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Use of detached tomato leaves to test for resistance to tomato mosaic virus

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Tomato mosaic virus (ToMV) is a member of the tomatovirusgroup (Hollings and Huttinga, 1976). Its wide occurence can cause serious losses in greenhouse and open field production of tomatoes (*Lycopersicon esculentum* Mill.) (Broadbent, 1960). Several resistance genes have been introduced into tomato cultivars to protect the crop against ToMV infection (Pelham, 1966). However, new strains of ToMV have appeared that kan overcome these resistance (Rast, 1985). The so called Tm-2^2 gene, originally found in *L. peruvanium* 128650 (Pelham, 1966) has been proven to confer stable resistance to ToMV strains in greenhouses. Therefore, this gene is introduced in all new hybrid variaties worldwide. For resistance screening of adult plants, a disease test is applied, which shows symptoms in susceptible cultivars after 5 to 8 weeks. Besides being time and space consuming, this test also involves inoculation of all plants of interest. If sensitive plants carry a useful trait, it will be difficult to evaluate that trait or to propagate the plants when at the same time, these are inoculated for resistance screening and become infected.

In a research programme to evaluate regenerated plants, we are interested in various agricultural traits including resistance to ToMV. To determine the susceptibility to ToMV, without infecting the tomato plants, a disease resistance test was developed with inoculation of detached leaves. In a recent publication, Rufty et al. (1987) described a TMV resistance test on *N. tabacum* with detached leaves. We adapted this method for ToMV resistance testing in tomato. As symptom expression on tomato leaves cannot be quantified, indirect methods of virus detection were applied.

Seeds of the sensitive tomato cultivar Moneymaker and of the resistant hybrid cultivar Sonatine were sown in seed boxes and after 10 days each of the seedlings was transplanted into 12 cm pots, 15 plants per genotype. The pots were placed in a greenhouse compartment where temperature was set at 20/15 °C, day/night. When the plants were 60 days old, a recently unfolded leaf (young) and one of the first true leaves (old) were detached. The top leaflet of each leaf was dusted with carborundum (600 mesh) and inoculated with a 100 times freshly diluted virus suspension in water (Dahlemense strain, stock A_{254} = 3-4, kindly provided by Dr A.Th.B. Rast, Glasshouse

Crops Research and Experiment Station, Naaldwijk, the Netherlands). Excess virus suspension was removed by rinsing the inoculated leaflet with tap water. For control, some leaves were treated in a similar way with water. The leaves were incubated on humid filter paper on a table in a plastic tunnel in the greenhouse at 22 °C for 6, 10 and 17 days. When direct sunshine was avoided, leaves could be kept in good condition for more than 3 weeks.

The trial was in five replicates for the virus-inoculated leaves and in two replicates for the control leaves. At 6 and 10 days after inoculation, the first, second and third leaflets from the top (labelled L1, L2 and L3, respectively) were harvested and stored in plastic bags in a cold room at 4 °C. For the control leaves only second leaflets were collected. At 17 days after inoculation, extracts were prepared from the leaflets, which were stored in the cold room, and from leaflets of the leaves kept in the greenhouse for 17 days.

Extracts were prepared by grinding 0.5 g of each leaflet in a Pollähne Press with a 1 : 20 volume of phosphate-buffered saline solution (PBS) with 0.05% Tween 20 (Clark and Adams, 1977). Virus presence in the extracts was determined by enzime-linked immunosorbent assay (ELISA) and by bioassay. For ELISA, methods of Clark and Adams (1977) with some modifications described by Maat en de Bokx (1978) and Dijkstra et al. (1987) were used. The alkaline phosphatase-conjugated β-globulin fractions of the ToMV antiserum were provided by Ing. D.Z. Maat, Research Institute for Plant Protection, Wageningen, the Netherlands. Bioassay was carried out by inoculation of detached leaves of 7-weeks-old *Nicotiana glutinosa* plants with the same extracts as used for ELISA. These leaves were incubated in a plastic tunnel in the greenhouse at 20 °C. After 5 days the number of local lesions per leaf was scored according to a scale of 0=0, 1=1-2, 2=3-10, 3=11-50, 4= >50 lesions.

An analysis of variance (ANOVA) was carried out on the results of ELISA and bioassay analyses (not shown). The greatest effect could be attributed to the varieties used. Smaller though still significant effects were found for the time between inoculation and virus assessment and for leaflet position. The amount of virus detected in the inoculated leaflet (top leaflet) differed significantly from the uninoculated leaflets (second and third). No significant differences were observed between young and old leaves (leaf age).

In Table 1 the results of the ELISA and bioassay analyses are presented. In ELISA, virus was already detected in the susceptible cultivar Moneymaker 6 days after inoculation with highest amounts in the first leaflet and lower in the second, but virus was not found in the third; after 10 days, virus was also detected in the third leaflet and after 17 days, the amount was high in all the leaflets examined. In contrast, in the resistant cultivar Sonatine, no virus was detected at 6 or 10 days after inoculation; after 17 days only the first leaflet showed a higher value than the control.

Results of the bioassay on *N. glutinosa* leaves were in agreement with those obtained in ELISA. The bioassay was more sensitive, as after 6 days virus was detected in all leaflets of 'Moneymaker' and after 17 days all leaflets of 'Sonatine' showed the presence of minor amounts of virus.

In summary, great differences between the susceptible and the resistant genotype were already detected 6 days after inoculation, especially in the top leaflet. These differences were also present in the other leaflets at 10 days after inoculation. At 17

Table 1. ELISA (E) values and amount of lesions (N) on *N. glutinosa* leaves of extracts from tomato leaflets (L1, L2, L3) at different times after inoculation with tomato mosaic virus. The results with young and old leaves are averaged.

Days after inoculation	Tomato cultivar											
	Moneymaker						Sonatine					
	L1		L2		L3		L1		L2		L3	
	E[1]	N[2]	E	N	E	N	E	N	E	N	E	N
6	0.79	3.5	0.35	1.7	0.05	0.9	0.06	0.0	−0.02	0.1	−0.02	0.2
10	0.96	3.3	0.44	2.4	0.46	2.4	0.04	0.1	0.03	0.0	−0.03	0.0
17	0.78	3.9	0.82	3.5	0.77	3.5	0.20	0.8	0.03	0.5	0.02	0.2

[1] The ELISA values refer to the mean extinction (A_{254}) corrected for the controls (approximately 0.15).

[2] The amount of lesions refers to the mean number of lesions according to a scale of 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-50, 4 = >50 lesions. The control values were generally 0; only occasionally 1 lesion was observed.

days after inoculation all leaflets of a leaf of 'Moneymaker' showed similar amounts of virus. 'Sonatine' showed some virus in the inoculated second leaflet, but much less than in a similar leaflet of 'Moneymaker'.

Bioassay proved to be more sensitive than ELISA, but both were found to be satisfactory. The choice of the analysis assay will mainly depend on the equipment available. ELISA is a good choice when laboratory facilities and experience with this technique are present. ELISA is also preferred for large scale experiments. Bioassay will be preferred when good greenhouse facilities are available.

The homozygous gene Tm-2^2 has been shown to inhibit the multiplication of ToMV, while in the plants with the heterozygous Tm-2^2 gene sometimes a low concentration of virus could be detected (Pelham, 1972). In mesophyll protoplasts, this gene does not cause reduction of ToMV mutiplication (Motoyoshi and Oshima, 1977), indicating that the resistance is only expressed at the plant level. In our trial, virus was detected in the resistant leaves relatively late after inoculation. We have shown that the virus could not only be detected in the inoculated leaflets, but also in leaflets next to the latter. Although the amount of virus was low, this indicated that the virus multiplied and spread. As the virus detection in our trial was not quantitative, no conclusion could be drawn about the quantitative distinction between susceptible and resistant leaves.

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Samenvatting

Het gebruik van afgesneden tomatebladeren voor het toetsen op resistentie tegen tomatemozaïekvirus

Een methode werd ontwikkeld om de resistentie van in de kas geteelde tomateplanten tegen tomatemozaïekvirus (ToMV) te bepalen. Bladeren van een vatbare en een resistente cultivar werden afgesneden en geïnoculeerd met ToMV. Na 6, 10 en 17 dagen werden de geïnoculeerde bladeren getoetst op de aanwezigheid van virus met ELISA en door inoculatie van bladeren van *Nicotiana glutinosa*. Met beide toetsmethoden kon de virustoename in de vatbare cultivar al vroeg na inoculatie duidelijk worden aangetoond. In de bladeren van de resistente cultivar was een zeer kleine hoeveelheid virus pas laat na de inoculatie aantoonbaar. Met deze methode is het mogelijk om de resistentie tegen ToMV te bepalen, tevens zaad te winnen en landbouwkundige eigenschappen te evalueren, zonder de plant te infecteren.

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