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IMPROVEMENT LEVEL OF NEMATODE RESISTANCE IN POTATO FACING CHANGES IN POPULATION OF *GLOBODERA* SPP

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ABSTRACT

Both *Globodera rostochiensis* and *G. pallida* are considered the most economically important pests affecting potato production. The spread of *G. pallida* in nematode populations in Europe have been observed in recent years. Annual regional surveys conduct in Poland in the years 2009-2013 show the presence of pathotype Ro1 of *G. rostochiensis* in central and northern Poland and a new pathotype Ro5 was detected in three separated provinces: małopolskie, lubuskie and lubelskie. The abundance of cysts in those locations suggests that there is a threat of further spread of Ro5 in the country. Consequently, only the growing resistant potato varieties should keep nematode population below damaging level. The assessment of the set of commonly grown Polish potato varieties for the resistance to nematodes shows insufficient amount of varieties resistant to Ro5 (4 out of 49 tested). The more resistant varieties may be developed by using parental lines in commercial breeding programs. Within such prebreeding program focused on multiple nematode resistances, the parental lines were obtained, which have unique resistances to all pathotypes of *Globodera* spp.

Keywords: PCN, *Globodera rostochiensis*, potato breeding, multiple resistance.

INTRODUCTION

Potato cyst nematode (PCN): *G. rostochiensis* (Wollenweber, 1923), the golden cysts nematode and *G. pallida* (Stone, 1973), the white cyst nematode, belong to quarantine pests attacking potato. The heavy infection with *Globodera* spp. causes the decrease of tubers yield up to 50% (Nicol *et al*., 2011). Both species are subject to strict regulations and quarantines in many countries, even where they have not already occurred. Nonetheless, today *G. rostochiensis* is widely distributed in Europe. The first report of its occurrence in Poland comes from 19th century. Fedorko (1987) suggested that it was territory of northern Poland and the pest spread towards south-west. In Poland, the pathotype Ro1 of *G. rostochiensis* is most known, although European populations are divided into five groups of pathotypes Ro1, Ro2, Ro3, Ro4, Ro5 based on their different pathogenic characteristics (Kort *et al*., 1977). The possibility of emergence other pathotypes of *Globodera* spp. on Polish potato fields significantly increases due to easy transfer of potato tubers across European Union countries. The nematodes pose a real threat for potato production, because of fast distribution on a new territory and long-term survival of cysts in soil.

The eradication of cysts is troublesome and ineffective and as so far planting of resistant potato varieties is the best way to limit the pest population in fields. The most frequently used source of resistance against nematodes is *Solanum tuberosum* ssp. *andigena*, the source of *H1* gene, which confers resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis* (Dellaert and Hoekstra 1987). This gene is present in many currently grown potato varieties (Milczarek *et al*., 2011; Asano *et al*., 2012). However, the wide spread cultivation of varieties resistant to Ro1 can affect the multiplication of other *Globodera* pathotypes as it was observed in England and Wales (Minnis *et al*., 2002). Nowadays, the multiresistant varieties are scarce and the use of different resistant varieties in the succession may prevent the further replacement of species or pathotypes of *Globodera*. Therefore, the cultivation of potato varieties with multiple resistance for many pathotypes of *Globodera* ssp. may influence the growth of entire PCN population.

The main goal of our study was to assess the distribution of PCN in Poland facing the *Globodera* spp. changes observed in Europe. Furthermore, the set of commonly grown Polish potato varieties was screened for resistance to detected pathotypes of nematodes. This evaluation of resistances to nematodes in currently available set of cultivars is supplemented with data from assessments of resistance present in breeding lines to show, what improvement in resistance might be achieved. These lines are being developed within the frame of parental lines breeding aimed at obtaining potato clones having multiple resistances to various pathotypes of *Globodera* spp.

MATERIALS AND METHODS

Collection and processing of soil samples: After harvest of potato tubers, 10 kg of soil samples were collected from the fields by inspectors of State Plant Health and Seed Inspection Service (PIORIN). The samples delivered to the laboratory were dried for a several days to become in coherent and mixed thoroughly on set of 6 sieves. In next step, cysts were extracted using the flotation method (Spears, 1968) with an automatic cyst extractor according to Seinhorst (1962). In the years 2009-2013, 65 soil samples were analyzed.

Species and pathotypes identification: To distinguish *G. rostochiensis* from *G. pallida*, multiplex PCR analysis was performed according to Fullaondo *et al*. (1999). DNA from mix of 30 cysts per sample was extracted with DNeasy Blood and Tissue KIT (Qiagen). Gel analyses were conducted by capillary electrophoresis with a QIAxcel Advanced System (Qiagen).

The pathotype determination of *G. rostochiensis* was done according to international PCN pathotype scheme proposed by Kort *et al*. (1977). In 15 samples an insufficient numbers of cysts were found. These cysts were multiplied on susceptible potato variety Desiree twice or three times. Tests were conducted in controlled glasshouse conditions with temperature 16 - 26°C, average humidity of 65 % and photoperiod 16/8 h day/night within 2.5-3 months. Tests for each sample were performed in three replications.

Plant materials: The development of potato parental lines with multiple nematode resistance was carried out in Młochów Research Center and started by the crossing program involving varieties Sante (resistant to Ro1, 2, 3 and 4), Sissi (resistant to Ro1 and 5), Palladia and Dorett (both resistant to Ro1-5 and Pa2/3) (www.europotato.org), and the breeding clone 07-VIII-27 resistant to Ro1-5 and Pa2. Fifty progeny genotypes were tested for resistance to all pathotypes of *Globodera* spp. Simultaneously, a set of 49 commonly grown Polish potato varieties was tested for resistance to pathotypes Ro1 and Ro5.

Test for resistance to nematodes: Bio-tests were conducted with the used of reference pathotypes of PCN recommended by EPPO standard (PM 3/68): Ro1 – Ecosee and Ro5 – Harmerz. Bio-tests were performed in one liter pots of soil containing nematode cysts of each pathotype of *Globodera* spp. separately. Concentration of nematode inokulum consists 10 eggs/ml of soil. The assessments were performed in two seasons. Each combination of PCN pathotype and tested potato variety was tested in 27 replicates and each combination of PCN pathotype and tested progeny clone was tested in 15 replicates. The relative susceptibility of tested varieties and breeding lines was calculated according to the formula:

(Pf of tested sample)/(Pf of susceptible standard variety) × 100%

where:

Pf – the mean number of cysts determined by counting all cysts from all replicates; cv. Desirée was a susceptible standard.

The values of relative susceptibility were converted into a 9-grade scale, where score 9 indicates the highest level of resistance according to the EU Council Directive 2007/33/EC. The potato clone or variety was regarded as resistant, when the score was higher than 5 (EPPO, 2006).

RESULTS AND DISCUSSION

Species and pathotype identification: In all tested samples, the multiplex PCR analysis identified cysts of *G. rostochiensis*. Pathotype identification was evaluated by Bio-test, in which the nematode's ability to multiply on a number of potato genotypes expressing specific resistances was tested. According to International PCN pathotype scheme recommended by Kort *et al*. (1977), five pathotypes of *G. rostochiensis* are numbered Ro1-Ro5 and three of *G. pallida* Pa1-Pa3. In this scheme different clones with related plant resistance codes (Ro1,4; Ro1,2; Ro1,2,3; Ro1,2,3,4; Ro1,2,3,4,5; Pa1; Pa1,2; Pa1,2,3) are presented. They are still in use in the description of resistance of commercial potato cultivars to PCN (Grubisic *et al*., 2007). The results of pathotype identification are presented in Table 1. The highest infestation by the pathotype Ro1 was observed in northern and east Poland and in two provinces of southern part of the country. As it was previously reported (Przetakiewicz, 2013) pathotype Ro5 appeared in three separated provinces: małopolskie, lubuskie and lubelskie.

Resistance to nematodes: Almost all tested potato varieties were highly resistant to Ro1. But only four were resistant to Ro5. The level of resistance to Ro1 and Ro5 in tested potato varieties is shown in Table 2.

All tested breeding lines were highly resistant to Ro1 and 49 of them were resistant to Ro3. The resistance to Ro2 and Ro4 was observed in 47 breeding lines, whereas 41 ones were resistant to Ro5. The resistance to pathotypes Pa2 and Pa3 of *G. pallida* were found in 39 and 41 breeding lines respectively. Although the resistance to Pa1 was not determined for parents used in crosses, it appeared in 28 genotypes from all progenies. Twenty three clones were resistant to all pathotypes of *Globodera* spp. and 12 out of them were highly resistant (Table 3).

European populations of *Globodera* spp. are divided into eight groups of pathotypes based on their differential pathogenic characteristics (Kort *et al*., 1977).

The pathotype Ro1 of *G. rostochiensis* was detected on potato field in Poland in 1946 for the first time (Jasińska, 1955). Thereafter annual national surveys conducted in every province of the country confirmed the presence of Ro1 in almost all new outbreaks of the disease (PIORIN, unpublished results). The highest infestation by the pathotype Ro1 was observed in north and east of Poland and in two provinces of southern part of the country. As it was previously reported (Przetakiewicz, 2013), a new pathotype Ro5 appeared in three separated provinces.

The possibility of emergence foreign pathotypes of *Globodera* spp. significantly increases due to easy transfer of potato tubers across European Union countries. For example, cysts of *G. pallida* were identified in potatoes imported to Poland from Cyprus (Karnkowski *et al*., 2012a). Although *G. pallida* was not observed in our research, it had been identified in one field in province opolskie by Karnkowski *et al*. (2012b). The local outbreaks of *G. pallida* were also observed in Czech Republic (Zouhar *et al*., 2003), Croatia (EPPO, 2004), Ukraine (Pylypenko *et al*., 2005), Serbia (Radivojević *et al*., 2006), Finland (EPPO, 2012), Slovenia (Širca *et al*., 2012), Denmark (EPPO, 2013) and Bosnia and Herzegovina (Nježić *et al*., 2014). So far *G. pallida* was not found in Lithuania, Latvia, Estonia, Belarus and the European part of Russia (Limantseva *et al*., 2014). In other European countries, both species were reported. However, the precise information about the spread of each pathotype of PCN is not available.

Table 1. Location of infested fields and results of pathotype identification (2009 – 2013).

Province	Number of tested samples	Detected pathotype	Mean no of cysts/100g of soil
kujawsko-pomorskie	6	Ro1	1.2
Lubelskie	11	Ro1	2.3
	3	Ro5	3.3
Lubuskie	1	Ro1	2.0
	1	Ro5	5.7
Łódzkie	2	Ro1	1.2
Małopolskie	2	Ro1	2.1
	1	Ro5	3.5
Mazowieckie	9	Ro1	1.2
Opolskie	2	Ro1	1.4
podkarpackie	2	Ro1	2.2
Podlaskie	1	Ro1	2.6
Pomorskie	8	Ro1	1.8
świętokrzyskie	4	Ro1	1.2
warmińsko-mazurskie	8	Ro1	1.3
wielkopolskie	4	Ro1	1.3

Table 2. Resistance of tested potato varieties to *G. rostochiensis* pathotype Ro1 and Ro5.

Resistance	Resistance score		Variety
	Ro1	Ro5	
Resistant to Ro1 and Ro5	9	8	Etiuda
	9	7	Syrena
	9	6	Promyk, Rumpel
Resistant to Ro1, susceptible to Ro5	9	4	Drop , Skawa,
	9	3	Etola, Eugenia, Jasia, Jubilat, Pasat
	9	2	Bogatka, Boryna, Bosman, Bryza, Bursztyn, Cedron, Czapla, Finezja,Gustaw, Gwiazda, Harpun, Hubal,Ignacy, Kaszub, Malaga, Madea, Oberon, Oman, Owacja
	9	1	Ametyst, Cekin, Cyprian, Denar, Flaming, Gandawa, Gawin, Ibis, Igor, Irga, Jutrzenka, Legenda, Niagara, Stasia
Susceptible to Ro1 and Ro5	1	1	Adam, Glada, Ikar, Irys, Ślęza

Table 3. Resistance of 12 clones highly resistant to all pathotypes of *Globodera* spp.

Cross	Clone	*G. rostochiensis*					*G. pallida*		
		Ro1	Ro2	Ro3	Ro4	Ro5	Pa1	Pa2	Pa3
Sante × Palladia	10-VIII-63	9	9	9	9	9	8	9	9
Sante × Dorett	10-VIII-98	9	9	9	9	9	8	9	9
	10-VIII-122	9	9	9	9	9	9	9	8
	10-VIII-130	9	9	9	9	9	8	9	8

	10-VIII-416	9	9	9	9	9	9	9	9
	10-VIII-421	9	9	9	9	9	8	9	9
Dorett × Palladia	10-VIII-143	9	9	9	9	9	9	9	9
	10-VIII-151	9	9	9	9	9	9	9	9
	10-VIII-211	9	9	9	9	9	9	9	9
	10-VIII-437	9	9	9	9	9	8	9	9
Dorett × 07-VIII-27	11-VIII-74	9	9	9	9	9	8	9	9
	11-VIII-77	9	9	9	9	9	9	9	9

Soil sampling provides information about the nematode pathotypes existed in the fields and allows selecting for growing potato varieties with proper resistance. The providing such information should be a part of an integrated protection program planned for potato growers. The varieties Etiuda and Syrena (highly resistant to Ro5) are recommended to grow in fields infested with pathotype Ro5.

Considering the limited availability of resistant varieties and the possibility of introduction of new pathotypes of *Globodera* spp., the breeding of resistant varieties seems to be an important strategy preventing fields from infestation by foreign pathotypes. The result of parental line breeding program focused on multiple resistance to nematodes was obtaining the set of clones very highly resistant to all pathotypes of *Globodera* spp.

In conclusion, this study provided an insight into changes in population of *Globodera* spp. observed in Poland and showed the lack of potato varieties resistant to pathotype Ro5 on the Polish market, which indicate the need of breeding new varieties with multiple nematode resistances. In the commercial breeding programs, this may be realized by using the presented clones with the unique set of resistances as the parental lines.

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REFERENCES

Asano, K., A. Kobayashi, S. Tsuda, M. Nishinaka, and S. Tamiya (2012). DNA-marker assisted evaluation of potato genotypes for potential resistance to potato cyst nematode pathotypes not yet invading into Japan. Breed Sci. 62(2): 142-150.

EPPO Bulletin (2006). Testing of potato varieties to assess resistance to *Globodera rostochiensis* and *Globodera pallida*. 419-420.

EPPO Reporting Service (2004). Situation of several quarantine pests in Croatia in 2003.

EPPO Reporting Service (2012). Situation of *Globodera pallida* in Finland in 2011.

EPPO Reporting Service (2013). First report of *Globodera pallida* in Denmark.Dellaert, L.M.W., and R. Hoekstra (1987). Resistance to potato cysts nematodes, *Globodera* spp., in wild and primitive *Solanum* species. Potato Research 30 (4): 579-587.

Fedorko, J. (1987). Controlling of potato cyst nematode. Manual of the Institute of Potato 2/87.

Fullaondo, A., E. Barrena, M. Viribay, I. Barrena, A. Salazar, and E. Ritter (1999). Identification of potato cyst nematode species *Globodera rostochiensis* and *G. pallida* by PCR using specific primer combinations. Nematology (1): 157–163.

Grubisic, D., L. Ostrec, T. Gotlin Culjak, and S. Blumel (2007). The occurrence and distribution of potato cyst nematodes in Croatia. J. Pest Sci. (80): 21-27.

Jasińska, A. (1955). Mątwik ziemniaczany. Przegląd Ogrodniczy (32): 15–16.

Karnkowski, W., R. Dobosz, A. Kaczmarek, A. Obrępalska-Stęplowska, and D. Kierzek (2012a). Occurrence of the white potato nematode *Globodera pallida* (Stone, 1973) Behrens, 1975 (Nematoda: Heteroderiae) in two consignments of table potatoes moved to Poland from Cyprus. Progress in Plant Protection (51): 1545–1549.

Karnkowski, W., A. Kaczmarek, R. Dobosz, P. Wieczorek, and A. Obrępalska-Stęplowska (2012b). Occurrence of the white potato nematode *Globodera pallida* (Stone, 1973) Behrens, 1975 (Nematoda: Heteroderiae) on the territory of Poland. Progress in Plant Protection (52): 1087–1092.

Kort, J., H. Ross, H. J. Rumpenhorst, and A. R. Stone (1977). An international scheme for identifying and classifying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*. Nematologica (23): 333–339.

Limantseva, L., N. Mironenko, O. Shuvalov, O. Antonova, A. Khiutti, L. Novikova, O. Afanasenko, D. Spooner, and T. Gavrilenko (2014). Characterization of resistance to *Globodera rostochiensis* pathotype Ro1 in cultivated and wild potato species accessions from the Vavilov Institute of Plant Industry. Plant Breed. (133): 660–665.

Milczarek D., B. Flis, and A. Przetakiewicz (2011). Suitability of molecular markers for selection of

potatoes resistance to *Globodera* spp. Am. J Pot. Res. (88): 245-255.

Minnis, S.T., P. P. J. Haydock, S. K. Ibrahim, I. G. Grove, K. Evans, and M. D. Russell (2002). Potato cyst nematodes in England and Wales - occurrence and distribution. Annals App. Biol. (140): 187–195.

Nicol, J. M., S. J. Turner, D. L. Coyne, L. den Nijs, S. Hockland, and Z. Tahna Maafi (2011). Current nematode threats to world agriculture. In "Genomics and molecular genetics of plant-nematode interactions" (ed: Jones J., Gheysen G., Fenoll C.) Springer (Berlin) 2011, pp 21–43

Nježić, B., B. G. Stare, S. Širca, and N. Grujic (2014). First Report of the Pale Potato Cyst Nematode *Globodera pallida* from Bosnia and Herzegovina. Plant Disease (98): 575–575

Przetakiewicz, A (2013). The First Report of *Globodera rostochiensis* pathotypes Ro5 occurrence in Poland. Plant Disease (97): 1125–1125

Pylypenko, L.A., T. Uehara, M. S. Phillips, D. D. Sigareva, and V. C. Blok (2005). Identification of *Globodera rostochiensis* and *G. pallida* in the Ukraine by PCR. European J Plant Pathol. (111): 39–46

Radivojević, M., D. Krnjaić, N. Grujić, V. Oro, S. Gladović, and M. Madani (2006). The first report of potato cyst nematode *Globodera pallida* (Stone, 1973) from Serbia. In: International Symposium on Crop protection (58th). Gent, Belgium, 23rd May, Book of Abstracts (1). 73 p.

Seinhorst, J. W. (1962). Modifications of the elutriation method for extracting nematodes from soil. Nematologica (8): 117–128.

Širca, S., B. G. Stare, P. Strajnar, G. Urek, and I. M. Lautar (2012). First report of the pale potato cyst nematode *Globodera pallida* from Slovenia. Plant Disease (96): 773–773.

Spears, J. F. (1968). The golden nematode. Agr. Handbook U.S. Dept. Agric. (353). 81 p.

Zouhar, M., P. Rysanek, and V. Gaar (2003). First Report of the Potato Cyst Nematode (*Globodera pallida*) in the Czech Republic. Plant Disease(87): 98–98.