doi: 10.26114/pja.iung.520.2023.52.11

Polish Journal of Agronomy
2023, 52, 97–109

Possibilities of using *Nicotiana* species in breeding for virus resistance

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Abstract. Tobacco (*Nicotiana tabacum* L.) is an important industrial crop. Among the diseases causing losses in tobacco cultivation, viral ones are of particular importance due to the very low effectiveness of chemical protection allowing only for vector control. The greatest threat to tobacco cultivation in Poland and in many countries of the world is posed by potato virus Y (PVY), tomato spotted wilt virus (TSWV) and, in recent years, the increasingly frequent tobacco mosaic virus (TMV). The genetic resources studies carried out in the genus *Nicotiana* have shown a wide variation in species resistance, assessed biologically, serologically and molecularly, depending on the virus used and, in the case of PVY, also on the specific isolate. The results of the assessment of resistance in *Nicotiana* accessions presented in this paper, gathered from literature data as well as from our own research, allow us to broaden and systematise our knowledge on the sources of resistance to viral diseases. This is the only such an extensive study in this field. It provides an excellent information base for the appropriate selection of accessions for use in resistance breeding.

Keywords: *Nicotiana*, tobacco, tobacco mosaic virus, tomato spotted wilt virus, potato virus Y, resistance to viral diseases

INTRODUCTION

The cultivation of *Nicotiana tabacum* L. is exposed to many diseases, so these issues occupy a very important place in research on this species. Particular attention is paid to research related to resistance to pathogens, i.e. bacteria, fungi and viruses, which cause diseases limiting yield and impairing quality. Insects, which are both pests and vectors transmitting viral diseases, are also an important threat.

Bacterial diseases of tobacco are mainly caused by *Pseudomonas syringae* and *Pseudomonas angulata*. In order to protect plants from infection by these bacteria, appropriate prevention is foremost important, especially at the seedling production stage through the use of disinfected seed, pathogen-free substrate, as well as the disinfection of tools (Doroszewska, Berbeć, 2021).

A very broad group are the fungal pathogens of tobacco which include: *Peronospora tabacina*, *Alternaria alternata*, *Cercospora nicotianae*, *Berkeleyomyces basicola* (formerly *Thielaviopsis basicola*), *Botrytis cinerea*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The best way to avoid fungal diseases is to use cultivars that are resistant to the pathogens in question if they are available, in addition to appropriate prevention and agrotechnique and, in the event of an infestation, to use appropriate fungicides (Doroszewska, Berbeć, 2021).

It is definitely more difficult to counteract viral diseases which is basically done by reducing the vector population in the case of viruses transmitted this way. However, limiting mechanically transmitted viral diseases poses an even greater challenge. Therefore, there is a need for breeding work towards resistance to viral diseases (Doroszewska et al., 2013). In order for resistance breeding to be effective, accurate knowledge of the sources of resistance as well as the causative agents of the disease is necessary. The subject of this review are the results of the evaluation of resistance in *Nicotiana* plants to three viral diseases causing huge losses in tobacco cultivation.

MAIN PATHOGENS CAUSING VIRAL DISEASES OF TOBACCO

The most important viral diseases of tobacco in Poland and in many countries of the world are tobacco veinal necrosis, tomato spotted wilt and tobacco mosaic. The viruses causing them differ considerably in many respects, including structure, biology or the way they infect plants. They also cause different disease symptoms and there are different ways to reduce the disease. However, a common

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problem in the fight against viral diseases is the lack of effective chemical protection, making it necessary to identify the main viruses and to verify available sources of resistance.

Potato virus Y (PVY)

A very important pathogen causing major economic losses in tobacco cultivation is potato virus Y belonging to the genus *Potyvirus* and the family *Potyviridae*. PVY genome is constructed from a single sense RNA strand of 9700 nucleotides in length. The 5' end of the RNA molecule contains a covalently bound veinal protein genome linked (VPg), while the 3' end is polyadenylated. The entire virus is surrounded by a coat protein (CP) (Robaglia et al., 1989; Thole et al., 1993). PVY infects many crops in the Solanaceae family, particularly potato, tobacco, tomato and pepper, as well as some wild plant species. On tobacco, PVY causes veinal necrosis, the visible symptoms of which are initially clearing of veins and then their necrosis (Fig. 1a), which reduces the transport of water and mineral salts to the leaf tissues, and chlorotic spots (Fig.1b) of the leaf blade, which reduce the surface area and assimilative capacity and gas exchange. This results in stunted plant growth and sometimes complete dieback (Wen et al., 1999; Scholthof et al., 2011).

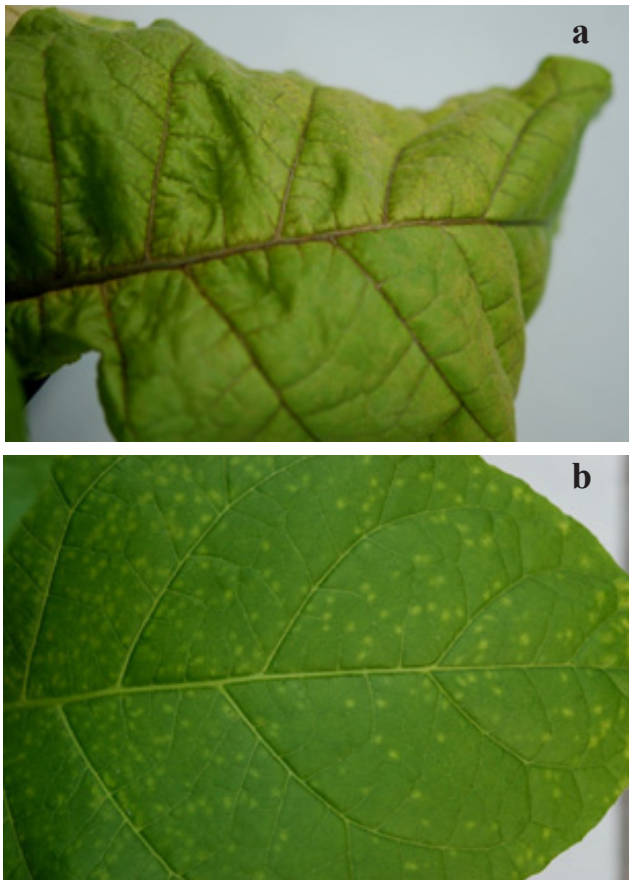

Figure 1. Necrosis of veins (a) and chlorotic spots (b) caused by potato virus Y on tobacco. Author – A. Depta.

The classification of PVY is based on biological, serological and molecular features. Three main groups of this virus have been identified. The first group includes the strain PVY^{O}, which is common and causes mainly mosaic discolouration, both in most potato cultivars and in tobacco (Hane, Hamm, 1999). The second group is the strain PVY^{C}, which causes streakiness in potato cultivars carrying the *Nc* gene and non-necrotic symptoms on tobacco (Doroszewska, 2004). The last group is the strain PVY^{N}, which causes necrotic symptoms on tobacco and diverse symptoms on potato. For this reason, it has been divided into two subgroups: $PVY^{N}W$ and PVY^{NTN}. $PVY^{N}W$ isolates cause weak mosaic symptoms on potato leaves, while PVY^{NTN} isolates can cause tuber necrosis (Chrzanowska, 1994; Le Romancer et al., 1994). Distinguishing between these subgroups of isolates is possible using two types of antibodies produced by Bioreba (Gugerli, Fries, 1983). An important feature of PVY is the high variability resulting from point mutations and recombination between isolates (Drake, 1993; Przybyś et al., 2013). It should be noted that isolates PVY^{N} have the ability to overcome existing sources of resistance which is the result of mutations at positions 105, 101 or 108 of the virus VPg protein (Masuta et al., 1999; Przybyś et al., 2013; Janzac et al., 2014). Furthermore, PVY is transmitted by aphids in a non-persistent manner (Crosslin, 2013) which makes the chemical protection ineffective.

Tomato spotted wilt virus (TSWV)

An extremely important pathogen that infects tobacco, as well as other species belonging to 85 botanical families, is tomato spotted wilt virus, also known as *Lycopersicum virus* 3 (Parella et al., 2003). It belongs to the genus *Orthotospovirus* and the family *Tospoviridae* (Francki et al., 1991; Wijkamp et al.,1993; Adams et al., 2017). It is transmitted by thrips (Jones, 2005), including tobacco thrips (*Thrips tabaci*), with the sap of infected plants, with the thrips in the larval stage only taking up the virus from the plant and only the infected adults causing infection of tobacco in spring after overwintering. Infestation can occur at any stage of tobacco development, but young plants are most susceptible, which can result in the elimination of entire plantations. Protection of tobacco against TSWV consists of chemical control of this vector and should be carried out during the growing season as well as after harvest and before planting in the field (Doroszewska et al., 2013). All commercial tobacco cultivars grown in Poland and worldwide are susceptible to this virus. The most characteristic symptoms of TSWV infestation include chlorotic and necrotic spots and vein clearing (Fig. 2a), which often

Figure 2. Vein clearing (a) and plant growth inhibition (b) caused by tomato spotted wilt virus on tobacco. Author – A. Depta.

occur on one side of the midrib, as well as stunted plant growth (Fig. 2b) and a characteristic bending of the tip at 45° (Mumford et al., 1996).

Tobacco mosaic virus (TMV)

A virus posing a major threat to tobacco cultivation, especially in recent years, is tobacco mosaic virus. It belongs to the genus *Tobamovirus* and is constructed from a single strand of RNA with sensible polarity and a length of 6395 nucleotides (Goelet et al., 1982). It is highly thermostable and can survive in dry material for up to 50 years. The virus is highly pathogenic and causes mainly severe mosaic symptoms (Fig. 3), but necrosis, stunting, leaf curl and yellowing of plant tissues can also occur. Symptoms depend on the age of the infected plant, its genotype, virus strain and environmental conditions. Infection by TMV results in reduced yield and deterioration of raw material quality (Scholthof, 2000; Knapp, Lewandowski, 2001). TMV occurs worldwide and is transmitted mechanically, so chemical protection is completely impossible. For this reason, breeding work towards resistant cultivars is necessary.

Figure 3. Symptoms of severe mosaic caused by tobacco mosaic virus on tobacco. Author – A. Czubacka.

SOURCES OF RESISTANCE AND POTENTIAL USE OF SPECIES OF THE GENUS *NICOTIANA* IN RESISTANCE BREEDING FOR VIRAL DISEASES

Sources of PVY resistance within the genus *Nicotiana* and opportunities for their use

Within *Nicotiana tabacum* cultivars, resistance to PVY is conditioned by a single recessive *va* gene resulting from a deletion in the *Va* susceptibility gene located on chromosome 21 (Julio et al., 2015). PVY-induced tobacco infection is possible when the product of the *Va* gene, which is the eukaryotic translation initiation factor 4F (eIF4E), interacts with the viral VPg protein (Wittmann et al., 1997; Robaglia, Caranta, 2006). A deletion within the *Va* gene was first obtained by X-ray mutagenesis in the susceptible cultivar Virgin A (Koelle, 1958) and subsequently transferred to other tobacco cultivars by breeding. Molecular studies using RAPD markers showed that the deletion in the VAM cultivar (Virgin A Mutant) is 1 Mbp in size (Noguchi et al., 1999). The high resistance of the VAM cultivar is due to the deletion of the entire *elF4E-1* gene derived from *N. sylvestris* Spegazzini et Comes and the overexpression of the *elF4E-2* gene derived from *N. tomentosiformis* Goodspeed. The absence of the *elF4E-1* gene prevents the viral VPg protein from binding to the eukaryotic translation initiation factor while the *elF4E-2* gene encodes a protein with which the virus forms non-functional connections (Takakura et al., 2018; Michel et al., 2019).

Determination of the nature of resistance of tobacco cultivars to PVY, identification of cultivars having *va*-type resistance and evaluation of the durability of this resistance depending on the PVY isolate used was the subject of the work of Depta et al. (2020). Based on molecular analyses of the 25 cultivars tested, 16 were identified in which the applied markers specific for the *Va* susceptibility gene were not amplified which may indicate a recessive form of this gene. However, the varied responses of these accessions to the used PVY isolates were noticed. The highest resistance was in case of the cultivar VAM, which was not affected by the weak and medium isolates, but responded with vein necrosis to the other two, described as strong. The results of other studies showed high resistance of the VAM cultivar to Japanese PVY-T isolates (Masuta et al., 1999) and Polish isolates belonging to the groups $PVY^{N}W$ and PVY^{NTN} (Doroszewska, Czubacka, 2008). Within the remaining cultivars with *va*-type resistance tested by Depta et al. (2020), six of them, including the cultivar Wiślica and V. SCR, were resistant to the weak isolate, and nine were affected by all PVY isolates. Literature data indicate that the VAM cultivar shows high resistance due to the possession of two recessive genes, *va1* and *va2*, which limit both the movement of the virus from cell to cell and its accumulation (Acosta-Leal, Xiong, 2008). The cultivar Wiślica has a slightly weaker *va1* allele (Verrier, Doroszewska, 2002), while the cultivar V. SCR was shown to have the *va2* allele (Lacroix et al., 2010). It was also demonstrated in the study by Korbecka-Glinka et al. (2017b) where, after the application of 10 PVY isolates, the cultivar VAM showed the highest resistance followed by Wiślica and the least satisfactory results were obtained for the cultivar V. SCR. Julio et al. (2015) conducted a detailed study of 163 *Nicotiana tabacum* accessions combining their molecular analysis and inoculation with the isolate PVY^{N}. The accessions, that showed disease symptoms, had the amplification product of all markers used, including S10760, which is associated with the susceptibility gene *Va*. On the other hand, the accessions considered resistant were divided into three groups. The first included 45 cultivars that did not amplify any marker which may be due to a large deletion within the *Va* susceptibility gene. The second group included four cultivars that amplified the markers used, with the exception of S10760, which may be due to a smaller deletion. The amplification product for all markers was observed for 13 subjects that were simultaneously symptomless. A detailed study using sequencing showed that they had a deletion of two base pairs at position 478-479. From among the resistant accessions studied by Julio et al. (2015) four groups differing in mutation length were selected and inoculated with nine PVY isolates of varying virulence (Michel et al., 2019). This study allowed determine the durability of PVY resistance. Belonging to the first group (so-called LD – Large Deletion), the cultivars VAM, Wiślica, TN86 and PBD6 had a large deletion on chromosome 21. The cultivar VAM had the highest resistance in this group and PBD6 – the weakest. The cultivars selected for the study with a smaller deletion on chromosome 21 (so-called SD – Small Deletion), cultivars with a deletion of two base pairs, as well as mutants derived as a result of ethyl methanesulphonate treatment showed significantly less resistance to PVY than the cultivars in the first group.

The PVY resistance presented above, which is conditioned by the recessive *va* gene, although it does not provide immunity, is the most commonly used in breeding and consequently most commercially grown cultivars have it. Unfortunately, this results in an increasing number of virulent PVY isolates, especially from the group PVY^{NTN}, which break this resistance (Lacroix et al., 2010; Lacroix et al., 2011; Verrier, Doroszewska, 2018).

The *NtTPN1* gene responsible for tolerance is also present within the cultivated cultivars of *N. tabacum*. It is located on chromosome 13 and arose from a point mutation at position 497 of the same gene (Michel et al., 2018). The results of the study by Depta et al. (2020) identified a group of five cultivars, considered as tolerant, which showed only vein clearing and chlorotic spots, and their growth and development was similar to healthy plants, despite the presence of the virus in the tissues. A characteristic feature of these plants was the absence of necrosis regardless of the PVY isolate used. At the same time, the presence of the susceptibility gene *Va* was demonstrated in these plants. Also from the 163 cultivars tested by Julio et al. (2015) 10 were identified with tolerance to all PVY isolates used.

Molecular studies (Depta et al., 2020) confirmed the presence of the *Va* gene in susceptible cultivars, which reacted with vein necrosis to all PVY isolates used. This gene was also detected in the Węgierski Ogrodowy cultivar whose response to PVY varied. This cultivar was resistant to the weak isolate IUNG 23, while it reacted to the medium isolate IUNG 17 with the occurrence of tolerance in the form of chlorotic spots and vein clearing. In contrast, the strong isolates resulted in the occurrence of vein necrosis. The variation of response depending on the PVY isolate may suggest that the resistance of cv. Węgierski Ogrodowy has a genetic basis other than the *va* gene making this cultivar, due to its unique nature, a valuable source of resistance in tobacco breeding.

The use of *Nicotiana tabacum* cultivars as a source of PVY resistance genes is often insufficient due to the increased threat of virulent PVY isolates that overcome *va*-type resistance. However, another species belonging to the genus *Nicotiana* are a valuable source of resistance to various pathogens including PVY. For this reason, an assessment of the resistance of wild tobacco species to various PVY isolates was carried out (Doroszewska, Depta, 2011). These studies were broadened by testing the resistance of the relatively recently discovered species *Nicotiana mutabilis* Stehmann et Samir (Depta et al., 2021). Three of the studied species: *Nicotiana raimondii* Macbride, *N. knigh-*

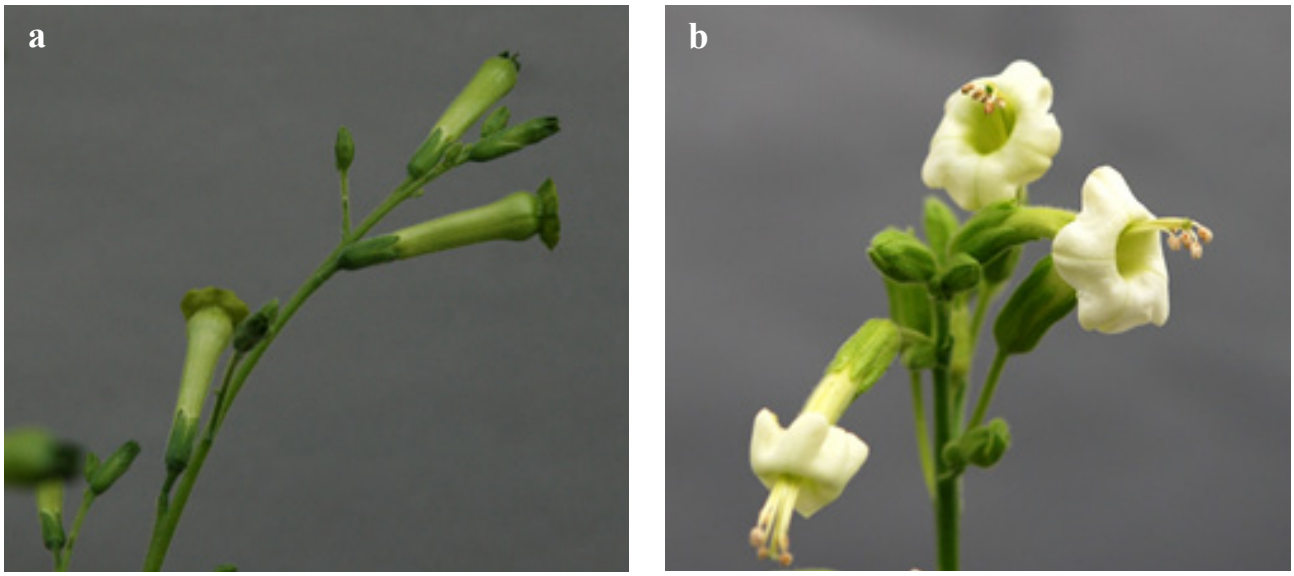

Figure 4. *Nicotiana knightiana* (a) and *Nicotiana africana* (b) – species with potato virus Y resistance. Author – A. Depta.

tiana Goodspeed (Fig. 4a) and *N. africana* Merxmüller et Buttler (Fig. 4b) showed immunity to all PVY isolates used (Doroszewska, Depta, 2011). Earlier studies by Sievert (1972) and Głażewska (1977) also identify *N. raimondii* and *N. knightiana* as PVY resistant species. In contrast, the use of American isolates PVY^{MM} resulted in the appearance of mosaic lesions in *N. knightiana* species (Burk et al., 1982). Breeding work using *N. knightiana* and *N. raimondii* to transfer PVY resistance to cultivated tobacco was carried out by Berbeć (1987a). However, as a result of high genetic instability, the obtained hybrids were characterised by completely non-viable pollen.

Immunity of *N. africana* species has been confirmed in previous studies using PVY isolates of varying virulence (Lucas et al., 1980; Doroszewska, Chrzanowska, 2001; Doroszewska, 2004; Doroszewska, Czubacka, 2008). Consequently, breeding work was undertaken to transfer PVY resistance from *N. africana* to *N. tabacum*. Obtained by Wersman (1992), by crossing the susceptible tobacco cultivar McNair 944 and the wild species *N. africana*, the NC152 additive line had a pair of homologous chromosomes from *N. africana* and showed only partial protection against PVY. This line was a component for further crosses with the susceptible cultivar Petit Havana, which resulted in generation BC_1F_1 resistant to the isolate PVY NN (Lewis, 2005). Further crosses with the susceptible cultivar K326 yielded lines with varying resistance, depending on the PVY isolate used (Lewis, 2007), and this trait was partially dominant (Lewis, 2005). Doroszewska (2010) performed a cross between the susceptible cv. BP-210 and *N. africana* as the paternal parent. The resulting BPA line was tolerant, as evidenced by weak disease symptoms in the form of vein clearing and chlorotic spots combined with an absence of necrosis, even after application of highly virulent isolates PVY^{NTN} (Doroszewska, Czubacka, 2008; Doroszewska, 2010; Korbecka-Glinka et al., 2017b; Czubacka, 2022). The resistance of the BPA line is recessive (Korbecka-Glinka et al., 2017a) and this line also has the *Va* susceptibility gene as confirmed by molecular studies using the S10760 marker (Korbecka-Glinka et al., 2017b). The work presented above concerned the use of susceptible tobacco cultivars in the establishment of breeding lines with *N. africana*. Depta and Doroszewska (2019) used two resistant cultivars of *N. tabacum* for crossing with *N. africana*: VAM and Wiślica, possessing the recessive *va* gene, whose hybrid forms are the subject of current research for PVY resistance. Due to the large phylogenetic distance the resulting hybrids *N. tabacum* x *N. africana* exhibited a barrier to intercrossing in the form of hybrid seedling dieback which was caused by browning of the root system (Tezuka et al., 2010). For this reason, it was necessary to use *in vitro* cultures to obtain viable hybrids. This was possible through cotyledon organogenesis and plant regeneration using appropriate media (Doroszewska, 1994; Depta, Doroszewska, 2019). Another problem was the infertility of the amphihaploid generation due to low chromosome conjugation. Obtaining fertile forms was possible by spontaneous doubling of chromosome number as a result of extended tissue culture time (Doroszewska, Berbeć, 2000) or using pith stem culture (Depta, Doroszewska, 2019). So far, it has not been successful to obtain immune hybrids from crossing *N. tabacum* x *N. africana*. An explanation for this could be the polygenic nature of PVY resistance in *N. africana* or the adverse influence of genetic factors derived from *N. tabacum* (Lewis, 2005; Doroszewska, 2007).

Other accessions belonging to the genus *Nicotiana* showed a varied resistance response depending on the species and PVY isolate used. The species *N. glauca* Graham was highly resistant (Sievert, 1972; Głażewska, 1977), but in a study by Doroszewska and Depta (2011), only the tetraploid form of this species proved to be resistant to all PVY isolates used. Meanwhile, the diploid form was infected by the isolate PVY^{NZ} with no disease symptoms observed but the presence of the virus was demonstrated by DAS-ELISA tests. A similar situation occured with *N. benavidesii* Goodspeed, where only the isolate PVY^{NZ} resulted in chlorotic rings. An attempt was made to cross the tetraploid form of *N. tabacum* cv. BP-210 and *N. benavidesii* which resulted with partially fertile sesquidiploid forms. The hybrid lines of successive generations obtained by self-pollination were characterised by resistance to PVY (Berbeć, Głażewska, 1988; Czubacka, 2022). Slightly less resistance, to three of the six isolates used, was observed in case of two species from section *Paniculatae* (*N. solanifolia* Walpers*, N. cordifolia* Philippi), two species from section *Tomentosae* (N. *otophora* Grisebach*, N. setcheli* Goodspeed) and one species from section *Noctiflorae* (*N. petuniodes* (Grisebach) Milan) (Doroszewska, Depta, 2011). In a study by Sievert (1972), using two PVY isolates, *N. solanifolia* and *N. cordifolia* were found to be susceptible. Simultaneously, previously considered as resistant species *N. thyrsiflora* Bitter ex Goodspeed (Sievert, 1972) and *N. paniculata* L. (Głażewska, 1977) proved to be susceptible to six isolates in a study by Doroszewska and Depta (2011). The cultivars of *N. rustica* L. showed great variation in resistance to PVY, of which *N. rustica* var. *brasilia* retained resistance to three isolates, *N. rustica* var. *pumila* to two, and *N. rustica* var. *neuchestii* to one. Disease symptoms on the remaining *N. rustica* cultivars included mainly vein clearing and chlorotic spots.

Among the tested *Nicotiana* accessions (Doroszewska, Depta, 2011), 26 species were identified that responded to all used PVY isolates with weak disease symptoms indicating tolerance. Tolerant species also include the species *N. mutabilis* and related species from the section *Alatae*: *N. alata* Link et Otto and *N. forgetiana* Hort ex Hemsley which did not develop vein necrosis but only weak disease symptoms after application of two isolates $PVY^{N}W$ and PVY^{NTN} (Depta et al., 2021). Cardin and Moury (2008) tested 30 *N. mutabilis* plants using the isolate PVY^{C} to which the species responded with mosaic lesions and vein necrosis on the leaves. Molecular testing of *N. mutabilis*, *N. alata* and *N. forgetiana* revealed the presence of the NsylelF4E1 marker, which means that these species do not possess the PVY susceptibility gene (Depta et al., 2021). Such a result indicates a different genetic basis for the tolerance of wild species compared to the tolerance of cultivars of *N. tabacum* in which a susceptibility-related marker is amplified (Michel et al., 2018; Depta et al. 2020).

Sources of resistance to TSWV within the genus *Nicotiana* and opportunities for their use

All cultivars are susceptible to TSWV to varying degrees, as there is no resistance to this virus within the species *Nicotiana tabacum* (Opoka, 1974; Laskowska, 2008). Due to its high harmfulness, it is very important to carry out resistance breeding preceded by knowledge of the sources of resistance. Therefore, Laskowska et al. (2013) conducted a study of *Nicotiana* accessions for resistance to TSWV in combination with the use of SCAR markers developed by Moon and Nicholson (2007) where differential resistance response to TSWV was demonstrated. The evaluation of 24 species belonging to the genus *Nicotiana* was previously performed by Opoka (1969) and Jankowski (1980) but their studies were conducted only under strong TSWV pressure in a field experiment. According to these studies, TSWV-resistant species included *N. alata*, *N. glauca* and *N. noctiflora* Hooker, while *Nicotiana* x *sanderae* was weakly susceptible. *N. langsdorfii* Weinmann, *N. longiflora* Cavanilles, *N. trigonophylla* (*N. obtusifolia*) M. Martens et Galeotti and *N. palmerii* Gray were also listed as resistant species. Species identified as moderately susceptible were *N. tomentosiformis*, *N. debneyi* Domin, *N. exigua* Wheeler, *N. longiflora* and *N. megalosiphon* Heurck et Mueller Arg. In contrast, *N. acuminata* (Graham) Hooker, *N. bigelovii* Pursh, *N. excelsior* Black, *N. glutinosa* L., *N. maritima* Wheeler, *N. miersii* Remy, *N. paniculata*, *N. pauciflora* Remy, *N. plumbaginifolia* Viviani, *N. repanda* Willdenow ex Lehmann, *N. rotundifolia* Lindley*, N. sylvestris* and *N. suaveolens* Lehmann proved to be highly susceptible (Opoka, 1969). Observations by Gajos (1978) showed resistance of three species to TSWV: *N. alata*, *N. affinis* and *N. langsdorfii.* In contrast, in the study by Laskowska et al. (2013) only *N. alata* and *Nicotiana* x *sanderae* were found to be resistant to TSWV while the other species were susceptible to varying degrees. Weak disease symptoms, only vein clearing and chlorotic spots, occurred in the species *N. petunioides*, *N. benavidesii*, *N. velutina* Wheeler, *N. otophora*, *N. setchelii, N. obtusifolia*, *N. palmerii*, *N. arentsii* Goodspeed and *N. wigandioides* Koch et Fintelmann. The remaining 71 species showed more severe infection symptoms that led to plant dwarfing and even death. The discrepancy in the results for TSWV resistance is due to both the different ways of inoculation and the methods used to identify the virus in plants (Opoka, 1969; Jankowski, 1980; Gajos, 1978, Laskowska, 2013). The variation in the assessment of resistance in some species may also be related to the growth rate of these plants. It has been observed that for perennial species, that grow slowly, the symptoms of infestation are weaker (Laskowska, 2013). Parella et al. (2003) refer a detailed list of species susceptible to TSWV belonging to different botanical families including 60 species belonging to the genus *Nicotiana*. This list includes 7

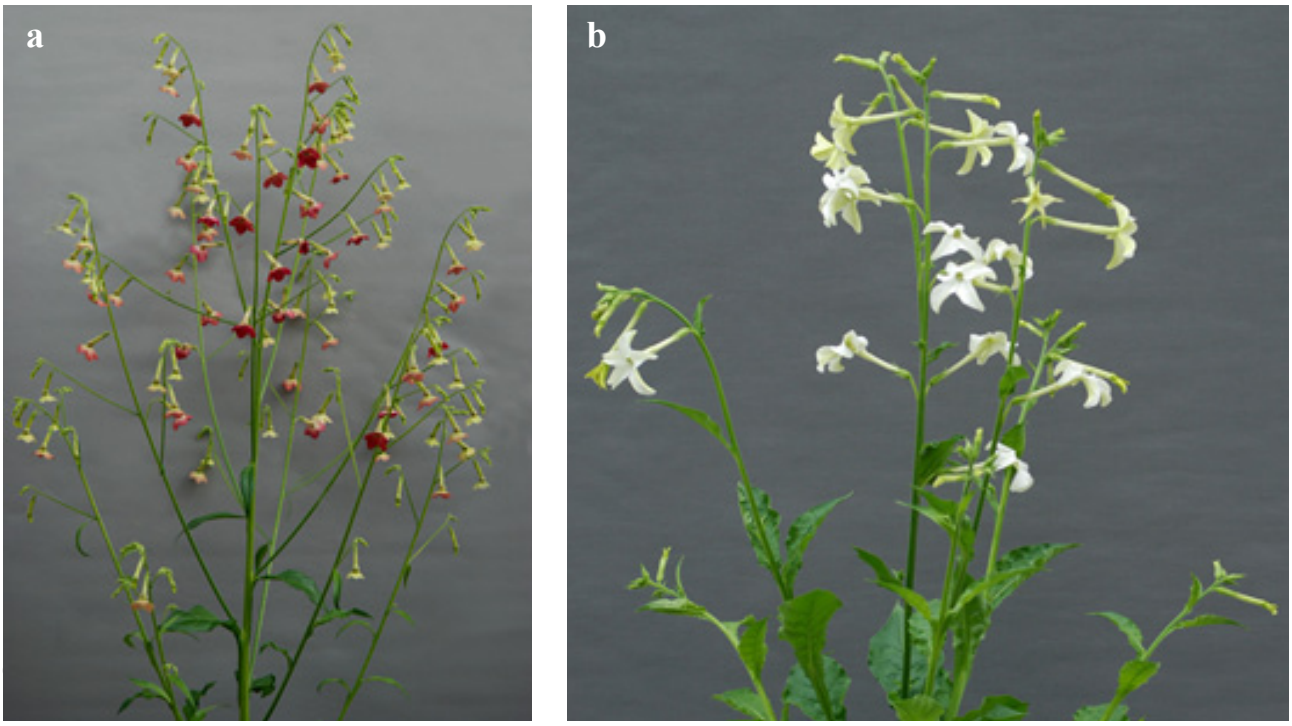

Figure 5. *Nicotiana mutabilis* (a) and *Nicotiana alata* (b) – species with tomato spotted wilt virus resistance in the genus *Nicotiana.* Author – A. Depta.

accessions not studied by Laskowska et al. (2013): *Nicotiana* x *calyciflora*, *N. clevelandii* Gray, *N. cycliflora*, *Nicotiana* x *edwardsomii*, *N. fragrans* Hooker, *N. bonariensis* Lehmann and *N. tomentosa* Ruiz et Pavon. In contrast, in the work of Parella et al. (2003) lacks information on the 10 susceptible species reported by Laskowska et al. (2013). This was complemented by studies on TSWV resistance of the species *N. mutabilis* which, like *N. alata*, belongs to section *Alatae* (Depta et al., 2021). *N. mutabilis* (Fig. 5a) was shown to respond with hypersensitivity to TSWV inoculation, with systemic infection possibly occurring for some of the plants tested. On the first test date, 4 weeks after inoculation, for 39 plants out of 40 tested there was a hypersensitive response on the lower leaves, for 30 plants also on the middle leaves and in 10 plants the virus was also transported to the upper leaves. On the second date, 8 weeks after inoculation, the middle leaves were infected in 31 plants and the upper leaves in 5 plants. However, 4 months after inoculation, 12 plants showed symptoms of infestation on the upper leaves indicating that systemic infection can occur long after inoculation. The hypersensitive response occurring for this species after TSWV inoculation may be an indication for its use in breeding work.

The results of many authors show clearly that TSWV-resistant species with a hypersensitive response are *N. alata* (Fig. 5b) and *Nicotiana* x *sanderae* (Opoka, 1969; Gajos, 1978; Jankowski, 1980; Laskowska et al., 2013; Depta et al., 2021). However, detailed studies of several populations of these two plants (Laskowska et al., 2013) reveal the possibility of a systemic hypersensitive response (SHR) to some of the tested plants of a given population. Only in two out of seven *N. alata* populations all plants reacted merely with a hypersensitive response (HR) while in the others the virus caused a systemic hypersensitive response which ranged from 6.3 to 50% of a given population. Similarly, one population of *Nicotiana* x *sanderae* was completely resistant and three were partially affected ranging from 16.7 to 50%. The lack of complete resistance may be related to the lack of total homozygosity of the two accessions due to their self-incompatibility. A similar phenomenon of SHR instead of HR is quite common in interactions of avirulent viruses with resistant species. Mandal et al. (2002) observed SHR in 17.6–46.7% of *Arachis hypogaea* 'C11-2-39' plants that initially developed HR. The species *N. forgetiana,* closely related to *N. alata,* initially showed a hypersensitive response after TSWV inoculation. However, systemic symptoms developed over time for all tested plants of this species (Laskowska et al., 2013; Depta et al., 2021).

Due to the high losses caused by TSWV numerous attempts have been made to transfer resistance from *N. alata* to *N. tabacum*. Obtaining a hybrid between the two species is difficult because of the lack of chromosome homology resulting from the large phylogenetic distance (Goodspeed, 1954).

Study on crossing the species *N. alata* with *N. tabacum* was undertaken by Gajos (1976). The amphihaploid plants obtained by the breeding process were treated with

colchicine and the resulting amphidiploid plant with male-fertile flowers was back-pollinated with pollen from tobacco. This yielded sesquidiploid BC_1 plants which showed a hypersensitive response after TSWV inoculation. In further work, Gajos (1979) used a bridge species, *Nicotiana otophora,* and obtained hybrids that could be backcrossed with tobacco and subsequently self-pollinated. Evaluation of the resistance of hybrids to TSWV in subsequent generations was carried out by artificial inoculation and the resulting proportions of segregation into resistant and susceptible plants were indicative of the dominant nature of the TSWV resistance gene (Gajos, 1981). The work of Gajos (1987) resulted in a cultivar in the dark cigarette type Polalta and a cultivar in the light cigarette type Wiktoria (Gajos, 1993; Czubacka, 2022). Study on obtaining hybrids with *N. alata* was also carried out by Berbeć (1987b) who used the tobacco cultivar Nadwiślański Mały for crosses in two forms: di- and tetraploid. The amphihaploids resulting from crossing the diploid form of *N. tabacum* with *N. alata* were completely infertile. In turn, the use of tetraploid form of tobacco resulted in obtaining sesquidiploids which admittedly showed low fertility but subsequent backcrosses allowed an increase in the degree of viability.

Testing of Polalta for TSWV resistance showed a hypersensitive response in all plants tested (Yancheva, 1990; Laskowska, 2013). Unfortunately, backcrossing Polalta with the susceptible cv. Wiślica resulted in hybrid plants with severe morphological deformationes such as abnormal ribbon-shaped leaves with thickened veins as well as tumours on the inflorescences and dwarfing of the plants (Laskowska, Berbeć, 2010). Breeding work between cultivar K326 and Polalta confirmed the linkage of morphological deformations with the resistance trait (Moon, Nicholson, 2007). Only the application of androgenesis and regeneration of doubled haploids under *in vitro* culture conditions allowed the selection of three resistant PW lines with reasonably good morphology (Laskowska, Berbeć, 2010; Czubacka, 2022). The selected PW line was crossed with the *Berkeleyomyces basicola* (formerly *Thielaviopsis basicola)* resistant line WGL3. Further breeding work led to lines with a medium degree of deformation and resistant to both TSWV and *B. basicola* (Trojak-Goluch et al., 2011).

A great support in breeding for TSWV resistance are the molecular markers developed by Moon and Nicholson (2007). The markers were detected in accessions that gave a hypersensitive response (*N. alata*, *N.* x *sanderae*, *N. mutabilis*, *N. tabacum* cv. Polalta) or a systemic hypersensitive response (*N. forgetiana*). These markers were not amplified in TSWV-susceptible accessions. A large variation in resistance was shown within the cv. Wiktoria plants tested, where only 4 out of 19 showed a hypersensitive response and in these accessions markers related to the resistance factor were amplified while the remaining plants were susceptible (Laskowska et al., 2013; Depta et al., 2021).

Sources of resistance to TMV within the genus *Nicotiana* and opportunities for their use

The assessment of resistance to tobacco mosaic virus by Depta et al. (2018) showed a large variation among accessions of the genus *Nicotiana.* The obtained results made it possible to distinguish four groups. The largest group, comprising mainly *N. tabacum* cultivars, consisted of susceptible accessions in which mosaic discolouration was observed and the virus was present in plant tissues. The second group included tolerant accessions in which no disease symptoms were observed in plants growing at 22°C but an increase in temperature to 30°C resulted in mosaic lesions as well as stunted growth and yellowing of the plants. At the same time, the DAS-ELISA tests used indicated the presence of the virus in plants regardless of temperature. The Colombian cultivar Ambalema and three wild species: *N. glauca*, *N. wigandioides* and *N. africana* were included into this goup. Information on the tolerance of Ambalema indicates that this trait is conditioned by two recessive genes (*mt1*, *mt2*) and several modifying genes (Nolla, Roque, 1933). For this reason, it is difficult to use in breeding programmes (Valleau, 1952). An asymptomatic response after TMV inoculation has been observed previously in several wild species: *N. glauca* and *N. tomentosiformis* (Valleau, 1952) and *N. wigandioides* (Holmes, 1946).

The third group consisted of treatments showing a hypersensitive response in the form of necrotic spots on the lower leaves. According to literature data, species reacting with hypersensitive response to TMV included: *N. glutinosa, N. repanda, N. gossei* Domin, *N. rustica, N. langsdorfii, N. benthamiana* Domin*, N. maritima, N.* x *sanderae, N. velutina, N. acuminata, N. goodspeedii* Wheeler*, N. forgetiana, N. nesophila* Johnston and *N. stoctonii* Brandegee (Holmes, 1929; Valleau, 1952; Burk, Heggestad, 1966; Gwynn, 1977; Yuan et al., 2015). The studies on TMV resistance have been also conducted with the use of *N. tabacum*. Most cultivars tested were susceptible to TMV and responded with mosaic lesions (Holmes, 1938; Lucas, 1975; Trancheva, 2005; Chen et al., 2014). Lucas (1975) lists as cultivars with hypersensitive response: Sota 27, Diubek 566, Newrokop 261 and Immunnyj 580. The results of Trancheva's (2005) work also identify the cultivars Diubek 566, Newrokop 261 and Immunnyj 580. Of the 22 cultivars tested by Chen et al. (2014) only Coker 86 and Jiyan 5 were found to be resistant. However, the papers presented do not provide complete information on the inoculation methods and TMV isolate used. In contrast, a study by Depta et al. (2018) indicate that a hypersensitivite response is present in 7 species of the genus *Nicotiana* (*N. glutinosa*, *N. maritima*, *N. goodspeedii*, *N. repanda*, *N. benthamiana*, *N. langsdorfii* and *N. gossei*) and in 14 cultivated tobacco cultivars, including the previously mentioned cultivars: Sota 27, Diubek 566, Newrokop 261 and Immunnyj 580.

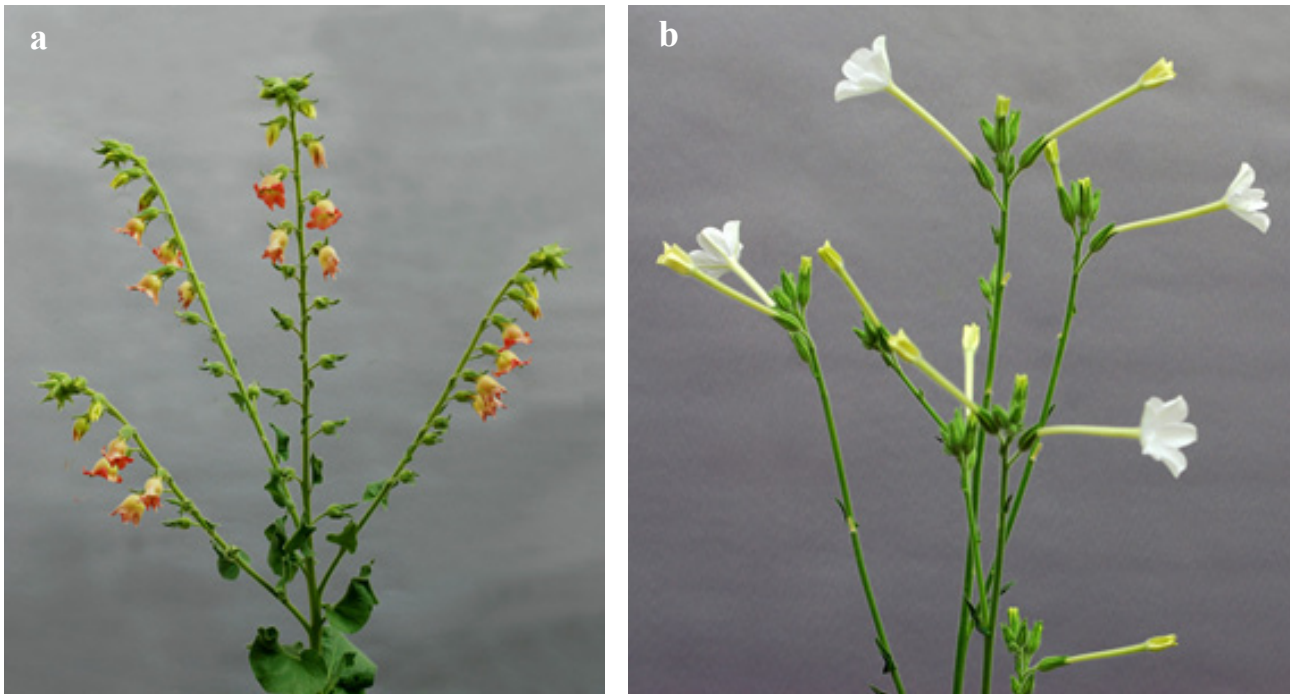

Figure 6. *Nicotiana glutinosa* (a) and *Nicotiana gossei* (b) as sources of resistance to tobacco mosaic virus. Author – A. Depta.

Molecular studies using E and N primers (Lewis et al., 2005) showed that all the tobacco cultivars tested by Depta et al. (2018) have TMV resistance conditioned by the *N* gene, derived from *N. glutinosa* (Fig. 6a). This is the single dominant gene responsible for the hypersensitive response (Holmes, 1938; Gerstel, 1943). In contrast, this gene was not identified in species of the genus *Nicotiana* exhibiting the hypersensitive response. This is because they have genes homologous to the *N* gene, which are not detected with the primers used in this study (Depta et al., 2018). According to Stange et al. (2004) a large proportion of species in the genus *Nicotiana* have homologous genes *NH* that have 82.6% nucleotide identity with the *N gene*, while Zhang et al. (2009) described a homologous *CN* gene from *N. rustica* that was 93.63% compatible with the *N* gene.

The two temperature ranges used by Depta et al. (2018) allowed to assess the durability of resistance to TMV. The resistance response of plants containing the *N* gene, as well as some species with homologous genes, was temperature-dependent. In plants at temperatures above 28°C virus multiplication and movement to higher parts of the plant occurred resulting in a systemic hypersensitive response (SHR). The observed resistance conditioned by the *N* gene is probably related to the presence of thermosensitive proteins (IVR) that inhibit virus replication (Gera et al., 1993). The *N. rustica* species showed a systemic necrotic response in both temperature ranges. Due to the warming climate and the consequent increase in the prevalence of TMV worldwide, the use of *N* gene-conditioned resistance may be unreliable. The results of the obtained study (Depta et al., 2018) showed that the resistance of the *N. gossei* species (Fig. 6b) is independent of temperature, so it may represent a more effective source of resistance to TMV.

SUMMARY

Species of the genus *Nicotiana* show great variation in their resistance to viral diseases. Complete resistance to all PVY isolates tested was found in: *N. glauca, N. raimondii, N. knightiana* and *N. africana,* while 26 species showed symptoms of tolerance. The other accessions showed a varied immune response depending on the isolate used. Based on molecular studies, within *Nicotiana tabacum* cultivars, resistance to PVY was found to be conditioned by a deletion in the susceptibility gene *Va*. Cultivars with *va*-type resistance showed varying levels of response to PVY infection which may reflect the different length of the deletion in the susceptibility gene among the cultivars tested. The VAM cultivar showed the highest resistance. Tolerant cultivars were also selected among the cultivars which reacted to PVY infection with vein clearing and chlorotic spots but did not show necrosis. At the same time they had the susceptibility gene *Va* which may indicate a different than *va*-type genetic background for tolerance.

Testing of the accessions for resistance to tomato spotted wilt virus indicates that resistance of the hypersensitive type takes place in the species *N. alata* and *N. mutabilis* and in the hybrid *Nicotiana* x *sanderae* and two tobacco

cultivars (Polalta and Wiktoria). The resistance to TSWV of the other collection accessions varied. Tolerant symptoms were observed in nine wild species but definitely more numerous was group of 71 accessions showing much stronger infection symptoms.

Tobacco mosaic virus resistance of the tested *Nicotiana tabacum* cultivars is conditioned by the *N* gene coming from the species *N. glutinosa*. It takes the form of hypersensitivity and is temperature-dependent. In the genus *Nicotiana,* an alternative source of TMV resistance is the species *N. gossei*, which lacks the *N* gene but exhibits a hypersensitive response. The resistance of this species is independent of temperature.

Accessions that are resistant to particular viruses are valuable gene sources for resistance breeding and could be the subject of further research. There are usually problems in breeding which are caused by the large genetic distance between wild species and tobacco cultivars and therefore the varying number and structure of chromosomes. However, in spite of difficulties at the crossing stage and the transmission of the desired trait, the breeding study is being undertaken because of the lack of other sources of resistance.

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Opracowano w ramach zadania 1.5.3 pt. "Upowszechnianie wiedzy o wynikach uzyskiwanych w ramach zadania (hodowla i nasiennictwo tytoniu)" z dotacji budżetowej przeznaczonej na realizację zadań MRiRW w 2023 r.

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received – 10 October 2023
revised – 8 November 2023
accepted – 15 November 2023

Authors declare no conflict of interest.