest yields were obtained from the control plants which may have become infected with the mutant. It should be noted that in these experiments the plants were the same age.

Meanwhile many tomato growers had been given the opportunity to try seedling infection with the mutant in their own crops. Their experiences were so favourable that a considerably increased demand for inoculum is anticipated.

Prior to large scale production of inoculum it was found that when the original isolate of the mutant contained in dried tobacco and tomato leaves was used for inoculation of *N. glutinosa*, followed by the isolation of single lesions for multiplication on 'Samsun nn.', only 7 out of 40 plants remained symptomless whereas the others developed mosaic symptoms. From this observation it was concluded that the original symptomless mutant was probably only one component in a mixture of strains and with storage had been inactivated to a much greater extent than the symptom producing components. By repeated single lesion isolation the proportion of the mutant in the strain mixture has gradually increased. Nevertheless the inoculum currently issued to infect tomato seedlings still causes mosaic symptoms on about three percent of the plants within two weeks after inoculation. Further attempts are being made to separate the mutant from the contaminant strains.

Mass production of the mutant has been made possible by recent modifications in the column chromatographic method of virus purification as developed by Venekamp and Mosch (1964). The simplifications include the use of leaf sap clarified by low-speed centrifugation and a column of cellulose powder mixed with coarsely ground filter paper held in a plastic container (Venekamp and Mosch, in preparation). From 4 kg of infected tomato leaves about 1 l of buffered virus suspension is prepared which is then distributed in vials each containing 5 ml. This quantity makes an effective inoculum when diluted with water up to 5 l and 100 g carborundum has been added as an abrasive. Because manual inoculation involves the risk of spreading TMV strains either from infected seedlings or contaminated seed coats, the use of a spray gun (working pressure 1.5 to 2 atmosphere, working distance 20 to 30 cm) is recommended. It was found that 5 l of inoculum is sufficient for a near to 100% infection of about 10,000 tomato seedlings, provided these are spaced 5 cm apart and the spray inoculation is done thoroughly.

Samenvatting

M II-16, een kunstmatig verkregen symptoomloze mutant van het tabaksmozaïekvirus voor kiemplantbesmetting van tomaat

De bruikbaarheid van een door inwerking van salpeterigzuur verkregen, symptoomloze mutant van het tabaksmozaïekvirus (TMV) is gebleken uit een volledige bescherming van planten tegen een infectie met andere stammen van het virus en uit bevredigende proefresultaten onder praktijkomstandigheden. Het probleem doet zich voor dat de mutant in feite deel uitmaakt van een stammenmengsel, waarbinnen de verhoudingen echter ten gunste van de mutant zijn te beïnvloeden.

Acknowledgment

The author is greatly indebted to Dr J. T. Fletcher of the Agricultural Development and Advisory Service, Leeds, for his valuable suggestions and for correcting the English text.

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Address

Proefstation voor de Groenten- en Fruitteelt onder Glas, Zuidweg 38, Naaldwijk, the Netherlands