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ASSESSMENT OF POTATO RESISTANCE TO *SYNCHYTRIUM ENDOBIOTICUM*

ABSTRACT

In Poland the Plant Breeding and Acclimatization Institute - National Research Institute (IHAR-PIB) is responsible for officially assessing the resistance of potato breeding lines and cultivars to *Synchytrium endobioticum*. In the official assessment of wart resistance the modified Glynne-Lemmerzahl method is used. A full cycle of assessment of the wart disease resistance requires 42 - 45 tubers per cultivar/breeding line. Forty two tubers are used in laboratory tests. To complete the laboratory tests, another 10 tubers are inoculated with winter sporangia of the fungus, using ring test. The final results are available after 3 years of investigation. If necessary, the full cycle of resistance tests to *S. endobioticum* can be performed during one year on 15 tubers in each of the 3 replications (45 tubers in total).

Molecular verification of potato genotypes resistance to pathotype 1(D1) is possible with the use of SCAR marker Nl25-1400. Nevertheless, an official phenotypical assessment of advanced breeding lines, as the final verification of their resistance, is required.

Key words: potato wart disease, resistance, molecular markers

INTRODUCTION

Synchytrium endobioticum is considered the most important quarantine pathogen of the cultivated potato in almost all countries were potatoes are grown. As chemical control of *S. endobioticum* in field conditions is not possible, the only strategy to restrict the disease is to cultivate resistant cultivars. The wart resistance is a very important trait in new potato cultivars. In Poland, from 1953 until 2014, registration and cultivation of potato cultivars susceptible to *S. endobioticum* was completely prohibited. The Glynne-Lemmerzahl method was chosen in Poland as the most reliable in distinguishing wart resistant and wart sus-

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ceptible potato clones/cultivars, and it is applied within the scope of official variety testing as well as resistance assessment of advanced potato breeding lines. The Glynne-Lemmerzahl method requires the availability of fresh warts with summer sporangia as inoculum. Although, screening for resistance to *S. endobioticum* is labor-intensive and time-consuming, still remains the method of choice.

Thanks to the availability of effective source(s) of resistance against pathotype 1 (D1), the conventional breeding programs were successful in controlling potato wart disease through the development of resistant cultivars early in the twentieth century. The resistance of the majority of these cultivars was probably based on the presence of gene *Sen1*. This gene was mapped to potato chromosome XI, near DNA marker Nl25 (Hehl *et al.*, 1999). This marker can be successfully used to identify potato genotypes/cultivars resistant to pathotype 1 (D1), which possess the *Sen1* gene (Gebhardt *et al.*, 2006). Potato cultivars resistant to virulent pathotypes of *S. endobioticum* occur very rarely and their resistance is of polygenic determination mainly. A new, highly effective source of monogenic resistance against a broad spectrum of *S. endobioticum* pathotypes has recently been identified at IHAR-PIB, Młochów Research Center, but the work on its introduction into tetraploid potato breeding lines still is in progress (J. Plich – personal communication). To date, there is no effective system of marker assisted selection (MAS) of potato genotypes resistant to virulent pathotypes of potato wart fungus.

MATERIALS AND REAGENTS

Phenotypical assessment

1) Vaseline (Pharma cosmetic)
2) Miedzian® Extra 350SC
3) Distilled water
4) $CaCl_2$ (Alchem grupa Sp. Z o.o.)
5) Sterilized river sand
6) Tubers of cv. Evora, cv. Spunta (HZPC Holland B.V.) and cv. Irga (Pomorsko- Mazurska Hodowla Ziemniaka Spółka z o.o.) for multiplication of fresh warts
7) Plastic boxes (PZT PRYMAT Sp.j.)

Marker Assisted Selection

1) PCR reagents
   a) DreamTaq PCR Master Mix (2X) (ThermoFisher Scientific, cat. No K1072)
   b) Distilled water
   c) Primers: Nl25f: TATTGTTAATCGTTACTCCCTC; Nl25r AGAGTCGTTTTACCGACTCC
   d) Template DNA isolated from tested potato cultivar/clone

2) Electrophoresis reagents:
   a) Agarose (Sigma-Aldrich, cat. No A9539)
   b) TBE buffer
   c) Ethidium Bromide (Sigma-Aldrich, BioReagents for molecular biology, cat. No E1510-10ML)
   d) GeneRuler 100 bp Plus DNA Ladder ready to use (ThermoFisher Scientific, cat. No SM0323),

EQUIPMENT

Phenotypical assessment

1) Climatic chambers (POL-EKO-APARATURA Sp.j.)
2) Refrigerators (Liebherr)
3) Stereoscopic microscope(Motic®)
4) Light microscope (Delta Optical-GSO)
5) Autoclave (De Ville Biotechnology)
6) Analysette 3 Pro (FRITSCH GmbH)
7) Centrifuge (MPW MED. INSTRUMENTS Sp. Pracy)

Marker Assisted Selection

1) Thermocycler for performing PCR (Eppendorf, Mastercycler epgradient)
2) Equipment for agarose gel electrophoresis (BioRad, PowerPac Basic, cat. No 1645050)
3) Wide Mini-Sub Cell GT System, cat. No 170-4468)
4) UV lighter for visualization of obtained products

PROCEDURE

Phenotypical assessment

A full cycle of resistance assessment to wart disease requires 42 - 45 tubers per one genotype in laboratory tests. The laboratory tests begin in late autumn and continue until late spring. Before testing the tubers are stored in low temperature (2 - 4ºC) to inhibit the development of sprouts. Each tuber is marked by waterproof marker to eliminate possible mistakes.

For breeding lines a full cycle of tests for *S. endobioticum* requires 3 years:

1) The first year of testing - 2 tubers per line (the same line will be tested again in the second year, if it has positively passed the assessment tests for resistance to *S. endobioticum*),
2) The second year of testing - 10 tubers per line (the same line will be tested again in the third year, if it positively passed the assessment of resistance to *S. endobioticum*),

3) The third year of testing - 30 tubers per breeding line.

For foreign cultivars a full cycle of tests to *S. endobioticum* requires 1 year, when three independent laboratory tests are performed (in total 45 tubers) in the winter/spring season.

For domestic breeding materials, additional 10 tubers per each resistant breeding line are tested with winter sporangia, using the ring test (Przetakiewicz, 2016).

Assessment of the resistance to *S. endobioticum* in a mass test is carried out, according to the Glynne-Lemmerzahl method as described in the EPPO standard PM 7/28 (Anon., 2004) with modifications. The modifications are described below.

Glynne-Lemmerzahl method for assessment of resistance to S. endobioticum

1) Tuber preparation

The tubers are washed in warm tap water, dried and incubated at room temperature in darkness or dim light to promote sprouting of up to 0.5-2 mm sprout length. Then, on whole tubers, a warm vaseline ring is made around the sprout using a syringe without a needle. The tubers are then cooled at 4°C for a few minutes or overnight so that the vaseline solidifies, and water tightness of the ring is subsequently checked.

2) Inoculation and incubation

At inoculation, the tubers are placed into plastic boxes lined with irrigation mats, and the vaseline rings are filled with distilled water. Fresh warts are cut into pieces and placed directly into the rings. The tubers are incubated at 10°C for 48 h. After 24 h, the warts are moved from one ring to another one within a box to align the infection level. After this period, the warts and rings are removed and the tubers are treated with the fungicide Miedzian® Extra 350SC (copper oxychloride). Subsequently, the tubers are incubated in boxes covered with a lid at 12°C/22°C (alternately 12 h/12 h), and sprayed regularly with water. The tubers are incubated without any peat or sand covering them.

3) Scoring

After two weeks of incubation, disease symptoms are scored for the first time, according to the modified classification scheme established by Langerfeld and Stachewicz (1994), using a stereo microscope at 40-80X magnification (Table 1, Photo 1). Reaction types 1 and 2 are included in the resistance group R1. The tubers scored at rank 3, 4 and 5 are incubated in plastic boxes for an additional 2 weeks. The tubers are covered with a thin layer of moist sand and incubated at 16°C in the dark. This cover mixture is moistened with water every second or third day during the incubation period. The final reaction of the sprouts is evaluated after 4 weeks of incubation (Photo 2). Before scoring, the sprouts are carefully cleaned. Reaction type 3 corresponds to the resistance group R2. The susceptible reactions of type 4 correspond to S1 while those of type 5 correspond to S2. Since differentiation between the reaction types 3 and 4 is often difficult, thin tissue sections are prepared and inspected under the microscope for the presence of winter sporangia. If winter sporangia are present, the sprouts are classified into reaction type 4. Cultivars show-

ing resistant and susceptible reaction types (on different eye fields/tubers) at the same time are classified as susceptible (S1 or S2).

Tabele 1

Classification of reaction types according to Langerfeld and Stachewicz (1994) with modifications

Reaction type	Group	Classification	Description
1	R1	Extremely resistant	Early defence necrosis; no visible sorus formation
2	R1	Resistant	Late defence necrosis; sorus formation partially visible, sori immature or necrotic before maturity
3	R2	Weakly resistant	Very late defence necrosis; single ripe sori or sorus fields developed, but completely surrounded by necrosis; up to five non-necrotic sori, clear necrosis in other zones of the same tuber piece, high attack of the control cultivar is essential. No tumours or resting sporangia.
4	S1	Slightly susceptible	Scattered infections; sori or sorus fields non-necrotic, few in number; late necrosis can be present on other infection sites on the sprout; the sprout can be slightly malformed (thickened). Resting sporangia are presented.
5	S2	Extremely susceptible	Dense infection fields, numerous ripe non-necrotic sori and sorus fields, fields with dense non-necrotic infection sites, predominant tumour formation.

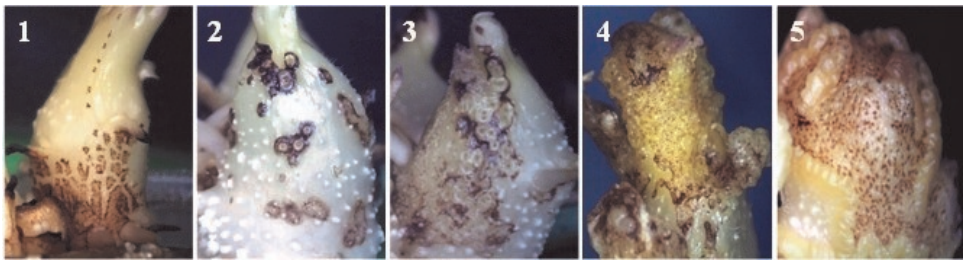

Photo 1. Symptoms of the reaction types 1 to 5 (from left to right) according to the scoring method used at the IHAR-PIB 14 days after inoculation

Photo 2. Symptoms of the reaction types 3 to 5 (from left to right) according to the scoring method used at the IHAR-PIB 28 days after inoculation

Marker assisted selection (MAS)

At IHAR-PIB, Młochów Research Center, the first molecular verification of wart resistance of breeding lines is based on the use of SCAR marker Nl25-1400 (the primers sequences: Nl25f: TATTGTTAATCGTTACTCCCTC; Nl25r AGAGTCGTTTTACCGACTCC (according to Gebhardt *et al.*, 2006)).

The reaction is performed in standard PCR reactions (20 µl of reaction mixture containing: DreamTaq PCR MasterMix 2x ThermoScientific (10 µl), primers (2 µl of 10 µM solution), template DNA (1 ng/µl), water (to 20 µl)) with the use of thermocycler program: 93°C – 3 min, 35× (93°C – 2 min, 93°C - 45s, 72°C – 1.5 min), 72°C – 10 min.

The PCR products are separated in a 1% agarose gel and stained with ethidium bromide.

An example of PCR products obtained for seven resistant cultivars (Jasia, Wawrzyn, Zeus, Neptun, Innovator, Robijn and Palladia) and five susceptible cultivars (Deodara, Tomensa, Bintje, Sarpo Mira and Eersteling) is shown on Photo 3. This molecular test confirms results of an official phenotypic evaluation of potato wart resistance of these cultivars. The marker Nl25-1400 is present in all resistant cultivars and is absent in all susceptible ones. Although the use of marker Nl25-1400 was fruitless in screening for resistance among wild *Solanum* species (Khiutti *et al.*, 2012), it is regarded as a useful tool for selecting potato breeding lines which possess the *Sen1* gene (Gebhardt *et al.*, 2006). The *Sen1* gene is located about 6 cM from marker Nl25 and recombinants between the marker and the gene can be observed in segregating populations. Therefore, phenotypical assessment of advanced breeding lines, as the final verification of their wart resistance, is highly recommended.

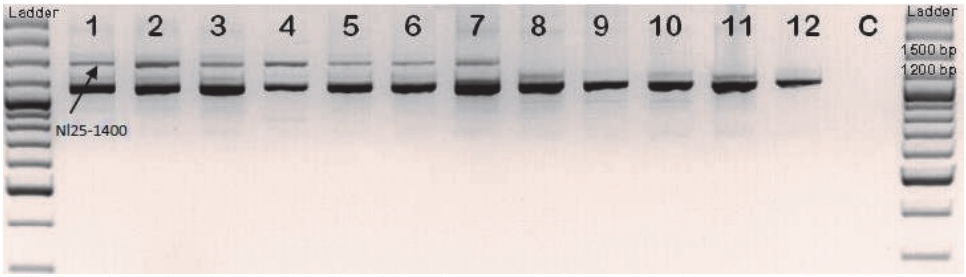

Photo 3. Electrophoretic separation of products of Nl25 marker in resistant cultivars (1 – Jasia, 2 – Wawrzyn, 3 – Zeus, 4 – Neptun, 5 – Innovator, 6 – Robijn, 7 – Palladia) and susceptible cultivars (8 – Deodara, 9 – Tomensa, 10 – Bintje, 11 – Sarpo Mira, 12 – Eersteling). C – control. Product Nl-1200 is present in all tested cultivars, while product Nl-1400 is present only in resistant cultivars.

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