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Reaction of *Nicotiana* Species and Cultivars of Tobacco to *Tobacco mosaic virus* and Detection of the *N* Gene that Confers Hypersensitive Resistance

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Abstract

Depta A., Kursa K., Doroszewska T., Laskowska D., Trojak-Goluch A. (2018): Reaction of *Nicotiana* species and cultivars of tobacco to *Tobacco mosaic virus* and detection of the *N* gene that confers hypersensitive resistance. Czech J. Genet. Plant Breed., 54: 143–146.

Tobacco mosaic virus (TMV) causes large losses in tobacco cultivation. Sixty-two cultivars of tobacco and eleven species of *Nicotiana* were evaluated for resistance to TMV. Biological tests at two temperature ranges, DAS-ELISA and molecular markers were applied to assess the resistance to TMV. Most cultivars of tobacco were susceptible (S) to TMV, two were tolerant (T), while others revealed a hypersensitive response (HR). Hypersensitivity, determined by the *N* gene, occurred only at a temperature below 22°C. At a temperature above 28°C, all the cultivars showed mosaic discolorations or extensive necrosis. The reaction of the *Nicotiana* species was dependent on growth conditions. At 22°C, the reactions of susceptibility, tolerance and hypersensitivity to TMV were all observed, whereas above 28°C the species showed systemic necrotic symptoms. *N. gossei* was an exception in that hypersensitivity occurred regardless of the thermal conditions. The resistance of this species was not conditioned by the *N* gene, which suggests that *N. gossei* could be an additional genetic resource for tobacco breeding.

Keywords: genetic resources; *Nicotiana gossei*; *Nicotiana tabacum*; TMV

Tobacco mosaic virus (TMV) is a serious problem in tobacco cultivation in Poland (data not published) and in other countries in a warm or moderate climate zone. The lack of resistance to TMV in the Virginia type tobacco is becoming increasingly problematic due to the consistent propagation of the virus under field conditions combined with its high durability and ease of spread. Tobacco breeders previously used the Ambalema cultivar as a source of resistance to TMV. Its resistance was and still is controlled by two recessive alleles and a number of modifying genes (Clayton *et al*. 1938). However, plants with Ambalema-type resistance were not virus-free and exhibited mosaic symptoms at temperatures above 28°C. The species *Nicotiana glutinosa*, characterized by the hypersensitive response (HR), conditioned by a single *N* gene with a dominant resistance allele, is an alternative source of resistance to TMV (Gwynn 1977). However, at temperatures above 28°C the HR resistance of this species stops functioning and plants become systemically infected (White & Sugars 1996). Only a few resistant cultivars of the Virginia type were grown based on *glutinosa*-type resistance, because the occurrence of the *N* gene negatively affects the physical and chemical properties of the leaves (Lewis *et al*. 2005). Within the genus *Nicotiana*, excluding for *N. glutinosa*, the potential sources of TMV resistance are *N. repanda*,

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N. gossei, *N. langsdorfii*, *N. rustica* (Valleau 1952; Gwynn 1977; Yuan *et al*. 2015) and *N. benthamiana*, *N. maritima*, *N. sanderae*, *N. velutina*, *N. acuminata*, *N. goodspeedii*, *N. forgetiana*, *N. nesophila*, *N. stocktonii* (Van Dijk & Cuperus 1989; Yuan *et al*. 2015). However, these reports do not provide the names of the TMV strains used for plant inoculation or the temperature settings of the experiments. Moreover, the evaluation of resistance was performed only on the basis of the presence or absence of disease symptoms.

In our study a comprehensive assessment has been conducted on a wide group of tobacco cultivars, combined with molecular detection of the *N* gene and the effect of temperature on plant resistance. Additionally, resistance tests were done for cultivars and *Nicotiana* species where contradictory or incomplete results had been found.

The plant material consisted of 11 wild species of *Nicotiana* and 62 cultivars of *N. tabacum* belonging to the oriental, dark-cured, Burley and Virginia types of tobacco. The influence of temperature on the immune response of plants to TMV was evaluated. For this purpose an isolate of the TMV-vulgare (PV-0107) CMI/AAB. Descr. Pl. virus was multiplied in the susceptible cv. Wiślica. Sap from the infected Wiślica cv. was rubbed onto the carborundum dusted leaves of six plants of each accession. Plants were placed in two growth chambers, one with a low temperature: day 22°C for 14 h and night 20°C for 10 h; and one with a high temperature: day 30°C for 14 h, and night 28°C for 10 h. Tobacco plants were inoculated at the 5–6 leaf stage. The disease symptoms were observed 5, 10, and 14 days after inoculation (DPI). Then DAS-ELISA tests using antibodies from BIOREBA were applied. Samples were taken from inoculated leaves and the upper parts of plants.

The presence of the *N* gene was detected using a PCR reaction. DNA was extracted by the CTAB method in accordance with the modified protocol described by Czubacka & Doroszewska (2010). DNA amplification was carried out using a PCR reaction with two pairs of E (5'-ACCAGAATGATATGTTCCAC-3' and 5'-GGACTCAACGTTAATTCTCTG-3') and N (5'CGTCGACACATTATGCCATC-3' and 5'-GAGGGGTCTTACCCCATTGT-3') primers previously described by Lewis *et al*. (2005). The PCR reaction mixture was prepared in 20 µl and contained 1µl of plant DNA and 2 µl of PCR buffer (10×), 1.6 µl of 25 mM $MgCl_2$, 0.6 µl of 10 mM dNTPs, 0.5 µl of each primer (10 µM each), and 0.4 µl of Taq DNA polymerase (5 U/µl). The PCR reaction consisted of initial denaturation at 94°C for 1 min followed by 35 cycles: 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final elongation step of 72°C for 7 min.

There were four types of responses to the virus, depending on the tested accession and the conditions of the experiment. The most frequent reaction among the cultivars of the Virginia type and the oriental types was susceptibility (S). ELISA absorbance values obtained in the samples from these plants ranged from 2.234 to 3.096. This type of reaction was observed in the majority of the tested cultivars of *N. tabacum* under each of the applied temperatures (Table 1). Another group of accessions, the Ambalema 1 and Ambalema 2 cultivars and the *Nicotiana* species *N. glauca*, *N. wigandioides* and *N. africana* exhibited a varied reaction to TMV that depended on the air temperature. At the temperature 20/22°C they exhibited no disease symptoms in the bottom and upper leaves and simultaneously they had high absorbance values ranging from 1.938 to 2.367. At the temperature 28/30°C strong mosaic discolorations were detected and ELISA absorbance was within the range of 2.361 to 3.071, which is similar to that obtained in susceptible cultivars. These results suggest tolerance to TMV that is temperature sensitive. Due to the above facts, Ambalema 1 and 2 from our collection do not constitute any effective and stable sources of resistance to TMV. A hypersensitive response (HR), characterized by small necrotic spots around the place of infection, was recorded in 14 cultivars belonging mainly to the Burley and oriental types. In the group of the Virginia type only cv. Vamorr 50 showed HR resistance. Molecular analyses using E and N primers have shown that the resistance of all these cultivars was determined by the *N* gene derived from *N. glutinosa*. However, the number of cultivars showing HR response was dependent on thermal conditions. Temperatures above 28°C promoted the multiplication and translocation of the virus to the upper levels of plants and the occurrence of systemic reactions. This was probably due to a decrease in the level of the thermosensitive proteins (IVR) that prevent virus replication (Gera *et al.* 1993). This indicates that using the *N* gene for developing resistance in tobacco breeding may not be a sufficient solution, especially under conditions of global warming and the increasing occurrence of TMV worldwide. The most frequent reaction among the *Nicotiana* species was hypersensitivity. Species reacting to TMV with necrotic spots included:

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N. maritima, *N. goodspeedii*, *N. repanda*, *N. gosseii*, *N. benthamiana*, *N. langsdorfii* and *N. glutinosa* (Table 1). Serological tests revealed the presence of the virus only in the lower leaves within the necrotic spots. The upper leaves without any visible symptoms did not contain the virus (ELISA values 0.063–0.114). However, the occurrence of HR was recorded only with temperatures not exceeding 22°C. With temperatures above 28°C, plants developed a systemic response which was characterized by extensive necroses and shoot top withering. ELISA of these plants confirmed the presence of the virus in both the lower and the upper leaves. The obtained results are consistent with the observations of Valleau (1952); Gwynn (1977) and Yuan *et al.* (2015), who screened *Nicotiana* species using TMV and stated that they may be used in tobacco breeding. Molecular analyses gave an overview of the occurrence of the *N* gene among selected species of *Nicotiana*. PCR products were obtained only in the case of the hypersensitive *N. glutinosa*. In the other species, no DNA fragment was found proving the presence of the *N* gene.

Among the tested species, of special interest is *N. gossei* which showed a hypersensitivity reaction to

Table 1. The assessment of the resistance of tobacco cultivars and *Nicotiana* species under inoculation with TMV

Cultivar	Temperature (°C)				$P_{N\text{-gene}}$	
	20/22		28/30			
	R_{TMV}	$N_{i/t}$	R_{TMV}	$N_{i/t}$	E	N
Oriental type						
Basma 153, CNR 12450, Diubek, Diubek 44, Djebel 139, Newrokop 1561, Newrokop 5 (T.130), Newrokop 5 (T.167), Ustina 2, Samsun, Samsun 27, Samsun 417, Samsun 935, Samsun de Katherini, Samsun Odskok, Talgar 30, Trapezund 245, Trapezund 2578, Trapezund 41, Trapezund Puławski 93, Trapezund WITIM 93	S	6/6	S	6/6	–	–
Diubek 556 (T.743), Diubek 556 (T.803), Newrokop 261 (T.677), Newrokop 261 (T.740), Samsun 155, Samsun H	HR	0/6	SNR	6/6	+	+
Dark-cured type						
Polalta	S	6/6	S	6/6	–	–
Ambalema (1)	T	6/6	S	6/6	–	–
Ambalema (2)	T	6/6	S	6/6	–	–
Immunnyj 580	HR	0/6	SNR	6/6	+	+
Burley type						
Burley 165, BY 103, Burley Har.Velvet, TN 86	S	6/6	S	6/6	–	–
(ms 21 × Ky 10) × Ky 10, Bu 563, Burley 21, Ky 10, KY 908, Sota 27	HR	0/6	SNR	6/6	+	+
Virginia type						
Vamorr 50	HR	0/6	SNR	6/6	+	+
974 (Hicks Resistant), AC Gayed, Broad Leaf Hicks, Coker 140, Coker 347, Hicks Fixed A2, Hicks Resistant (T.581), Hicks z Ontario, Mc Nair 1040, Mc Nair 944, V. Gold Dollar, V. Golta, V. Sun Cured, Weneda, Wenus, White Mammoth, Wiera, Wisana, Wiślica	S	6/6	S	6/6	–	–
Nicotiana **species**						
N. glauca, *N. africana*, *N. wigandioides*	T	6/6	S	6/6	–	–
N. maritima, *N. goodspeedii*, *N. repanda*, *N.benthamiana*, *N. langsdorfii*	HR	0/6	SNR	6/6	–	–
N. glutinosa	HR	0/6	SNR	6/6	+	+
N. gossei	HR	0/6	HR	0/6	–	–
N. rustica	SNR	6/6	SNR	6/6	–	–

R_{TMV} – response to *Tobacco mosaic virus* (TMV); $N_{i/t}$ – number of plants per each accession containing TMV in upper leaves (a high antibody titre in ELISA)/tested plants; $P_{N\text{-gene}}$ – presence of *N* gene confirmed with primer pairs E or N; SNR – systemic necrotic response; T – tolerance; HR – hypersensitive response; S – susceptibility

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TMV. Necrotic spots were formed on the inoculated leaves, but systemic spread of the virus was suppressed. TMV was not detected in the upper leaves even at the high temperatures. No previous studies have shown that the resistance of *N. gossei* is stable and effective regardless of the thermal conditions. Furthermore, our studies demonstrated that it is not determined by the *N* gene, which indicates the presence of a new mechanism of resistance in the genus *Nicotiana*.

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