P L A N T  B R E E D I N G  A N D  S E E D  S C I E N C E
Volume 54 2006

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# RESISTANCE TO *TOMATO SPOTTED WILT VIRUS* (TSWV) IN *NICOTIANA ALATA* AND *N. SANDERAE* AND IN HYBRIDS BETWEEN *N. TABACUM* AND *N. ALATA*

## ABSTRACT

Seven populations of *N. alata*, 4 populations of *N. sanderae* and several hundred plants belonging to three generations of the hybrid (*N. tabacum* cv. TB-566 tetra × *N. alata*) × *N. alata* were tested for resistance to TSWV. Tested plants were artificial inoculated with TSWV under greenhouse conditions. Out of 11 accessions of *N. alata* and *N. sanderae* 7 were found to be composed entirely of plants hypersensitive to TSWV. In the remaining four accessions there were from 16 to 25% of TSWV susceptible plants. The hybrids (*N. tabacum* cv. TB-566 tetra × *N. alata*) × *N. alata*, regardless of their cytogenetical status, showed hypersensitive response to inoculation with TSWV. The local necrotic lesions that developed upon inoculation were identical to those observed in TSWV-inoculated plants of *N. alata*. Bioassay involving the susceptible species *N. glutinosa* detected no virus in the tissues of either hypersensitive or symptomless plants. Hypersensitive response to TSWV was faithfully expressed in the hybrids (*N. tabacum* cv. TB-566 tetra × *N. alata*) × *N. alata*, and unlike heretofore obtained resistance (cv. Polalta) did not seem to be associated with morphological abnormalities. It may have a potential as a source of resistance in commercial tobacco cultivars.

*Key words:* artificial inoculation, disease resistance, hybrids, hypersensitivity, *N. alata*, *N. sanderae*, *N. tabacum*, *Tomato spotted wilt virus* (TSWV),

## INTRODUCTION

*Tomato spotted wilt virus* (TSWV) is a tospovirus (Francki *et al.* 1991) of a very wide host range and worldwide distribution. More than a thousand different plant species are known to be susceptible to TSWV (Parrella *et al.* 2003). It is one of the most pathogenic and aggressive plant viruses currently known and the causal agent of one of the most devastating diseases of tobacco. Disease symptoms are often severe and include stunting and necrosis of stems and leaves (German *et al.* 1992). TSWV is transmitted from plant to plant and replicates by its vectors. At least seven thrips species spread this virus (Wijkamp *et al.* 1993; Sin *et al.* 2005). Among those *Thrips tabaci* Lind. is the only TSWV vector on tobacco in Central and East Europe and in Poland (Zawirska *et al.* 1983). In principle, the virus is not transmissible through direct plant-to-plant contact although it can be transmitted easily with the

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sap and this property is exploited for experimental purposes (plant inoculation). Genetic resistance to TSWV in the plant kingdom is rare and has been positively confirmed for *Capsicum chinense* (Black *et al.* 1991; Cebolla-Cornejo *et al.* 2003), *Lycopersicon peruvianum* (Stevens *et al.* 1992) and *Lactuca sativa* (O'Malley and Hartmann 1989). In *Nicotiana*, the only species reported as showing resistance to TSWV of the local lesion response (hypersensitive) type are *N. alata* and the closely related synthetic species *N. sanderae* (Gajos 1978, Jankowski 1980, Pop 1979). However, the reports on the resistance of those species are not consistent (Ruter and Gitaitis 1993, Winokurowa 1971). In this study an attempt was made to screen several populations of *N. alata* and *N. sanderae* (both outcrossing species) maintained in the *Nicotiana* Collection of Institute of Soil Science and Plant Cultivation at Puławy for the hypersensitive response to TSWV. The aim of the study was also to examine the evidence of the TSWV resistance agent in amphihaploid and amphidiploid hybrids of cultivated tobacco *N. tabacum* cv. TB-566 with *N. alata*.

## MATERIALS AND METHODS

One set of experiment material for this study comprised 7 outcrossing populations of *N. alata* and 4 populations of *N. sanderae* belonging to the section *Alatae* and each having a haploid complement of 9 chromosomes. All those germplasm accessions were of different origin.

Another set was made up of hybrids derived from crossing *Nicotiana tabacum* L. cv. TB-566 with the wild species *N. alata* Link et Otto:

— vegetatively cloned, self and cross-sterile (*N. tabacum* cv. TB-566 tetra x *N. alata*) x *N. alata* $BC_1$ hybrid. Cytological data of this hybryd (2n=35) suggest that it was a near-allohaploid with two *N. alata* chromosomes in disomic condition

— $R_1$ generation of partly self-fertile near-amphidiploids, obtained by culturing pith fragments of the former hybrid, each chromosome-doubled regenerant being cloned vegetatively to increase the number of individuals available for screening

— selfed $R_2$ generation of the near-amphidiploid hybrids

The derivation, detailed cytogenetics and nomenclature of the hybrids is described in the previous paper (Laskowska and Berbeć 2005).

The experiment plants were tested for TSWV resistance using the method described by Tsakiridis and Gooding (1972). The plants were grown in the greenhouse and were inoculated at the rosette stage (prior to the transition to the generative stage). The inoculum was prepared by squeezing out sap from the leaves of *N. tabacum* plants that showed typical well-developed systemic symptoms of TSWV infection and stabilizing it with an addition of an anti-oxidant (Tsakiridis and Gooding 1972). After 12 days the inoculated plants were observed for TSWV symptoms. Three weeks after inoculation the presence of the virus in TSWV tested plants was confirmed by inoculating *N. glutinosa* used as a TSWV indicator species.

## RESULTS

### Resistance to TSWV in outcrossing populations of *N. alata* and *N. sanderae*

Of the seven populations of *N. alata* screened for TSWV resistance six were found to be composed entirely of plants that showed local lesion response (hypersensitivity) (Fig. 1a) and in one - three of the plants developed systemic symptoms. Among the populations of *N. sanderae* three were found to be partly susceptible and in one all plants showed localized lesion response (Table 1). The localized lesion of hypersensitive response consisted of necrotic spots 6-7 mm in diameter that appeared on inoculated leaves ca. 5 days after inoculation (Fig. 1a, b). In some cases local lesions were also accompanied by chlorotic spots. The systemic infection, at first manifested as spreading and coalescing chlorotic spots, ultimately led to leaf and apex distortion, stunted growth and death.

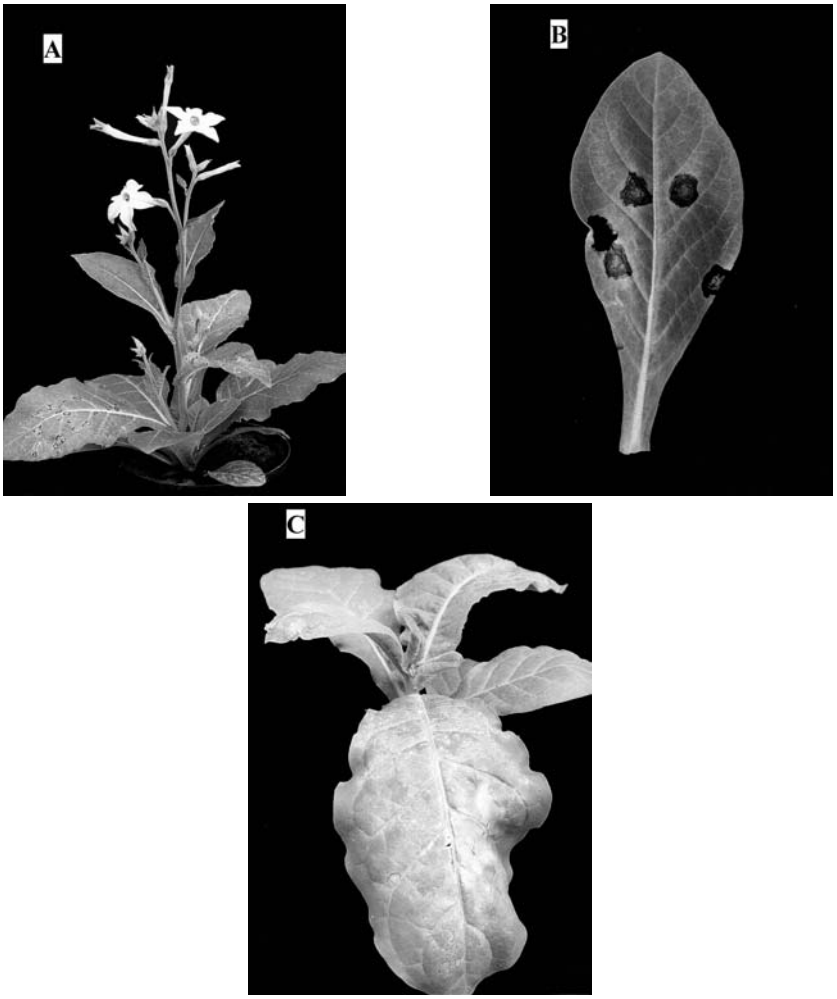


Fig. 1. Symptoms after inoculation with TSWV: a) localized lesion of hypersensitive on *N. alata* plant and b) *N. alata* leaf, c) systemic symptoms on *N. tabacum* cv. TB-566

Table 1

**Reaction to TSWV of the populations of *N. alata* and *N. sanderae***

| Genotype | Number of plants tested | Number of plants | | |
|---|---|---|---|---|
| | | Symptom less | Systemically affected | Hypersensitive |
| *N.alata* 89392 | 12 | 0 | 0 | 12 |
| *N.alata* D616 | 12 | 0 | 0 | 12* |
| *N.alata* D634 | 12 | 3 | 0 | 9 |
| *N.alata* var.*grandiflora* D112 | 12 | 1 | 0 | 11 |
| *N.alata* var.*grandiflora* D617 | 12 | 0 | 0 | 12 |
| *N.alata* var.*alba* D259 | 12 | 0 | 3 | 9 |
| *N.alata* Biały Narcyz D718 | 12 | 0 | 0 | 12 |
| *N.sanderae* D635 | 12 | 0 | 3 | 9 |
| *N.sanderae* D407 | 12 | 1 | 0 | 11 |
| *N.sanderae* D408 | 12 | 0 | 2 | 10** |
| *N.sanderae* D409 | 12 | 2 | 2 | 8 |
| *N.glutinosa* | 12 | 0 | 12 | 0 |
| *N.tabacum* cv. TB 566 | 12 | 0 | 12 | 0 |

* — 5 plants with localized necrotic spots and chlorosis
** — 2 plants with localized necrotic spots and chlorosis

The reference TSWV susceptible tobacco *N. tabacum* cv. TB-566 responded to inoculation by developing, five days after being inoculated, necrotic rings and small spots on the inoculated leaves that grew into large chlorotic areas. During the next several days the infection symptoms which included vein clearing, leaf distortions, and the characteristic curving of the apex spread to the upper portion of the plant (Fig. 1c). Stunting and death followed. The indicator plants of *N. glutinosa* died quickly following the inoculation because of extensive necrotic lesions. The effectiveness of inoculations was very high as all susceptible controls developed systemic symptoms.

**Resistance of *N. tabacum* × *N. alata* hybrids to TSWV**

A vegetatively increased generation of near-allohaploids ($BC_1$), near-amphidiploids ($R_1$), generatively reproduced selfed offspring of the near-amphidiploids ($R_2$) and the parental forms of the hybrid (*N. tabacum* cv. TB-566 tetra × *N. alata*) × *N. alata* were tested for resistance to TSWV (Table 2). Regardless of their derivation, all hybrid plants showed a hypersensitive response to inoculation in a manner similar to that observed in the species *N. alata* (Fig. 2). Within 5 days from inoculation round necrotic local spots appeared on inoculated

leaves. The spots were 2-3 mm in diameter in cloned near-allohaploids and were larger, 4 – 6 mm, in near-amphidiploids and its selfed derivatives. The symptoms did not spread to non-inoculated leaves nor were any other symptoms of systemic infection observed. Following each inoculation treatment some symptomless plants occurred which could be explained, in a given case, by insufficient inoculation efficiency. Under the same conditions, the plants of *N. tabacum* cv. TB-566 developed systemic symptoms.

Table 2

**Reaction to TSWV of the hybrids (*N. tabacum* cv. TB 566 tetra x *N. alata* ) × *N. alata* and their parental forms**

| Genotype | Number of plants tested | Number of plants | | |
|---|---|---|---|---|
| | | Symptomless | Systemically affected | Hypersensitive |
| $BC_1$ hybrid | 55 | 4 | 0 | 51 |
| $R_1$ generation: | | | | |
| Clone 2 | 42 | 8 | 0 | 34 |
| Clone 5 | 42 | 7 | 0 | 35 |
| Clone 9 | 24 | 6 | 0 | 18 |
| Clone 10 | 18 | 3 | 0 | 15 |
| Clone 13 | 25 | 0 | 0 | 25 |
| Clone 22 | 24 | 0 | 0 | 24 |
| Clone 34 | 14 | 4 | 0 | 10 |
| $R_2$ generation: | | | | |
| Line 1 | 12 | 2 | 0 | 10 |
| Line 2 | 9 | 0 | 0 | 9 |
| Line 3 | 2 | 1 | 0 | 1 |
| Line 4 | 14 | 2 | 0 | 12 |
| Line 5 | 10 | 3 | 0 | 7 |
| Line 6 | 6 | 0 | 0 | 6 |
| Line 7 | 5 | 1 | 0 | 4 |
| Line 8 | 1 | 0 | 0 | 1 |
| Line 9 | 2 | 0 | 0 | 2 |
| Parental forms: | | | | |
| *N.tabacum* cv. TB 566 | 69 | 9 | 60 | 0 |
| *N.alata* 89392 | 62 | 15 | 0 | 47 |

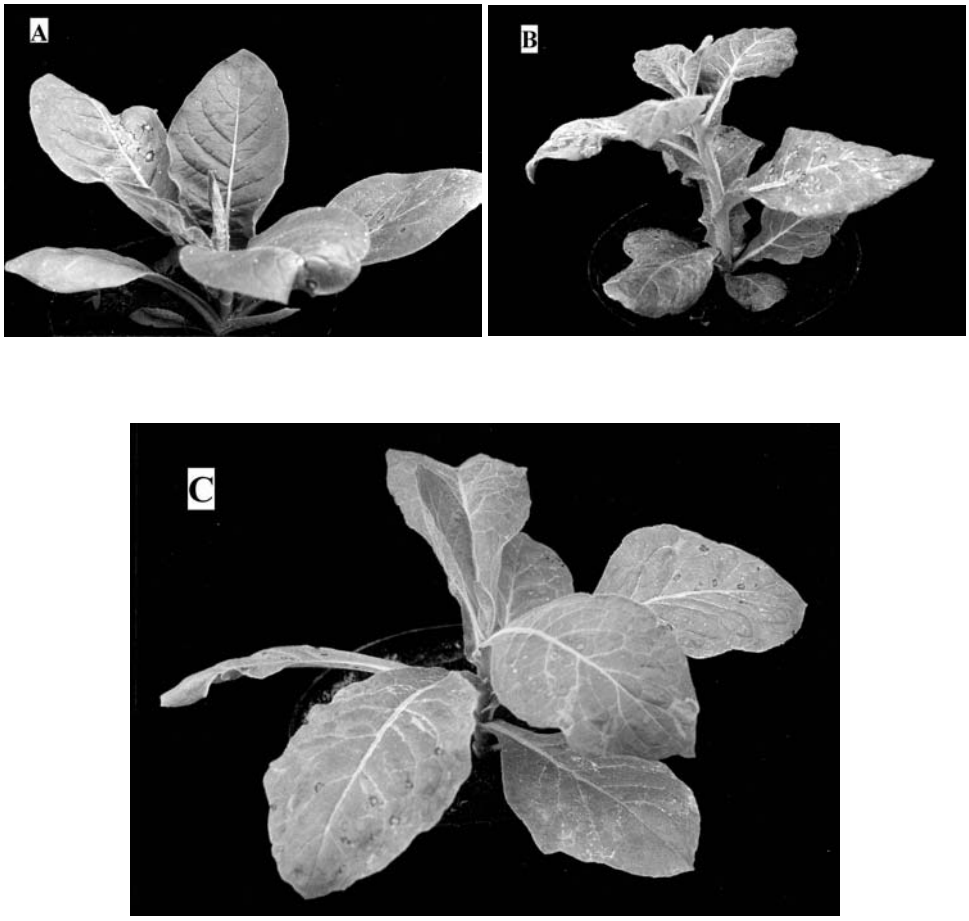


Fig. 2. Hypersensitive respons to TSWV inoculation of (*N. tabacum* cv. TB-566 tetra × *N. alata*) × *N. alata* hybrids: a) $BC_1$, b) $R_1$, c) $R_2$

**TSWV bioassays using *N. glutinosa***

Twenty one days after inoculation the selected plants of *N. alata*, *N. sanderae* and of the hybrids *N. tabacum* cv. TB-566 × *N. alata* were tested for the presence of TSWV using *N. glutinosa* as the indicator plant. In Table 3 are shown the results of the bioassay. When inoculated with the sap from inoculated plants showing localized necrotic spots the plants of *N. glutinosa* did not develop any TSWV symptoms. When the inoculum was collected from plants in which localized necrotic spots were accompanied with chlorotic spots *N. glutinosa* responded with chlorotic spots which after a few days turned necrotic, the development of the disease was usually arrested at that stage rarely leading to plant death and only on two *N. glutinosa* plants full systemic symptoms devel-

oped. The inoculation with the sap from plants with clear systemic symptoms (curving of the apex, distortions of the upper leaves) resulted in the development of full systemic symptoms on *N. glutinosa* (Fig. 3).

Table 3

**TSWV bioassays using *N. glutinosa* as the indicator plant**

| Genotype | Inoculation with the sap from plants: | | | |
|---|---|---|---|---|
| | Symptomless | Systemically affected | Hypersensitive | Localized necrotic spots and chlorosis |
| The bioassays reaction: Number of tested *N.glutinosa* plants/the result of the bioassay | | | | |
| *N. alata* 89392 | 0 | 0 | 10 / 10 - | 0 |
| *N. alata* D616 | 0 | 0 | 0 | 5 / 3 +, 2 ++ |
| *N. alata* D634 | 3 / 3 - | 0 | 3 / 3 - | 0 |
| *N. alata* D617 | 0 | 0 | 5 / 5 - | 0 |
| *N. alata* D.259 | 0 | 2 / 2 ++ | 3 / 3 - | 0 |
| *N. alata* D718 | 0 | 0 | 10 / 10 - | 0 |
| *N. alata*€ D635 | 3 / 3 - | 0 | 2 / 2 - | 0 |
| *N. sanderae* D635 | 0 | 3 / 3 ++ | 2 / 2 - | 0 |
| *N. sanderae* D408 | 0 | 0 | 3 / 3 - | 2 / 2 + |
| $BC_1$ hybrid | 0 | 0 | 10 / 9 -, 1 + | 0 |
| $R_1$ Clone 2 | 5 / 5 - | 0 | 5 / 5 - | 0 |
| $R_1$ Clone 5 | 0 | 0 | 5 / 5 - | 0 |
| $R_1$ Clone 10 | 0 | 0 | 5 / 5 - | 0 |

- — without symptoms
+ — slight chlorosis appearing on *N. glutinosa* plants ten days after inoculation
++ — chlorosis appearing five days after inoculation, after fourteen days *N. glutinosa* plant died

Fig. 3. Systemic symptoms on *N. glutinosa*

## DISCUSSION

There are some discrepancies in the literature regarding the resistance of *N. alata* to TSWV. Many investigators reported the species as resistant (Gajos 1978, Jankowski 1980, Pop 1979). However, in the tests performed by Winokurova (1971) 6,4% of *N. alata* plants became systemically defected, and in Ruter and Gitaitis investigations (1993) *N. alata* was susceptible. In this study some populations of *N. alata* and *N. sanderae* were composed entirely of hypersensitive plants whereas some other seemed to segregate for the hypersensitive response. A reason for that may have been different origins of *N. sanderae* and *N. alata* accessions. They had been maintained as relatively small populations and, as outcrossing species, may have undergone various changes in the frequency of genes due to genetic drift. Of the two species studied, *N. alata* seemed to be superior to *N. sanderae* as the source of resistance to TSWV. As a donor species to produce hybrids studied in this paper, a *N. alata* accession was selected in which hypersensitive response to TSWV was faithfully reproduced over generations.

Regardless of the cytological status of the (*N. tabacum* cv. TB-566 tetra × *N. alata*) × *N. alata* hybrids studied, the hypersensitive response of the *N. alata* parent was well expressed, an indication of the dominant nature of that particular trait. Failure of some of the plants to develop local necrotic lesions following inoculation was probably due to the ineffective treatment. None of the plants developed typical systemic symptoms, which may indicate that the factor/factors from *N. alata* may give good protection against TSWV when employed in the genetic milieu of *N. tabacum*. However, serious barriers to hybridization and to gene transfer via conventional mating encountered and described in our previous study (Laskowska and Berbeć 2005) indicate that methods other than conventional sexual transfer must be applied to this end.

Our attempt was not the first one to make use of *N. alata* as the source of resistance to TSWV in cultivated *N. tabacum* (Laskowska and Berbeć 2005). The most successful trial was that described by Gajos (1988, 1993) who managed to breed the hypersensitive response gene, reportedly from *N. alata*, into *N. tabacum* as a result of which TSWV resistant cultivars, Polalta and Wiktoria were developed although, in the latter cultivar the hypersensitive response seems to be rather poorly expressed and erratic. There are some differences involved regarding hypersensitivity to TSWV obtained by Gajos (1981) and that described in this study. Gajos (1981) did not report any difficulties in transferring the resistance over successive hybrid generations in contrast to the experience of other investigators who found such barriers either in the sesquidiploid (Chaplin and Mann 1961) or amphidiploid (Laskowska and Berbeć 2005) to be practically insurmountable. The hypersensitivity to TSWV of the type obtained by Gajos (1988) and bred into cv. Polalta is accompanied by morphological malformations of the leaves (Nielsen 1993), the two traits seemingly linked close enough to each other to make the practical use of Polalta-derived resistance very difficult. On the other hand, we did not find any such deformities in our TSWV-hypersensitive hybrids, although the comparison is risky at best because it involves hybrid derivatives of very different advancement level. The comparison of the two resistance factors (Polalta vs. *tabacum-alata* hy-

brids of this study) is further complicated by the fact that Gajos's description of the manner in which his Polalta was developed is sketchy and lacking in cytogenetical details. From his account it can be inferred that another species, *N. otophora*, was involved at some point in the origin of Polalta (Gajos 1981). So it is an open question whether Polalta-type factor and the response derived from *N. alata* described in this study are actually identical in origin.

## CONCLUSIONS

– Out of 11 accessions of *N. alata* and *N. sanderae* 7 were found to be composed entirely of plants hypersensitive to TSWV. In the remaining four accessions there were from 16 to 25% of TSWV susceptible plants. In some of the plants no symptoms developed following inoculation
– The hybrids (*N. tabacum* cv. TB-566 tetra × *N. alata*) x *N. alata*, regardless of their cytogenetical status, showed hypersensitive response to inoculation with TSWV. The local necrotic lesions that developed upon inoculation were identical to those observed in TSWV-inoculated plants of *N. alata*. In the remaining plants no symptoms developed.
– Bioassay involving the susceptible species *N. glutinosa* detected no virus in the tissues of either hypersensitive or symptomless plants.
– Hypersensitive response to TSWV was faithfully expressed in the hybrids (*N. tabacum* cv. TB-566 tetra × *N. alata*) × *N. alata*, and unlike heretofore obtained resistance (cv. Polalta) did not seemed to be associated with morphological abnormalities. It may have a potential as a source of resistance in commercial tobacco cultivars.

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