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Application of DNA markers linked to the potato *H1* gene conferring resistance to pathotype Ro1 of *Globodera rostochiensis*

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Abstract Ninety-one potato genotypes (cultivars and breeding lines) selected as resistant or susceptible to pathotype Ro1 of *Globodera rostochiensis* were screened for the presence of two PCR markers, 0.14 and 0.76 kb in length. Both PCR markers were linked with the *H1* gene, located at the distal end of the long arm of chromosome V, and were present in 88 to 100% of the resistant cultivars and breeding lines. The 0.76 kb PCR marker was detected in all resistant genotypes and in approximately 86% of susceptible breeding lines as well as in all susceptible cultivars. The 0.14 kb marker was detected in 88% of resistant breeding lines and in 94% of resistant cultivars. Most of the susceptible genotypes tested (91% of cultivars, but only 50% of breeding lines) did not show the presence of the 0.14 kb marker. We conclude that the 0.14 kb*H1* marker is likely to be useful for the proper selection of potato genotypes resistant to the Ro1 pathotype of *G. rostochiensis*.

Keywords *Globodera rostochiensis* · Marker · Pathotype Ro1 · PCR

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The discovery of resistance genes against potato cyst nematodes (*Globodera rostochiensis*, golden cyst nematode, and *G. pallida*, white cyst nematode) in *Solanum tuberosum* ssp. *andigena*, *S. spegazzinii* and *S. vernei*, and their subsequent incorporation by breeders into many potato cultivars is one of the most important achievements in European potato breeding. Since 2006 resistance to *G. rostochiensis* has been obligatory for new potato cultivars in Poland. Genetic mapping of resistance loci in potato is generally performed at the diploid level, but considerable progress has also been made in tetraploid genotypes. To date (Finkers-Tomczak et al. 2011) 14 potato cyst nematode resistance gene loci have been mapped on eight linkage groups in potato. Four resistance genes (*H1*, *GroVI*, *Gro1* and *Gpa2*) are responsible for nearly absolute resistance to one or more nematode pathotypes, while other genes confer only partial resistance (Bakker et al. 2004). The inheritance of nematode resistance ranges from dominant monogenic to polygenic alleles; it depends on the pathogen and the source of resistance. The genes *H1* and *Gro1*, conferring resistance to the potato cyst nematode *G. rostochiensis*, were localised on potato chromosome V and VII, respectively (Barone et al. 1990; Gebhardt et al. 1993). Those sources of resistance, are effective against all the major pathotypes of *G. rostochiensis*. *Gro1* is inherited as a single dominant allele conferring resistance to the *G. rostochiensis* pathotypes Ro1 and Ro5. The monogenic *H1* locus from *S. tuberosum* ssp. *andigena* is associated with resistance to the *G. rostochiensis* pathotype Ro1 and Ro4. The inheritance of Ro1 resistance from *S.*

spegazzinii and *S. vernei* is complex and based on several loci (Jacobs et al. 1996).

The aim of our study was to evaluate the usefulness of two PCR based markers linked to *H1* for rapid screening of Polish potato genotypes (cultivars and breeding lines) for resistance to *G. rostochiensis*. The potato cultivars and breeding lines originated from Polish Plant Breeding Stations (Strzekęcin, Krokowa and Dybowo) and were propagated in Swojec (Experimental Station of the University of Environmental and Life Sciences in Wroclaw, Poland). The plants were grown in the field and young leaves were taken for DNA analyses. The biological test for resistance to *G. rostochiensis* pathotype Ro1 was conducted for 3 years, according to the protocol of Stefan and Malinowska (2000). Plants with less than 3 cysts were considered as resistant (R) and those showing more than 3 cysts as susceptible (S). Plant DNA was extracted from flash-frozen leaves, using Jughans and Metzlaff (1990) or DNA Plant MiniKit (Qiagen) protocols. For PCR detection of markers linked to the dominant allele of *H1*, two pairs of primers were used. A product of 0.76 kb resulted from amplification with primers F (5′-GGCGTTACAGTCGCCGTAT-3′) and R (5′-GTTGA AGAAATATGGAATCAAA-3′), while primers TG689AlleleSpecific (5′-TAAAACTCTTGGTTATAGCCTAT-3′) and TG689indel12 (5′-CAATAGAATGTGTTGTTTCAC CAA-3′) amplified a 0.14 kb product. The conditions for PCR amplification with F and R primers, according to Niewöhner et al. (1995) and modified by Skupinová et al. (2002) were as follows: 6 ng total DNA, 0.05 U of Taq Platinum polymerase, 0.25 mM dNTPs and 1x Taq Platinum polymerase buffer containing 2 mM $MgCl_2$ in 25 μl total volume with 0.1 μM of each primer. The thermal profile was as follows: 96° C- 3 min., next 45 cycles of 93° C- 30 s, 51° C- 45 s and 72° C- 90 s. The last step included 72° C- 90 s. The conditions for PCR amplification of *H1* allele with TG689AlleleSpecific and TG689indel12 primers (as well as amplification of a 0.29 kb control fragment with primers designed against conserved regions of beta carotene hydroxylase: BCH-F2 [5′-CATGACA TAGTTTGAATTTTGAGTC-3′] and BCH-R2 [5′-GCTTTGGCGCTGCCGTAAGTT-3′] (Brown et al. 2006)) were as follows: 12 ng total DNA, 0.06 U of Taq Platinum polymerase, 0.25 mM dNTPs, 0.2 μM of each primer (except BCH primers- 0.08 μM) and 1x Taq Platinum polymerase buffer containing 3 mM $MgCl_2$ in 25 μl total volume. The thermal profile was as follows: 96° C- 3 min., next 50 cycles of 93° C- 70 s, 51° C- 60 s and 72° C- 90 s. Final elongation proceeded for 90 s at 72° C. All the PCR analyses were performed in two independent labs on Hybaid and Biometra thermocyclers. Because PCR is very sensitive to many factors, to rule out the possibility of failed PCR reactions, all assays (from at least three biological replicates) were performed in at least three technical replicates in this study. Amplification products were separated on 2% agarose gels and stained with ethidium bromide.

The results obtained with PCR markers developed for the *H1* locus (Niewöhner et al. 1995) are presented in Fig. 1 and in Table 1. Genotypes presented in Table 1 were chosen randomly. The 0.76 kb PCR marker was detected in all resistant genotypes and in approximately 86% of susceptible breeding lines as well as in all susceptible cultivars (Table 1). According to Skupinová et al. (2002) this marker detects only dominant *H1* allele in potato genotypes. Thus, this marker is not well-suited for fast resistance screening of Polish potato germplasm. The 0.14 kb PCR marker appeared much more useful. We found a positive, but not perfect, relationship between the presence of the 141 bp PCR marker and resistance to the Ro1 pathotype estimated phenotypically by biological tests. Eighty eight percent of resistant breeding lines and 94% of resistant cultivars tested displayed the presence of the 0.14 kb marker. Most of the susceptible genotypes tested (91% of cultivars, but only 50% of breeding lines) did not show the presence of the 0.14 kb marker (Table 1).

Experiments performed with molecular markers mapping system by Kreike et al. (1993) indicated that resistance to the pathotype Ro1 is not determined by a

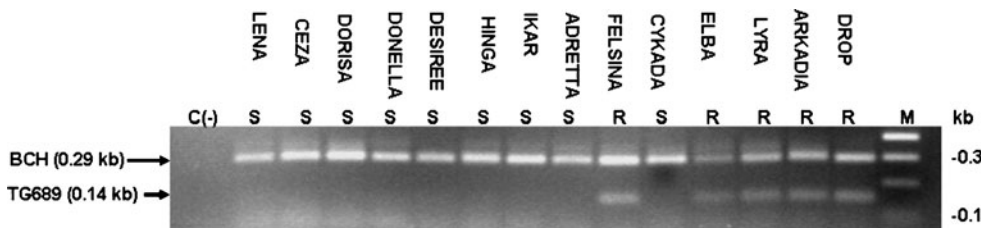

Fig. 1 Electrophoretical pattern (on 2% agarose gel) of analysed 0.14 kb marker (amplified with TG689 primers) for different potato genotypes. Amplification of a fragment of the beta carotene hydroxylase gene with BCH primers (giving 0.29 kb PCR product) served as a positive control for all PCR reactions, confirming the quality of isolated total DNA samples. R - resistant genotypes; S - susceptible genotypes; C(−)- negative control (containing H_2O instead of DNA); M- DNA molecular weight marker. The size of DNA fragments is indicated in kb

Table 1 The presence of markers linked to the *H1* locus in analysed potato genotypes

No.	Breeding lines	*H1* locus		R/S	No.	Cultivars	*H1* locus		R/S
		0.76 kb	0.14 kb				0.76 kb	0.14 kb	
1	B-54214	+	+	R	1	Accent	+	+	R
2	B-56018	+	+	R	2	Agria	+	+	R
3	B-56109	+	+	R	3	Albina	+	+	R
4	B-57022	+	–	R	4	Ania	+	+	R
5	B-57023	+	+	R	5	Anielka	+	+	R
6	B-57085	+	+	R	6	Arkadia	+	+	R
7	BU58.109	+	+	R	7	Arnika	+	–	R
8	K.95-1730	+	+	R	8	Bard	+	+	R
9	K.95-1731	+	–	R	9	Bóbr	+	+	R
10	K.95-1738	+	–	R	10	Bryza	+	+	R
11	K.95-1752	+	–	R	11	Cara	+	+	R
12	K.96-1357	+	+	R	12	Danusia	+	+	R
13	PS-113	+	+	R	13	Drop	+	+	R
14	PS-1216	+	+	R	14	Elba	+	+	R
15	PS-1611	+	+	R	15	Felsina	+	+	R
16	PS-1667	+	+	R	16	Fregata	+	+	R
17	PS-622	+	+	R	17	Gitte	+	+	R
18	PS-646	+	+	R	18	Grot	+	+	R
19	PS-647	+	+	R	19	Irga	+	–	R
20	S-39106	+	+	R	20	Jasia	+	+	R
21	S-39143	+	+	R	21	Koral	+	+	R
22	S-39176	+	+	R	22	Lyra	+	+	R
23	S-39186	+	+	R	23	Moreene	+	+	R
24	S-39207	+	+	R	24	Mors	+	+	R
25	S-39325	+	+	R	25	Obelix	+	+	R
26	S-39375	+	+	R	26	Oda	+	+	R
27	S-39376	+	+	R	27	Orłan	+	+	R
28	S-39382	+	+	R	28	Pamir	+	+	R
29	S-39392	+	+	R	29	Perkoz	+	+	R
30	S-39399	+	+	R	30	Rebecca	+	+	R
31	S-39403	+	+	R	31	Remarka	+	+	R
32	S-39415	+	+	R	32	Rustica	+	+	R
33	S-39425	+	+	R	33	Sante	+	+	R
34	B-54207	–	+	S	34	Adretta	+	–	S

Table 1 (continued)

No.	Breeding lines	*H1* locus		R/S	No.	Cultivars	*H1* locus		R/S
		0.76kb	0.14kb				0.76kb	0.14kb	
35	B-56004	+	+	S	35	Ceza	+	–	S
36	B-57038	+	–	S	36	Cykada	+	–	S
37	B-57060	+	–	S	37	Desiree	+	–	S
38	B-57107	+	–	S	38	Donella	+	–	S
39	B-57118	+	–	S	39	Dorisa	+	–	S
40	K.95-3227	+	+	S	40	Hinga	+	–	S
41	P.94-1162-4	+	–	S	41	Ikar	+	–	S
42	P.94-1188-2	+	+	S	42	Lena	+	–	S
43	PS-109	+	+	S	43	Liu	+	+	S
44	PW-153	–	–	S	44	Zarewo	+	–	S
45	PW-362	+	–	S					
46	PW-363	+	+	S					
47	S-39326	+	+	S					
Summary:									
Potato genotypes	R/S	No. of tested genotypes	No. of genotypes with the 0.76 kb marker present		% of genotypes with the 0.76 kb marker present		No. of genotypes with the 0.14 kb marker present		% of genotypes with the 0.14 kb marker present
Breeding lines	R	33	33		100		29		88
	S	14	12		86		7		50
Cultivars	R	33	33		100		31		94
	S	11	11		100		1		9

R - resistant genotypes; S - susceptible genotypes; + amplification product present; – amplification product absent

single locus, which is localised in chromosomes V or VII; it is rather a quantitative polygenic way of inheritance. Because tetraploid potatoes can carry resistance genes from many sources it can be difficult to use markers that reliably distinguish resistant and susceptible genotypes. Nevertheless, PCR-based methods using primers may be promising among many other approaches in those efforts (Leister et al. 1996). A potato genotype may be resistant to pathotype Ro1, for example, even though it does not contain the *H1* gene, for instance, by having resistance derived from *S. spegazzinii* (Kreike et al. 1993). In addition, markers like the 0.14 kb one described here can become separated from resistance by recombination. Therefore, bioassays are the only way to prove that potato markers represent resistance to the golden cyst nematode *G. rostochiensis*.

The conclusion emerging from our study would be the following. For breeders working with many different genotypes and multiple sources of resistance, using markers linked to *H1* provide the opportunity to track *H1* independently of any other resistance genes that might also be present, e.g. pyramiding resistance genes. In practice it may prove useful to test for the presence of *H1* markers before using bioassays, as the marker is much simpler to deploy. This approach will take less time and give more reproducible results than starting with biological tests. Working to develop additional markers linked to *H1*, as well as *Gro1*, may also prove productive.

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All experiments carried out in this study complied with Polish law.

The authors declared that they have no conflict of interests.

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