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EVALUATION OF POTATO CULTIVARS AND BREEDING LINES FOR RESISTANCE TO *GLOBODERA ROSTOCHIENSIS* AND *GLOBODERA PALLIDA*

ABSTRACT

Nematodes are among the most important agents affecting potato crops. Heavy infestations by *Globodera rostochiensis* and *G. pallida* can cause significant yield losses and limit the choice of potato cultivars that can be grown effectively (Oerke, 2006). Breeding of new potato cultivar resistant to *G. rostochiensis* and *G. pallida* is a long-term process. It is preceded by screening of potato breeding lines for resistance in repeated biotests, which seems to be the most effective and safest method of protection. Evaluation of nematode resistance is an important element of characterization of breeding lines and new cultivars. Resistance to *Globodera* spp. is evaluated in biological tests. The use of DNA markers for detecting nematode resistance genes may be an alternative approach to phenotypic evaluation of resistance degree of potato plants (Jena and Mackill, 2008). The goal of this report is focused on a description of resistance assessment procedure of breeding lines and varieties of potato to PCN and on comparison of biological and molecular methods of resistance evaluation. Presented information is addressed to both breeders and Polish inspection services.

Key words: golden nematodes, potato cyst nematodes (PCN), methods, resistance sources

INTRODUCTION

Potato cyst nematodes (PCN) are important quarantine pests of potatoes (Oerke, 2006). They damage the roots causing poor growth, wilting during periods of water stress and early senescence (van Riel and Mulder, 1998). This can mean up to 80% loss in tuber yield (Turner and Evans, 1998). There are two closely related species of PCN: *G. rostochiensis* (Stone, 1973a) and *Globodera pallida* (Wollenweber, 1923; Stone, 1973b). International scheme of potato genotype reaction to PCN separated these species

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of *Globodera* on five pathotypes of *Globodera rostochiensis* (Ro1-Ro5) and three pathotypes of *G. pallida* (Pa1-Pa3) (Kort *et al.*, 1977).

The potato cyst nematodes are pests of which chemical control involves unspecific and harmful pesticides. Cultivation of resistant varieties is the most effective and environmentally the safest method of protection of potato. Moreover cultivation of resistant cultivars seems to be an important strategy preventing fields from infestation by introduced foreign pathotypes.

Historically, the resistance against nematodes was introduced to potato cultivars from *Solanum tuberosum* ssp. *andigena*, *S. vernei* and *S. spegazzini* (Ross, 1979). Many other wild species of *Solanum* sp. were also found to be sources of PCN resistance genes (Ruiz de Galarreta *et al.,* 1998; Castelli *et al.*, 2003; Caromel *et al.,* 2005). Resistance to *Globodera* spp., which is conferred by already localized genes, could be expressed as partial or nearly full extreme. The most frequent source of resistance against nematodes among currently grown cultivars is the *H1* gene, conferring resistance to pathotypes Ro1 and Ro4 of *Globodera rostochiensis* (Milczarek *et al.*, 2011; Asano *et al.*, 2012).

Therefore evaluation of nematode resistance is an important element of characterization of breeding lines and new varieties. Most available assessment method of potato resistance to all pathotypes of *Globodera* is official scheme according to EPPO procedure PM 3/68(1). By including a standard or reference population of potato cyst nematodes and by comparing the nematode multiplication rate on a tested cultivar with that on a reference cultivar, it is possible to relate the new cultivar to internationally recognized level of resistance.

Unlike the phenotypic assessment of resistance to nematodes, MAS (marker-assisted selection) can be applied at early stages of selection. Two markers of the *H1* gene (TG689 and 57R) were previously applied and found to be useful in the selection of Polish breeding material resistant to pathotype Ro1 of *G. rostochiensis* (Galek *et al.*, 2011; Milczarek *et al.*, 2014).

MATERIALS

1) GenElute Genomic DNA Miniprep Kit (Sigma, cat. No G2N350)
2) Polymerase *Taq* (Novazym, cat. No N1000-10)
3) dNTPs (Invitrogen, cat. No 18427088)
4) Agarose (Sigma, cat. No A9539-500G)
5) Distillated water
6) Liquid nitrogen
7) Ethanol 99,8% (HPLC) (Avantor Performance Materials Poland S.A., cat. No 396483150)
8) Potato tubers
9) Universal soil (KronenErde)
10) Sterilized river sand
11) PCN (Collection of: IHAR-PIB, JKI, SASA)

EQUIPMENT

1) Fuchs-Rosenthal chamber (Sigma Aldrich, cat. No 719805)
2) Stereoscopic microscope (Bresser Science ETD-101, cat. No 1019077)
3) Mastercycler EpGradient S (Eppendorf, cat. No 5341-007966)
4) Centrifuge (Eppendorf, cat. No 5804R)
5) PowerPac Basic Bio Rad (Biorad, cat. No 1645050)

PROCEDURE

Bio-test procedure

1) Bio-test of resistance is conduct in quarantine condition in greenhouse's separated chambers (for particular pathotype).
2) Bio-tests are repeated twice or three times in combinations of potato cyst nematode population and tested potato breeding lines.
3) The tests are confirmed by additional bio-test performed in next year only for cultivars scored > 5 in 1-9 scale (Table 1).

Inoculum preparation

1) Inoculum is prepared from individual reference populations of PCN (Ro1-Ecosee; Ro5-Harmerz; Pa1-Scottish and Pa3-Chavornay) and local populations of *Globodera* identified with the aid of differential genotypes of potato according to Kort procedure (Kort *et al.*, 1977).
2) Nematode inoculum (Pi) consists of 5 to 10 infective eggs and juveniles per ml of soil. Density of inoculum is estimated with the aid of Fuchs-Rosenthal chamber (CE IVD 98/79 WE) by counting of mean number of juveniles and eggs in cyst (on sample of 100 cysts from 10 grams of soil). It is recommended that the number of nematodes to be used for inoculation of one ml of soil was determined in hatching experiments (Malec, 1980).
3) Potato tubers may be inoculated by cysts or by eggs and juveniles in a suspension, with viability at least 70%.
4) Test of cysts viability is carry out on potato tubers of variety Desiree - highly susceptible to all *Globodera* pathotypes. Tubers of potato are infected by cysts of particular pathotype of nematode in known concentration of inoculum (5 eggs × ml^{-1} of soil).
5) After three month of growth cysts from outer surfaces of soil clod taken out of the pot are counted. Comparison of contents of newly created cysts and cysts used to infection estimate percent of its viability.

Performance of the test

1) 50 eye plugs cut out from potato tubers of tested potato cultivar and 10 eye plugs from susceptible control cultivar (e.g. cvs: Desiree, Zebra or Alicja) are planted each in a pot containing at least one

liter of universal soil (KronenErde) mix with sterilized river sand in a ratio 3:1.

2) Plants are watered adequately for about 3 - 4 months.
3) The number of new developed cysts on roots of tested potato plants are counted on five outer surfaces of soil clod (four side and one bottom surfaces) under the light microscope at magnitude 10x, separately for each pot.
4) Relative susceptibility of the tested potato cultivar to the standard susceptible control is calculated according to the formula.

Pf of test variety/Pf of standard susceptible control cultivar × 100%,
where Pf (final population) specifies number of cysts on roots of potato plant.

5) Standard scoring notation is determined acc. to Table 1.

Table 1

Scoring of potato resistance to PCN based on the relative susceptibility in comparison to susceptible standard cultivar

Relative susceptibility (%)	Resistance score
< 1	9* (very high)
1.1 – 3.0	8 (high to very high)
3.1 – 5.0	7 (high)
5.1 – 10.0	6 (moderate to high)
10.1 – 15.0	5 (moderate)
1.1 – 25.0	4 (low to moderate)
25.1 – 50.0	3 (low)
50.1 – 100.0	2 (very low to low)
> 100.0	1 (very low)

* 1 – 9 scale, where 9 means the most resistant

The use of diagnostic PCR marker assay for presence of the H1 gene

1) Total genomic DNA is extracted from fresh or frozen leaves of potato varieties or breeding clones using the GenElute Plant Genomic DNA Miniprep Kit (Sigma, Germany).
2) 20 µl of PCR mixture contains: 40 ng x μl^{-1} genomic DNA, 0.2 mM dNTPs, 0.4 µM of each primer for TG689 or 57R, 0.2 mM of each primer for BCH (the marker of conserved regions of beta-carotene hydroxylase (Brown *et al.* 2006) added as an internal control for successful PCR in the reaction with marker TG689) and 1 U *Taq* DNA Polymerase in the reaction buffer provided by the manufacturer (Novazym, Poland).
3) The PCR temperature parameters, primer sequences for amplifying the markers and products size are shown in Table 2.
4) Amplified markers are detected in 1% agarose gel.

Table 2

The PCR temperature parameters and primers sequences for amplifying markers of *H1* gene

Marker	Product size [bp][1]	Primer sequence (5'-3')	Temperature parameters for PCR	Reference
TG689	141 (R)	FTAA AACTCTTGGTTATAGCCTAT CAATAGAATGTGTTGTTTCACCAA	→ 95ºC for 3 min initially Then 35 cycles of: → 94ºC, 20 s; → 55ºC, 20 s; → 72ºC, 30 s → 72ºC for 3 min finally	Walter De Jong (pers. comm.)
BCH2	290 (R, S)	CATGACATAGTTTGAATTTTGAGTC CGTTTGGCGCTGCCGTAAGTT		
57R	450 (R) 1500 (R, S)	TGCCTGCCTCTCCGATTTCT GGTTCAGCAAAAGCAAGGACGTG	→ 95ºC for 3 min initially, Then 35 cycles of: → 94ºC, 30 s; → 63ºC, 15 s; → 72ºC, 1 min → 72ºC for 3 min finally	Finkers-Tomczak *et al.,* 2011

[1] Size of the amplification products in resistant (R) and susceptible (S) plants
[2] Internal control for successful PCR in the reaction with marker TG689

The cost of using molecular markers for evaluation of clones having the *H1* gene (resistant to Ro1, Ro4) is two or three times lower than the cost of the phenotypic evaluation (Milczarek *et al.*, 2014). Furthermore the optimizing the MAS procedure for more markers could allow the simultaneous detection of more genes in the future.

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