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EVALUATION OF RESISTANCE TO *POTATO VIRUS Y* OF CULTIVARS IN PRE-REGISTRATION (STATUTORY) TRIALS

ABSTRACT

The paper describes testing potato cultivars for resistance to potato viruses in field and greenhouse conditions according to the scheme of the statutory trials. Seed tubers of tested cultivar are planted in the field in two replications with 30 tubers each. On both sides of each cultivar, 15 PVY-infected tubers, i.e. the source for PVY inoculation, are planted. The evaluation includes standard cultivars of known resistance levels to PVY. The progeny tubers are collected in autumn and growing-on testing is carried out in greenhouse conditions to estimate the number of plants infected with PVY. The other method is to evaluate the resistance of potato cultivars in greenhouse conditions. At present, the resistance of cultivars is evaluated only with respect to PVY, the most important potato virus. Two strains are used in the evaluation, i.e. PVY^{N-Wi} and PVY^{NTN}. For each testing cultivar, 10 plants are mechanically inoculated with each PVY strain. The analysis of the results makes it possible to classify the cultivars into four resistance groups on a 1-9 scale: highly resistant (with the resistance score of 8), resistant (with a value of 7), mid-resistant (with a value 5-6), and susceptible (with a value of 3-4). The ”9” rating is specified after the registration of the cultivars based on molecular tests, that validate the presence of a marker linked to extreme resistance (ER) gene Ry_{sto}.

Key words: field test, growing-on test, greenhouse test, potato cultivar, PVY

INTRODUCTION

Testing potato cultivars for resistance to potato viruses in field and greenhouse conditions according to the scheme of the statutory trials is done at Młochów Research Center of IHAR-PIB, at the request of the Research Center for Cultivar Testing (COBORU). The varieties reported by the breeders to COBORU before the registration in the Polish Varieties Catalog are evaluated for PVY resistance. Research includes two-year field experiments in natural

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infectious pressure. Conducting these tests in Młochów is justified by appropriate PVY infection pressure in this region. Besides field tests, an evaluation of resistance to PVY is conducted in greenhouse conditions after artificial inoculation with known strains of the virus in order to select the most resistant cultivars. The goal of evaluation of resistance to PVY of potato cultivars in greenhouse conditions is to select highly resistant forms, to characterize them for the type of resistance (extreme resistance ER, or based on hypersensitivity HR) and for the type of symptoms (necrotic reactions, mosaics etc.). The scheme of testing resistance to PVY in potato applied at IHAR-PIB Młochów Research Center is shown in Fig. 2.

MATERIALS

Field experiments

1) experimental field
2) potato seed tubers (60 tubers each cultivars)
3) PVY-infected tubers, about 1000 tubers (planted as the source of the virus for infection). These PVY-infected tubers are derived from the plants of susceptible potato cultivars that were mechanically inoculated with PVY in greenhouse conditions in the previous year
4) three standard cultivars of known resistance levels (resistant, medium resistant and susceptible) to PVY e.g. cultivars: Cekin (with a resistance score 5-6), Michalina (with a value of 7) and Satina (with a value of 5), (240 tubers of each cultivar).

Greenhouse experiment

1) insect-free greenhouse with natural lighting
2) potato seed tubers (20 tubers of each cultivars)
3) three PVY resistance standard cultivars e.g. cultivars Cekin (score 5-6), Michalina (score 7) and Satina (score 5) (20 tubers of each cultivar)
4) pots (Ø=16 cm)
5) mixture of: soil and horticultural peat + Fertilizer PG Mix (14-16-18) +Micro, about the proportions of ingredients: one part of peat, two thirds of soil, 0.5-0.8 kg PG Mix × m^{-3} substrate
6) silicon carbide powder
7) latex powder-free gloves “Protect clinic” (Semperit Technische Producte Gesellschaft)
8) mortar and pestle
9) inoculum - infected tobacco tissue with two strains of PVY: PVY^{N-Wi} a relatively mild strain and the most popular in Poland, and PVY^{NTN} - a severe strain able to develop potato tuber necrotic ringspot disease (PTNRD) in many cultivars (Chrzanowska and Doroszewska, 1997)
10) PVY monoclonal cocktail antibody (Bioreba IgG, cat. No 112911, Bioreba Conjugate cat. No 112921)

Growing-on tests

1) insect-free growth chamber with natural lighting
2) sprouting room 22°C, 24-hours light and high humidity
3) progeny tubers: 60 tubers of each cultivar, 240 tubers of each standard cultivar
4) gibberellic acid GA_3 (Sigma, cat. No G 7645)
5) thiourea (CHEMPUR, cat. No 118345701)
6) propanol (CHEMPUR, cat. No 427515001)
7) distilled water
8) mixture of: soil, horticultural peat and Fertilizer PG Mix (14-16-18) with Micro, the proportions of ingredients: one third of peat, two thirds of soil, 0.5-0.8 kg PG Mix × m^{-3} substrate
9) containers filled with peat
10) pots (Ø=8 cm)
11) PVY monoclonal cocktail antibody (Bioreba IgG, cat. No 112911, Bioreba Conjugate cat. No 112921)
12) latex powder-free gloves "Protect clinic" (Semperit Technische Producte Gesellschaft)

ELISA (enzyme-linked immunosorbent assay)

1) 96-well micro-ELISA plates (MEDLAB 39-096f-OS)
2) PBS (Phosphate-buffered saline) (pH 7.4)

a)	NaCl	8.0 g	(POCH: Polish Chemicals Reagents, cat. No 794121116)
b)	KH_2PO_4	0.2 g	(POCH, cat. No 742020112)
c)	Na_2HPO_4 12 H_2O	2.9 g	(POCH, cat. No 799280115)
d)	KCl	0.2 g	(POCH, cat. No739740114)
e)	NaN_3	0.2 g	(CHEMPUR, cat. No 117927704)

Adjust pH with either NaOH or HCl (POCH, cat. No 575283115) and make up with distilled water to 1 l

3) PBS-Tween
   a) add 0.5-1ml Tween 20 per 1 l PBS (SIGMA, cat. No P-1379-1L)
4) Coating buffer (pH 9.6)
   a) Na_2CO_3 1.59 g (POCH, cat. No 810570113)
   b) $NaHCO_3$ 2.93 g (POCH, cat. No 810530115)
   c) NaN_3 0.20 g (CHEMPUR, cat. No 117927704)

Make up to 1 l with distilled water

5) Extraction buffer (pH 7.4)
   a) PBS-Tween (as above) 20 g
   b) Polyvinylpyrrolidone (PVP) 2 g (Sigma, cat. No PVP 40T)
6) Conjugate buffer
   a) The same as the Extraction buffer (see above)
7) Substrate buffer (pH 9.8)
   a) Diethanolamine 97 ml (Sigma, cat. No D8885)

b) Distilled water 600 ml
c) NaN_3 0.5 g (CHEMPUR, cat. No 117927704)

8) Phosphatase substrate
a) powder (Sigma, cat. No P4744)

PROCEDURE

Field experiments

1. In field experiments, potato seed tubers are planted in two replications with 30 tubers each. Spacing between plants is 40 cm and between rows 67.5 cm. Planting dates 20^{th} -30^{th}, April.
2. In each replicate, each cultivar is planted in two rows, with 15 plants per raw. On both sides of each cultivar, 15 PVY-infected tubers are planted as the source of the virus for infection (Fig. 1). These PVY-infected tubers are derived from the plants of susceptible potato cultivars, that were mechanically inoculated with PVY in greenhouse conditions in the previous year. Standard cultivars of known resistance levels (resistant, medium resistant and susceptible) to PVY are included in the experiment, e.g., cultivars Cekin (scored 5-6), Michalina (7) and Satina (5). The reaction of the tested cultivars is compared to that of the standards.

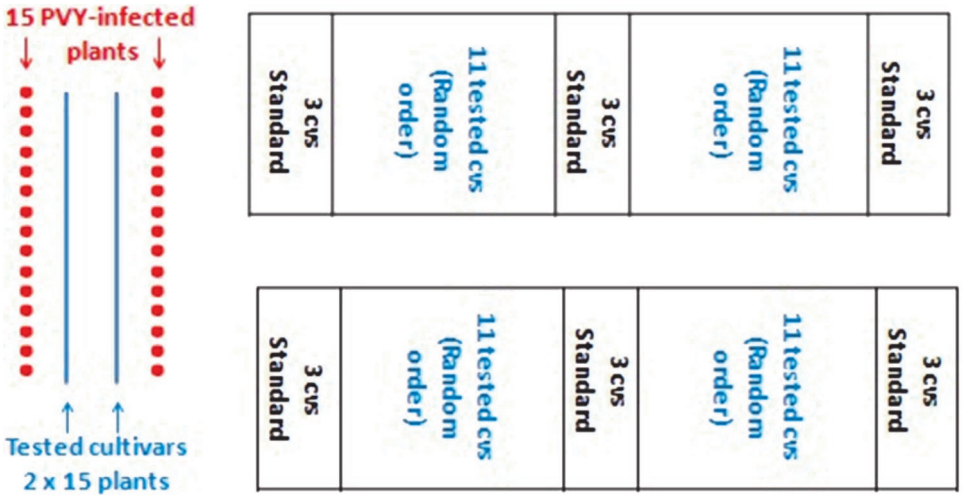

Fig. 1. The scheme of the field experiment for testing resistance to PVY. cvs: cultivars

3) From mid- to late- June field observations were made for abiotic and biotic abnormalities.
4) The progeny tubers are collected in autumn (one tuber from each plant), and growing-on testing is carried out in greenhouse conditions to estimate the number of plants infected with PVY in the field.

Growing-on tests

1) During autumn, after the harvest of the progeny tubers, growing-on tests are conducted in a greenhouse. The tubers just harvested in the autumn are in a physiological state of dormancy. In order to obtain plants at this time of the year, eyes with pieces of surrounding tuber tissue are cut out and soaked in a solution of gibberellic acid and thiourea to break down the dormancy period (Turska, 1971).
2) Solution:
3) 10 mg gibberellic acid dissolved in 0.5 ml propanol
4) 100 g thiourea dissolved in 10 l of water
5) The tuber pieces with eyes are kept in this solution for 15 min, then washed with tap water
6) Put into moist pit in boxes, which are placed in a sprouting room at 22° C, with 24h light and high humidity.
7) Sprouted eyes with tuber pieces (about 1.5 cm in size) are planted into pots Ø 8 filled with a mixture of soil and pit.
8) After 4 weeks, observation of symptoms is performed together with serological evaluation with ELISA. Monoclonal cocktail antibody that recognizes all known isolates of all groups/subgroups of PVY (Bioreba IgG, cat. No 112911, Bioreba Conjugate cat. No 112921) is used for detection.

Greenhouse experiment

1) For each tested and standard cultivar 10 plants are mechanically inoculated with each of the two PVY strains:
2) $PVY^{N\text{-}Wi}$ – a relatively mild strain and the most popular in Poland
3) PVY^{NTN} – a severe strain able to develop potato tuber necrotic ringspot disease (PTNRD) in many cultivars
4) The isolates are maintained in plants of tobacco cultivar Samsun in a insect-free growth chamber
5) The inoculum used is the infected tobacco tissue homogenized with mortar and pestle in the autoclaved distilled water
6) All plants of tested and standard cultivars are mechanically inoculated at the 6- to 10- leaf-stage. Before inoculation the leaves are sprinkled with silicon carbide powder and then an inoculum is applied with a sponge
7) After inoculation, each plant is rinsed with water to remove the excess inoculum from the leaves
8) Infection with PVY is assessed with ELISA three weeks post inoculation and the visual symptoms of PVY infection are described
9) PVY monoclonal cocktail antibody used for the ELISA assay are (Bioreba IgG, cat. No 112911, Bioreba Conjugate cat. No 112921)
10) Tubers from plants infected with PVY^{NTN} are collected and stored in room-temperature (20°C) condition for 6 weeks for visual observation of PTNRD symptoms.

ELISA (enzyme-linked immunosorbent assay) (acc. Syller, 2001)

1) Add 200 µl of γ-globulin diluted in coating buffer to each well of the plate
2) Incubate 2-4 h at 37°C
3) Remove γ-globulin solution and wash the plate carefully 3 times
4) Add 200 µl of plant extract diluted in extraction buffer to each well
5) Incubate overnight at 4°C
6) Remove plant extracts and wash the plate very carefully 3-4 times
7) Add 200 µl conjugate, diluted in conjugate buffer to each well
8) Incubation for 2-4 h at 37°C
9) Remove conjugate and wash the plate carefully 3 times
10) Add 200 µl of freshly prepared enzyme substrate solution (0.75-1.0 mg per 1 ml of substrate buffer)
11) Incubate at room temperature for 0.5 to 2 h
12) Read the absorbance values for each well at 405 nm with the ELISA reader (DYNATEX MRX II)

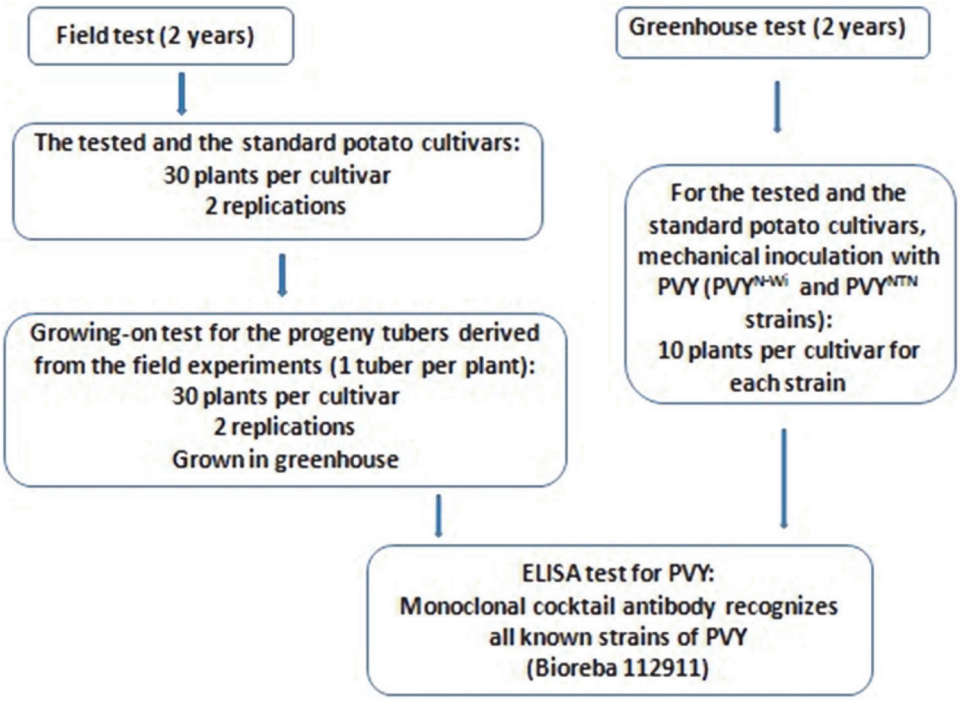

Fig. 2. The scheme of testing resistance to PVY in potato cultivars applied at IHAR-PIB, Młochów Research Center.

Data analysis

1) The level of infection of the tested cultivars is compared with that of the standard cultivars. The average infection rate of the standard cultivars is variable in years and depends on the infectious pressure of the virus in a given year. As shown in Table 1, depending on the year, the percentage of the infection rate of the standard cv. Michalina can range from 5.2 to 68.6%.

Table 1

The infection rate of the standard cultivars in growing–on tests in the greenhouse in different years

Cultivars	Resistance to PVY on a scale of 1-9*	Year				
		2012	2013	2014	2015	2016
Michalina	7	16.8	7.7	5.2	68.6	18.2
Cekin	5	78.9	28.8	36.6	93.1	57.3
Satina	5	nd	nd	nd	96.7	84.6

*Evaluation of resistance, where: 1 represents the least resistant, and 9 - the most resistant. nd: not determined.

2) To fit the percentages of PVY infection to normal distribution, a transformation of data is used according to Wójcik *et al.,* (1976) and Gabriel (1995):

$$y = log[230(1-p)^{-1} + 1]$$

where p = the rate of infection, as a decimal fraction (0-1).
To find significant differences between the mean y values of the tested cultivars and standards, the Tukey test is used.

3) Data analysis from the field experiment and laboratory test of obtained results makes it possible to classify the cultivars into four resistance groups on 1- 9 scale:
   a) highly resistant (with a resistance score of 8);
   b) resistant (with a value of 7);
   c) mid-resistant (with a value 5-6);
   d) susceptible (with a value of 3-4)
   e) The ”9” rating is definite after registration of the cultivar based on molecular tests, that validate the presence of a marker linked to the extreme resistance (ER) gene Ry_{sto} (Chrzanowska *et al.*, 2011).

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