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Inheritance of *Potato virus Y* tolerance introgressed from *Nicotiana africana* to cultivated tobacco

(Short communication)

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Abstract. *Potato virus Y* (PVY) is an important pathogen of tobacco. Growing resistant cultivars is the best way to prevent significant losses of the crop caused by this virus. However, the protection given by the commonly used resistance factor, called *va* gene, can be overcome by the highly virulent PVY strains. Therefore, tobacco breeding for resistance will benefit from introducing additional PVY resistance/tolerance factors. BPA is a tobacco breeding line with introgressed PVY tolerance from a wild species *N. africana*. This trait is effective against a wide range of PVY isolates, including the ones that overcome *va* resistance. Here, we describe the inheritance of PVY tolerance of BPA. We obtained F_1 and F_2 plants from reciprocal crosses between BPA and a susceptible tobacco cultivar BP-210. Then we performed mechanical inoculation tests using sap from PVY infected leaves on both generations of plants. Four weeks later we recorded disease symptoms and subjected all experimental plants to DAS-ELISA tests. All F_1 plants developed vein necrosis which confirmed their susceptibility to the virus. The proportion of susceptible and tolerant plants in the F_2 fitted 3:1 ratio which was expected under the assumption that the tolerance is determined by a single, recessive gene. Moreover, the proportion of the susceptible and tolerant individuals did not differ between two F_2 populations derived from crosses where BPA was used as a maternal plant or a pollen donor, hence cytoplasmic factors do not influence the tolerance of that breeding line.

Keywords: *Potato virus Y*, PVY tolerance, *Nicotiana tabacum*, *Nicotiana africana*

INTRODUCTION

Potato virus Y (PVY) belongs to a genus *Potyvirus* and it is an important pathogen of economically important solanaceous crops, such as: potato, tobacco, tomato and pepper (Scholthof et al., 2011). In tobacco, a necrotic strain of the virus (PVY^N) causes a necrosis of the vascular tissues reducing the transport of water and mineral salts to the leaves and leading to necrosis of the whole leaves (Doroszewska et al., 2013). These symptoms lead to a significant reduction of the crop and changes in chemical composition of the leaves (Verrier et al., 2001).

Chemical control of the disease is ineffective because PVY is transmitted by aphids in a non-persistent manner (Scholthof et al., 2011). Growing resistant cultivars is frequently proposed to be the best way to protect the crop. However, PVY resistance of most of the currently grown tobacco cultivars rely on a single, recessive resistance factor – *va* gene (Verrier and Doroszewska, 2002). Moreover, the appearance of PVY isolates capable of breaking *va*-resistance is recently frequently reported (Lacroix et al., 2010; Lacroix et al., 2011).

New sources of PVY resistance can be sought among wild *Nicotiana* species. For example, *N. africana* proved to be immune to this virus and at least two attempts were made to transfer PVY resistance from this species to cultivated tobacco (Doroszewska, 2010; Lewis, 2005). A breeding line called BPA, was derived from an interspecific cross between a susceptible tobacco cultivar BP-210 and *N. africana* by Doroszewska (2010). After PVY inoculation of that breeding line, the virus can be detected in the leaves using serological methods but necrosis of the tissues does not develop; only mild symptoms, such as chlorotic spots, can be observed (Fig. 1). PVY tolerance of BPA is effective against a wide range of virus isolates representing diverse recombinant strains, including the ones that overcome *va* resistance (Korbecka-Glinka et al., 2017). This introgressed trait can be combined with other factors of PVY tolerance/resistance in order to obtain a cultivar which is able to cope with a wider range of the virus strains. However, the mode of inheritance of PVY tolerance in BPA is unknown.

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Figure 1. Comparison of leaves of uninoculated control plant of BPA (left) and plant of the same breeding line inoculated with PVY isolate IUNG4 (right). Mild symptoms of chlorotoic spots and leaf veins with no necrotic changes are visible on the leaf of inoculated plant.

Antiviral resistance in most plant species is controlled by a single gene showing dominance (Kang et al., 2005). PVY tolerance of introgression lines acquired by Lewis (2005) is determined by a chromosome segment derived from *N. africana* and acting in a partially dominant fashion. Here, we ask if PVY tolerance introgressed in BPA, and derived from *N. africana* in another breeding program, shows the same mode of inheritance. We also test whether cytoplasmic factors influence this trait.

MATERIALS AND METHODS

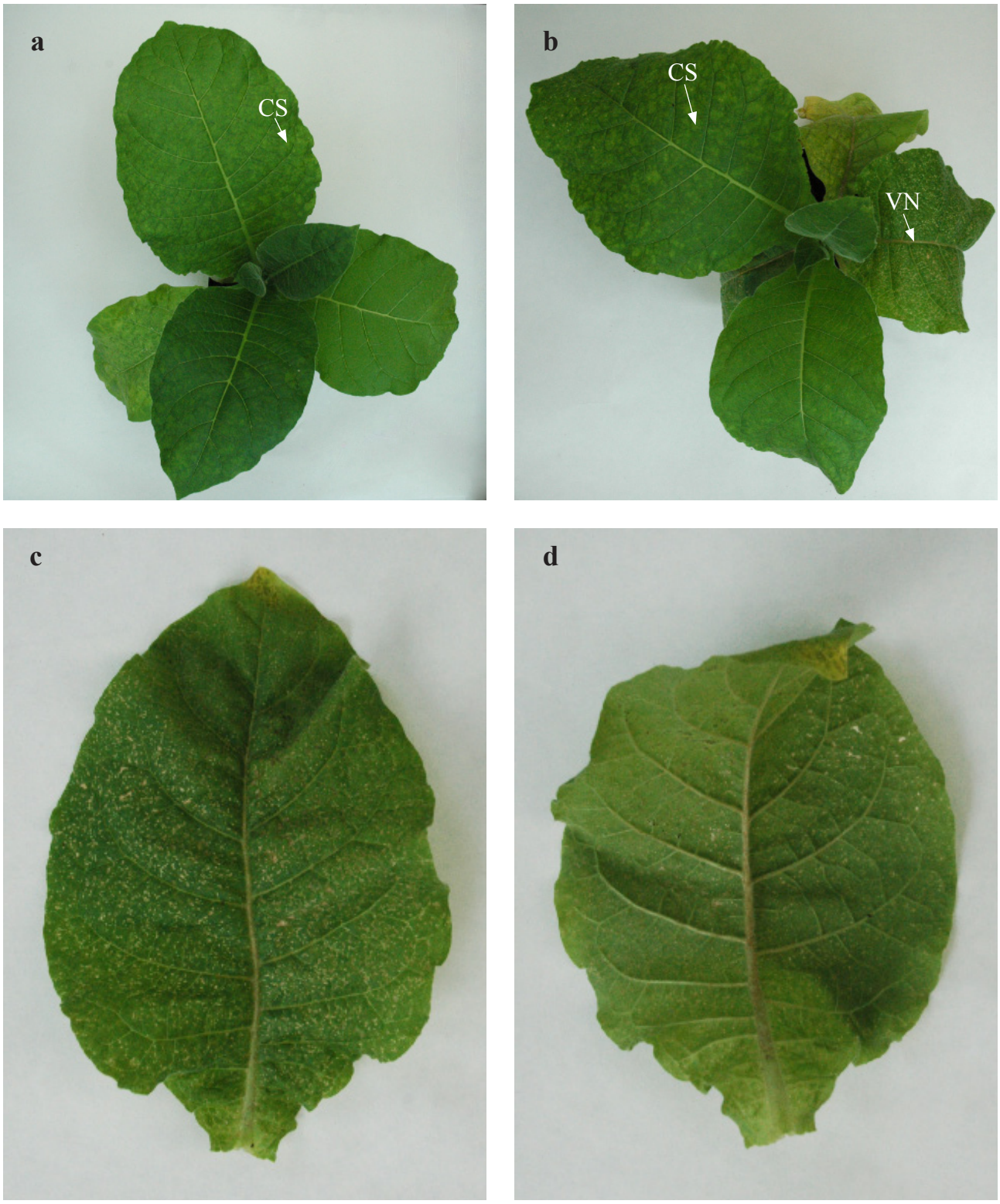
Plant material

BPA was previously obtained by Doroszewska (2010) from the interspecific cross between susceptible tobacco cultivar and *N. africana* accession from a germplasm collection of the Institute of Soil Science and Plant Cultivation (also stored at the National Center for Plant Genetic Resources in Radzików, Poland, accession no. PL167840). Here, BPA and a susceptible tobacco cultivar BP-210 were used for reciprocal outcrossing. Subsequent self-pollinations of single F_1 plants allowed to obtain two F_2 populations. We used following populations of plants in the PVY inoculation tests: (BPA x BP-210)F_1, (BP-210 x BPA)F_1, (BPA x BP-210)F_2 and (BP-210 x BPA)F_2. Seeds from all above-mentioned plant populations were germinated and seedlings were transplanted and grown until they reached a stage of 3–4 leaves. Then they were transplanted again into individual 0.6 L pots filled with peat based compost. PVY inoculations were performed on these plants when they developed 5–6 leaves. The whole experiment was carried out in a greenhouse and the experimental plants were kept spaced out on the greenhouse tables so that there was no physical contact between them. The mineral fertilizer was regularly applied to the plants to prevent nutrient deficiency.

Inoculation tests

PVY isolates, named IUNG5 and IUNG4, were selected for inoculations of F_1 and F_2 plants, respectively. The first isolate belongs to PVY^{NTN} recombinant group while the second one – to $PVY^{N}W$ group (Green et al., 2017; Przybys et al., 2013). Therefore, these two isolates differed with virulence, however recent study showed a similar response of BPA to isolates representing these two recombinant strains (Korbecka-Glinka et al., 2017).

The selected isolates were multiplied using PVY susceptible tobacco cultivar Samsun H which is resistant to *Tobacco mosaic virus*. Inoculum was prepared from leaves of this cultivar with visible disease symptoms. The leaves were ground with mortar and pestle. Then ground plant material was squeezed through gauze to obtain plant sap which was rubbed with a sponge into the leaves of experimental plants dusted with carborundum. The number of inoculated plants equalled 150 and 300 for each of the F_1 and F_2 population, respectively. In addition, BPA was used as tolerant control, while BP-210 as susceptible control. The number of plants used for each control type equalled 3 and 5 in inoculation tests of F_1 and F_2 populations, respectively. Inoculated plants were sprinkled with distilled water and sheltered from a direct sunlight for two days. Approximately four weeks after inoculation, disease symptoms were recorded. Then also all plants were sampled for DAS-ELISA tests which were done using monoclonal antibodies MoAbs antiY manufactured by Bioreba (catalogue no.: IgG112911). Positive DAS-ELISA tests



a – PVY tolerant F_2 plant with visible symptoms of chlorotic spots (CS).
b – PVY susceptible F_2 plant with visible symptoms of chlorotic spots (CS) and vein necrosis (VN).
c, d – Both sides of the leaf of PVY susceptible F_2 plant. Browning of the veins due to necrosis is visible especially on the lower side of the leaf.

Figure 2. Representative F_2 plants, derived from a cross between BPA and BP-210, four weeks after inoculation with PVY isolate IUNG4.

Table 1. Observed number of susceptible and tolerant plants recorded for two F_2 populations and controls. Expected numbers of susceptible and tolerant plants and chi-square tests are calculated to verify the hypothesis that these numbers in F_2 popualtions fit 3:1 ratio.

Tested plants	Type of value	No. of susceptible plants	No. of tolerant plants	Total no. of inoculated plants	χ^2	p-value
(BPA x BP-210)F_2	observed	217	83	300	1.138	0.286
	expected	225	75	300		
(BP-210 x BPA)F_2	observed	220	80	300	0.444	0.505
	expected	225	75	300		
BPA	observed	0	5	5	-	-
BP-210	observed	5	0	5	-	-

confirmed efficient inoculation, multiplication and spread of PVY within experimental plants. Distinguishing the tolerant and susceptible plants was then done depending on the absence/presence of vein necrosis. These symptoms are very characteristic of the studied tobacco disease and, to our knowledge, they do not develop as a response to any environmental factors. The observed ratios of numbers of susceptible and tolerant F_2 plants were tested against expected ratio 3:1 using χ^2 tests by means of the software STATISTICA v. 9.0. The same statistics was calculated to test if the numbers of tolerant and susceptible plants differed between two F_2 populations which were derived from crosses of BPA, in which it was used as a maternal plant or a pollen donor.

RESULTS

Inoculation tests on F_1 plants

All 300 F_1 plants were susceptible to PVY irrespective of their origin from the crossings where BPA was maternal plant or pollen donor, in plant populations: (BPA x BP-210) F_1 and (BP-210 x BPA)F_1. The symptoms observed on these plants included vein necrosis, chlorotic spots and mostly also vein clearing. Susceptible parental cultivar (BP-210), used here as control, showed the same symptoms. BPA plants, used as tolerant control, showed chlorotic spots and vein clearing but no vein necrosis. DAS-ELISA tests gave positive results for all F_1 and control plants.

Inoculation tests on (BPA x BP-210)F_2 and (BP-210 x BPA)F_2

In F_2 generation segregation of PVY tolerance could be observed. The plants classified as tolerant showed at most chlorotic spots and sometimes also vein clearing but no vein necrosis (Fig. 2a). DAS-ELISA tests gave positive results for these plants. The susceptible plants showed clear symptoms of vein necrosis (Fig. 2b-d). Proportions of susceptible and tolerant plants in both F_2 populations fitted 3:1 ratios which are expected if the tolerance is determined by a single recessive gene (Table 1). These proportions also did not differ between the two F_2 populations derived from the two crosses, in which BPA was used either as the maternal parent or as the pollen donor ($\chi^2 = 0.076$, df = 1, p = 0.783). Therefore, cytoplasmic factors present in BPA line had no influence on inheritance of PVY tolerance.

DISCUSSION

PVY inoculation tests, done on two generations of plants, showed that tolerance to this virus segregated in F_2 generation. Approximately, 25% of the F_2 plants were found to be tolerant, as expected for a trait determined by a single, recessive gene. The use of F_2 generation for such study allowed for a simple test of a large segregating population, but each of the tested plants was a unique genotype which could be inoculated only once. Moreover, the assessment of plant tolerance relied on detection of vein necrosis, which may be less visible on plants suffering from nutrient deficiency (T. Doroszewska – pers. comm.). Therefore, in our inoculation tests, we used plants which were well fertilized and maintained in good condition and the symptoms were recorded by a researcher experienced in tobacco disease diagnostics. Nevertheless, testing PVY tolerance of stable, homozygous lines derived from F_2 generation would be desirable to confirm results obtained in this study.

BPA shows PVY tolerance rather than resistance, even though this trait originates from *N. africana* which is completely immune to the virus (Doroszewska and Depta, 2011; Lucas et al., 1980). This suggests that this breeding line may contain only one of the genetic factors contributing to polygenic resistance in this wild species. A similar conclusion was reached in another breeding program aiming at the transfer of PVY resistance from *N. africana* to cultivated tobacco which was carried at the North Carolina State University (Lewis, 2007; Wernsman, 1992). Introgression lines obtained within that program carried *Nafr* factor protecting only against necrotic effects of severe PVY isolates. However, BPA and *Nafr* introgression lines clearly differ with the mode of inheritance of PVY tolerance. As we showed in this study, PVY tolerance of

BPA is a recessive trait, while *Nafr* gene is inherited in a partially dominant fashion. This difference is not likely to be explained by the fact that different resistance sources were used in both breeding programs, because *N. africana* accession included in the Polish germplasm collection and used to obtain BPA was acquired from North Carolina State University (A. Berbeć – pers. comm.). It is possible that different factors contributing to *N. africana* resistance were transferred from the wild species to these breeding lines and pyramiding genetic factors they carry may help to obtain a cultivar which shows a higher level of resistance.

Majority of known examples of natural recessive plant virus resistance genes are associated with resistance to viruses belonging to *Potyviridae* family. Resistance to this group of viruses in pepper, lettuce and pea, results from lost susceptibility due to mutations of the eukaryotic translation initiation factor 4E (eIF4E), which is involved in interaction with the viral genome-linked protein VPg (Diaz-Pendon et al., 2004; Kang et al., 2005). Recently, Julio et al. (2015) found a strong correlation between *eIF4E* expression and PVY susceptibility in tobacco. Moreover, they showed that mutations of this gene are responsible for recessive *va* resistance.

Despite the same mode of inheritance, PVY tolerance of BPA is likely to be independent of the above-mentioned transcription factor for two reasons. Firstly, BPA amplifies S10760 marker associated with susceptibility at *va* locus (Julio et al., 2015; Korbecka-Glinka et al., 2017). Secondly, PVY is capable of moving and multiplying within BPA plants to a level detectable using DAS-ELISA test which cannot be observed in the case of *va*-resistant cultivars inoculated with PVY isolates unable to break this resistance (Korbecka-Glinka et al., 2017). PVY tolerance factor present in BPA may be located in a different place than the *va* gene in tobacco genome which can be verified by genetic mapping of this trait in the future.

A virus tolerance reduces disease symptoms severity and may considerably decrease the loss of the crop, but it does not affect the virus epidemics (Lecoq et al., 2004). Tolerant breeding lines are used mainly in breeding only if resistant cultivars are not available or their resistance is not sufficient. The increase of frequency of PVY isolates breaking *va*-resistance creates the need to search for new ways of protecting the tobacco crop and PVY tolerance introgressed into BPA may useful for that purpose.

CONCLUSION

PVY tolerance of BPA breeding line is determined by a single, nuclear and recessive gene.

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