STUDIES REGARDING THE TRANSMISSION OF POTATO VIRUS Y (PVY) TROUGH SEVERAL MECHANICAL MEANS

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ABSTRACT

The aim of this studies was to investigate the transmission of PVY strains PVY^{o}, PVY^{N}, PVY^{NTN}, via tuber cutting and plant treated in susceptible cultivars. For the tuber cutting experiment, after one infected tuber was cut with a knife, four uninfected tubers were cut sequentially with the same instrument without disinfecting it between the cuts. In the other experiments, the virus transmission from infected to healthy plants was made by bouncing, brushing, hammering, squeezing and carborundum rubbing treatments. These treatments allowed exchange of sap between the healthy and infected material. Results revelead that seed cutting did not transmit the pathogen, whereas the other plant treatments caused varying level of PVY transmission, depending on the experiments variant. Plant bouncing was the least effective whereas hammering was the most effective variant.

Abbreviations: PVY potato virus Y

INTRODUCTION

Potato virus Y (PVY), genus Potyvirus, member of the *Potyviridae* family is a growing concern for potato crops across many areas of the world [3,13,15,16,17,19]. The increase in PVY levels and the emergence of different strains of PVY, including the tobacco veinal strain PVY^{N}, the recombinant N:O strain PVY$^{N:O}$ and the potato necrosis strain PVY^{NTN} have significant impact on both seed and commercial potato productions [6,7,10,11]. In recent years, some of the growers in the world observed unacceptable levels of current season spread of PVY, depending upon management practices [5,9]. PVY is transmited chiefly within the field by the movement and probing aphids. The significance of aphid-mediated current season spread of PVY has been well studied and documented [12,14]. However, information on current season spread of PVY through mechanical means is very limited. Recently, growers started asking questions about mechanical transmission of PVY because of quicker spread of some of the strains of PVY. Mechanical transmission may occur due seed-cutting and/or plant/tuber wounding in the standing crop. Sturz et al. (2000) reported that there was no mechanical transmission of PVY^{O} following the cutting of the infected tubers by hand or mechanical cutting [18]. In contrast, there are other reports suggesting mechanical transmission of PVY due to plant wounding and seed cutting [4]. Moreover, these observations were conducted mainly on PVY^{O} strains, wich has been replaced by PVY$^{N:O}$ and/or PVY^{NTN} in some potato production areas. Farm operations such as post-emergence tillage and frequent use of tractors for spraying of the pesticidesand mineral oils during the crop season may cause plant wounding and sap exchange between the healthy and the infected plants. In adition, bouncing of the infected plants against the healthy ones or vice versa by strong winds may cause plant wounding, wich may potentially lead to virus transmission.

The study aimed to investigate the transmission of several PVY strains due to tuber cutting and to the plants treatments (on behalf to sap exchange between the healthy and infected material by bouncing, metal brishing, hammering, carborundum rubbing and

squeezing). Our results demonstrated that PVY does not transmit via tuber cutting but it does via plant touch in certain situations.

MATERIAL AND METHOD

Plants material. *Solanum tuberosum L*. plantlets of varieties Hermes and Carrera were grown at the Biotechnology Department of National Institute of Research and Development for Potato and Sugar Beet Brasov, in summer of 2015 and tested for PVY strains. Plants tested positive were marked and tubers were harvested from individual plants and maintained separately. This material was used as source of the inoculum for mechanical PVY mean transmission.

Transmission trough seed tubers cutting

The experiment was carried out under greenhouse conditions. 45 tubers were used (3 infected tubers each of PYY^{O}, PYY^{N} and PVY^{NTN}, and 36 virus-free tubers, each of varieties Hermes and Carrera). After one tuber infected with a specific strain was cross-cut with a knife, four virus-free tubers from the same variety were cut sequentially with the same knife without using a disinfection solution. Both pieces from each tuber were planted separately in pots (in 2 rows of 5 plants). After every two rows, a row of five PVY-free tubers was planted in the pots and used as negative control. So, 30 tubers including 15 controls were studied for each PVY strain. There were 3 strains, 90 tubers were tested for each cultivar. The plants obtained from this biological material were tested using DAS ELISA, 21 and 42 days after planting.

Transmission through plant touching

Mechanical transmission of 3 PVY strains (PVY^{O}, PYY^{N} and PVY^{NTN}), due to plant touching was investigated. PVY-free tubers of cultivar Hermes were planted in pots and maintained in the greenhouse. After 3 weeks, these plants were tested again using DAS ELISA to make sure that all the material was PVY-free. These plants were then subjected to the following treatments:

- **Inclining and bouncing** healthy plants against PVY infected ones. The healthy and infected biological material were maintained in two separate rows. The healthy plants were bounced against the infected ones. Plants were bounced three times every week. These treatments had the intention to mimic the modality which could incline and bounce plants in the field.
- **Metal brushing** The leaf of an infected plant was brushed, followed by brushing on the leaf of the healthy plant; two leaves of the healthy plant were brushed. The brush had the sharp needles, which were supposed to exchange sap between the healthy and the infected leaflets.
- **Hammering** A leaf from an infected and a healthy plant was layered and pressed; 4 leaves from each healthy plant were hammered. Hammering could exchange sap between the healthy and the infected leaves.
- **Carborundum rubbing** The carborundum was put on the leaf of the healthy plant and then this leaf was rubbed by the leaf of the infected plant; 4 leaves of healthy plant were treated. Carborundum is an abrasive widely used for virus inoculations.
- **Squeezing** Twigs of the healthy plant and the infected were squeezed together. This may also exchange sap between the healthy and the infected leaves. Two twigs of the healthy plant were pressed using 2 rocks.

Therefore, 90 plants (3 strains x 6 treatments x 5 plants per treatment) were treated and tested.

Plants not treated were used as controls.

For each treatment, the touching procedure was repeated on a weekly basis three times. Two leaves from each plant were collected 7 days after the last touching and tested for PYY using DAS ELISA and PCR according to Lorenzen et al. (2006) [8]. In addition,

two daughter tubers from individual plants were also collected five weeks after the last touching treatment. Then, tubers from individual plants were composited, and tested for PYY using DAS ELISA to ensure translocation of virus from foliage to tubers. The experiment was repeated twice (experiment 1 starting in Mars 2015 and experiment 2 starting in Avril 2015).

DAS ELISA test. The plants were tested for the viruses using DAS-ELISA kits for PVY and according to the manufacturer's instructions with several exceptions (Bioreba, Switzerland). Also, the analysis was performed following the protocol described by [2] (100 μl per well). Microplates were filled with substrate solution (p-nitro phenyl phosphate) incubated 1 hour for PVY and the absorbance values were estimated at 405 nm (A_{405}) on Tecan reader (Magellan softwere). The samples having A_{405} values exceeding two times the average of healthy controls were considered virus infected. In the first stage, the material was tested for Potato virus Y (polyclonal antibodies) and then, this biological material was retested using monoclonal antibodies (mAb). The plates were coating with anti PVY-NOC mAb (Bioreba, Switzerland, antibodies that could recognize all the PVY strains excepting the PVY^O) and the virus was detected using alcalin phosphatase (AP) linked to anti–PVY-NOC mAb (Bioreba, Switzerland, specific for the strains PVY^N) [1].

RESULTS AND DISCUSSIONS

In the transmission through the seed tuber cutting experiment, none of the plants including daughter tubers of either Hermes and Carrera were positive except those used as sources of PVY inoculum (data not shown). This indicates that seed cutting is not effective in spreading PYY strains tested. These results are in close agreement with those of Sturz et al. (2000) [18], who reported no PYY^O transmission following the cutting of the infected tubers during seed multiplication. In contrast, Draper and Gudmestad (1992) [4] reported different degrees of transmission of PVY depending upon susceptibility of the cultivars and pattern of tuber cutting, such as infected eye to healthy eye, infected eye to healthy flesh, infected flesh to healthy flesh. They used cultivars Russet Norkotah, Shepody and Red LaSoda. Our study was conducted under greenhouse conditions, by using another cultivars and only one pattern of virus transmission (infected flesh to healthy flesh).

In the transmission through plant touching experiment, bouncing of healthy plants against the infected ones led to, out of five plants in each strain, two plants being infected with PYY^O and one plant with PVY^N in the first experiment, and one plant with PYY^O in the second experiment (Table 1). There was no transmission of PYY^{NTN} caused by bouncing. Out of five plants tested in each strain, metal brushing resulted in transmission of PVY^O in five plants in the first experiment and four in the second experiment, PVY^N in five plants in the first experiment and two in the second experiment and PYY^{NTN} in four plants in the first experiment and one plant in the second experiment. Hammering showed mechanical transmission of PVY in all the five plants tested in each strain in the first experiment and for PVY^N and PVY^{NTN} transmission, resulted four plants in the second experiment. Carborundum rubbing resulted in transmission of PVY in all the five plants tested in each strain in both experiments. Squeezing twigs of the healthy and infected plants together resulted in transmission of PVY^O in all five plants in the first experiment, and in four plants in the second experiment, PVY^N in two plants in the first experiment and four plants in the second experiment, and PVY^{NTN} in four plants in the first experiment and three plants in the second experiment. These results were also confirmed with RT-PCR (results not reported). The daughter tubers collected from positive plants (based on foliage testing) were also tested positive (data not shown), which confirmed that PVY spreads mechanically through different kinds of plant touching. The transmission of PVY strains due to hammering was identical to Carborundum rubbing in the first experiment and close

to Carborundum rubbing in the second experiment. Carborundum is most widely used for artificial disease inoculation. Thus, like Carborundum rubbing, hammering can also be used for artificial inoculation of PVY for experimental purposes.

Table 1

Mechanical transmission of PVY strains trough plant touching treatments

Treatments (mechanical means)	PVY strains	Experiment 1*		Experiment 2*	
		Number of plants tested	Number infected plants	Number of plants tested	Number infected plants
Bouncing healthy plants against PVY infected	PVY^{O}	5	2	5	1
	PVY^{N}	5	0	5	0
	PVY^{NTN}	5	0	5	0
Metal brushing PVY infected plants and then healthy ones	PVY^{O}	5	5	5	4
	PVY^{N}	5	5	5	4
	PVY^{NTN}	5	4	5	2
Hammering leaf of the healthy and infected plant together	PVY^{O}	5	5	5	5
	PVY^{N}	5	5	5	4
	PVY^{NTN}	5	5	5	4
Carborundum dusting on the healthy leaf plants + rubbing this with sap from infected leaf plant	PVY^{O}	5	5	5	5
	PVY^{N}	5	5	5	5
	PVY^{NTN}	5	5	5	5
Squeezing together twigs of healthy and infected plants	PVY^{O}	5	5	5	4
	PVY^{N}	5	2	5	4
	PVY^{NTN}	5	4	5	3
Controls (untreated healthy plants)	PVY^{O}	5	0	5	0
	PVY^{N}	5	0	5	0
	PVY^{NTN}	5	0	5	0

*Experiment 1 was started in March 2015 and Experiment 2 started in Avril 2015

The touching treatments used in this study would like to mimic field operations such as cultivation and spraying.

Usually, the pesticides and mineral oils are frequently sprayed in potato crops for the management of different pests. The equipment used for these practices may damage twigs and outer cells of infected plants and carry sap with them, introducing sap into the healthy plants. Thus, early rouging of PVY infected plants becomes important to minimize the risk of mechanical as well as insect mediated PVY transmission. PVY might be transmitted mechanically through wounds due to plant-to-plant contact from wind or by farm equipment operation. In the mechanical transmission through plant touching, the virus may move along with the sap. It may not happen that quickly in the mechanical transmission through tuber-cutting. In the same time, foliage usually shows higher viral titer than the tubers. Maybe, this is the reason because seed-cutting could not transmit PVY but plant touching did transmit it. Indeed, further research is required to know the mechanism of PVY transmission via plant touching.

CONCLUSIONS

The study showed that the seed cutting did not transmit the pathogen, whereas the other plant treatments caused varying level of PVY transmission, depending on the experiments variant. Thusly, different plant touching treatments (means that could mimic the field operations) induced mechanical transmission of PVY strains.

Regarding the efficiency of the plant treatment variants, the plant bouncing was the least effective whereas hammering was the most effective mechanical mean.

However, the study should be repeated under field conditions to confirm the results.

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