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Development of *Potato virus* Y (PVY) infection in susceptible and resistant tobacco cultivars

(Short communication)

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Abstract. *Potato virus* Y (PVY) is one of the most important pathogens affecting tobacco. Some cultivars, such as VAM and Wiślica, carry *va* type of resistance which can be broken by more virulent strains of PVY. Here, we compare effects of inoculation of resistant (VAM, Wiślica) and susceptible (Samsun H, Burley 21) cultivars using three PVY isolates differing with their virulence. We recorded symptoms of PVY infection two, three and four weeks after inoculation and confirmed the presence of the virus in the leaves using DAS-ELISA tests done four weeks after inoculation. We observed one-week delay in the development of the first disease symptoms on resistant cultivars compared to susceptible ones. However, four weeks after inoculation, DAS-ELISA tests confirmed the presence of the virus in the tissues with visible symptoms. At this time, disease symptoms were observed on all tested cultivars inoculated with two isolates (named IUNG 16 and IUNG 13). The least virulent isolate (IUNG 5) broke the resistance of Wiślica but not VAM, what contradicts results of previous inoculations tests using this isolate. Previously, IUNG 5 was unable to break the resistance of Wiślica. The observed change of virulence of isolate IUNG 5 was confirmed in subsequent inoculation tests: PVY from the infected Wiślica broke resistance of VAM.

Keywords: *Potato virus* Y, PVY resistance, *Nicotiana tabacum*

INTRODUCTION

Potato virus Y (PVY) is one of the most important viruses affecting important crops from *Solanaceae* family (such as potato, tobacco and pepper), thereby causing significant economic losses (Scholthof et al., 2011). PVY is transmitted by aphids in a non-persistent manner what limits effectiveness of pesticides in controlling the spread of disease (Lucas, 1975). Therefore, the best way of preventing infection with this virus is growing resistant cultivars. Tobacco breeding for PVY resistance often depends on finding new sources of resistance among available cultivars, breeding lines and wild *Nicotiana* species (e.g. Doroszewska and Depta, 2011). One of the most commonly used source of PVY resistance was produced from Virginia type of tobacco through X ray-induced mutagenesis (Koelle, 1958). Virgin A Mutant resulting from this treatment appeared to be resistant to many PVY isolates, and therefore VAM cultivar, derived from it, is cultivated and included in breeding programs (e.g. Brandle, 1995). Polish cultivar Wiślica shows a resistance with a comparable effectiveness to VAM, but origin of this resistance is not well documented. PVY resistance of both cultivars is determined by a single recessive gene, which is, by many researchers, assumed to be the same gene *va* (e.g. Julio, et al., 2006).

PVY isolates may differ with their effect on various tobacco cultivars as some of them are strong enough to break resistance of all available sources (Doroszewska and Czubacka, 2008). However, it is not documented if breaking the resistance results in the same development of symptoms as in case of infecting a susceptible cultivar. Therefore, here we tested three different PVY isolates differing with their virulence on four tobacco cultivars: two of which are known as susceptible and the other two carry a resistance gene *va*. We will describe development of the disease symptoms in three different points in time (two, three and four weeks after inoculation). We also tested lower, middle and upper leaves for the presence of the virus using DAS-ELISA tests.

MATERIALS AND METHODS

Plant material

Plants used in this study include four cultivars of *Nicotiana tabacum* differing with their susceptibility to PVY.

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Two of them (Samsun H and Burley 21) are susceptible and two other cultivars (Wiślica and VAM) carry a *va* type of resistance. Plants were germinated and then transplanted into individual pots filled with peat based compost when they reached a stage of 3–4 leaves. They were subjected to virus inoculations at a stage of 5–6 leaves. The whole experiment was carried out in a greenhouse under natural sunlight conditions in the spring of 2013.

PVY isolates

Three Polish PVY isolates were used as inoculum:

– Isolate IUNG 16 – isolated from a resistant cultivar, it overcomes *va* type of resistance; it can be detected with two kinds of antibodies produced by Bioreba (MoAbs antiY and MoAbs antiY^{N}), therefore it belongs to PVY^{N} serotype.

– Isolate IUNG 13 – isolated from a resistant cultivar, it overcomes *va* type of resistance; it can be detected with both kinds of antibodies produced by Bioreba (PVY^{N} serotype), sequencing of this isolate showed its similarity to a group of PVY^{NTN} (Przybys et al., 2013; GenBank accession number: JF927761).

– Isolate IUNG 5 – isolated from a susceptible cultivar, it does not overcome major resistance sources of *va* type and it is not detectable by monoclonal antibodies MoAbs antiY^{N}, therefore it is a serotype PVY^{O}; based on sequencing results, it can be classified as isolate belonging to a group PVY^{N}W (Przybys et al., 2013; GenBank accession number: JF927753).

All isolates were multiplied using Samsun H plants.

Inoculations

Three different methods of preparing inoculum from each isolate were tested on five plants of each cultivar. In the first method, plants were inoculated with undiluted sap from infected plants. In the second one, the plant sap from infected plants was diluted with water in such a way that sap from 1 g of fresh leaf tissue was combined with 5 ml of distilled water. In the third method, plant sap was diluted in the same proportion but using a phosphate buffer (9.078 g/l KH_2PO_4, 11.867 g/l Na_2HPO_4; Tsakiridis and Gooding, 1972) instead of water. Before inoculation, leaves of experimental plants were dusted with carborundum and then the above-mentioned types of inoculum were rubbed into leaves of experimental plants using a sponge. Inoculated plants were then sprinkled with distilled water and sheltered from a direct sunlight for 48 hours.

The three different methods of preparing inoculums appeared to be equally effective; they induced the same symptoms on experimental plants from the same cultivar inoculated with the same isolate. Therefore, for the qualitative analysis of results in this paper, we pooled together 15 plants of the same cultivar inoculated with the same isolate irrespective of how the inoculum was prepared.

Observations and DAS-ELISA tests

Observations of symptoms were carried out three times: two, three and four weeks after inoculation. Four weeks after inoculation, nine out of 15 inoculated plants from each unit of observation (plants of the same cultivar inoculated with the same isolate) were sampled for DAS-ELISA tests using monoclonal antibodies MoAbs antiY (catalogue number IgG112911, supplied by Bioreba) directed against diverse strains of PVY. For each tested plant, leaf tissue was sampled from: upper, middle and lower – inoculated leaves. Independent DAS-ELISA tests on these samples allowed for assessment of the virus spread within the plants.

RESULTS

Observation of symptoms

Susceptible cultivars showed symptoms (mainly vein clearing) already two weeks after inoculations. Then other symptoms like chlorotic and necrotic spots appeared on the

Table 1. Infection symptoms recorded two and four weeks after inoculation with three virus PVY isolates on four tobacco cultivars.

Virus isolate (isolate group)	Time after inoculation	Cultivar			
		VAM	Wiślica	Samsun H	Burley 21
IUNG 16 (PVY^{N})	two weeks	ns	ns	VC	VC or ns
	four weeks	CS, severe NS, VN	CS, VC, NS, VN	CS, VC, NS, VN	severe CS, and NS, VN
IUNG 13 (PVY^{NTN})	two weeks	ns	ns	VC or ns	VC
	four weeks	VC	CS, VC, NS, VN	CS, VC, VN	CS, severe NS, VN
IUNG 5 (PVY^{N}W)	two weeks	ns	ns	VC or ns	VC or ns
	four weeks	ns	CS or/and VC, VN	CS, VC, VN	CS, VC, VN

Every cell in this table summarizes symptoms of 15 experimental plants. Shaded cells of the table indicate positive results of DAS-ELISA test done four weeks after inoculation on leaves with visible symptoms (lower and middle leaves).

Abbreviations of the symptoms:

VC – vein clearing, CS – chlorotic spots, NS – necrotic spots, VN – vein necrosis, ns – no symptoms.

leaves followed by vein necrosis. Development of disease symptoms took longer in case of resistant cultivars (Wiślica and VAM) infected with isolates IUNG 16 and IUNG 13: first symptoms (vein clearing or chlorotic spots) could be observed only three weeks after inoculation. One week later, leaf vein necrosis was recorded on Wiślica infected with all three isolates and VAM infected with isolate IUNG 16 (Table 1). Isolate IUNG 13 caused only vein clearing on VAM four week after inoculation. However, since DAS--ELISA confirmed the presence of PVY in the leaves (see below), we assume that in this case, VAM resistance was broken as well. In the case of inoculation with the least virulent strain (IUNG 5), no symptoms were detected on VAM; however, contrary to results of previous inoculation tests, Wiślica developed all infection symptoms including leaf vein necrosis.

In most cases, when leaf vein necrosis appeared on the lower leaves, the middle leaves showed only chlorotic spots and upper leaves remained asymptomatic.

DAS-ELISA tests

Generally, DAS-ELISA tests confirmed the presence of PVY in the tissues with visible symptoms: the virus was detected in the lower and middle leaves and no virus was detected in asymptomatic upper leaves.

Despite slower development of symptoms in resistant cultivars (Table 1), four weeks after inoculation, ELISA tests gave positive results for all four cultivars in cases of inoculation with more virulent PVY isolates (IUNG 16 and IUNG 13). Inoculation of VAM with the least virulent isolate (IUNG 5) resulted in negative results of DAS-ELISA tests which confirmed resistance of this cultivar. On the other hand, positive result of these tests for Wiślica inoculated with IUNG 5 corresponds to the observed symptoms and confirms breaking resistance of this cultivar.

DISCUSSION

One of the aims of this study was to compare development of symptoms in time after inoculation with various PVY strains for both susceptible cultivars and cultivars with *va* type of resistance. In case of the more virulent isolates, we found that disease symptoms take longer to develop, but after four weeks both groups of plants developed disease symptoms and a high level of the virus in their tissues detectable in DAS-ELISA tests. Therefore, in contact with the most virulent PVY strains, having *va* resistance may only slow down the disease development which is beneficial only in case of late infections in field conditions.

The least virulent PVY isolate (IUNG 5) did not cause disease symptoms on VAM but broke resistance of Wiślica, which is a contradictory result to previous inoculation tests on this cultivar using the same isolate. We suspect that this isolate increased its virulence in our experiment due to mutation. In the subsequent greenhouse tests, inoculation with sap from these Wiślica plants with vein necrosis caused the same symptoms on VAM (data not presented here). Changes in the virulence of the virus isolates used for mechanical inoculation in greenhouse experiments is relatively common. Multiple passages on susceptible cultivar may lead to a loss of virulence of PVY isolate (T. Doroszewska – personal communication), while selective pressure caused by *va* type of resistance may cause mutations determining increased virulence of PVY (Lacroix et al., 2011).

Results presented here provide useful information for future PVY inoculation tests on tobacco. Firstly, disease symptoms develop slower on resistant cultivars. Therefore, it is advisable to prolong observations on these cultivars beyond four weeks or to confirm the presence of the virus in leaves using serological tests (as it was done in case of this study). Secondly, asymptomatic upper leaves from infected plants should not be taken for preparing inoculums, because they contain relatively low levels of the virus, not detectable by DAS-ELISA tests.

CONCLUSIONS

1. Disease symptoms develop slower on cultivars carrying *va* resistance (in cases where this resistance is broken) compared to susceptible cultivars.
2. Isolate IUNG 5 increased its virulence after inoculation of cv. Wiślica. Increased virulence of this isolate was confirmed in subsequent inoculations of VAM.
3. Asymptomatic leaves on infected plants may not contain PVY in a quantity detectable in DAS-ELISA.

REFERENCES

Brandle J.E., 1995. Resistance to a necrotic strain of *Potato virus* Y among *Nicotiana* species, somatic hybrids, and tobacco cultivars. Plant Disease, 79: 152-154.

Doroszewska T., Czubacka A., 2008. Ocena odporności odmian i linii hodowlanych tytoniu na wirusa Y ziemniaka (PVY). Studia i Raporty IUNG-PIB, 13: 29-41.

Doroszewska T., Depta A., 2011. Resistance of wild *Nicotiana* species to different PVY isolates. Phytopathologia, 59: 9-24.

Julio E., Verrier J.L., de Borne F.D., 2006. Development of SCAR markers linked to three disease resistances based on AFLP within *Nicotiana tabacum* L. Theor. Appl. Gen., 112: 335-346.

Koelle G., 1958. Versuche zur Vererbung der Krankheitsresistenz bei Tabak. 2 Mitt. Eine Rippen-bräune-resistente Virgin A--Mutante nach Anwendung künstlicher Mutationsaulösung durch Röntgenstrahlen. Tabak-Forschung, 24: 83-84.

Lacroix C., Glais L., Verrier J.L., Jacquot E., 2011. Effect of passage of a *Potato virus* Y isolate on a line of tobacco containing the recessive resistance gene *va*(2) on the development of isolates capable of overcoming alleles 0 and 2. Europ. J. Plant Pathol., 130: 259-269.

Lucas G.B., 1975. Diseases of tobacco. Biological Consulting Associates, Raleigh, North Carolina, USA.

Przybys M., Doroszewska T., Berbec A., 2013. Point mutations in the viral genome-linked protein (VPg) of *Potato virus* Y probably correspond with ability to overcome resistance of tobacco. J. Food, Agric. Environ., 11: 986-989.

Scholthof K.B.G., Adkins S., Czosnek H., Palukaitis P., Jacquot E., Hohn T., et al., 2011. Top 10 plant viruses in molecular plant pathology. Molecul. Plant Pathol., 12: 938-954.

Tsakiridis J.P., Gooding G.V.J., 1972. *Tomato spotted wilt virus* in Greece. Phytopathol. Mediterranea, 11: 42-47.