Segregation of DNA markers of potato (Solanum tuberosum ssp. tuberosum L.) resistance against Ro1 pathotype Globodera rostochiensis in selected F$_1$ progeny

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ABSTRACT

Marker assisted selection is the fast and objective method for detection of resistance major genes. This method is practical for identification of some candidate genes of quantitative resistance. Genetic markers based on Polymerase Chain Reaction (PCR) were used for evaluation of F$_1$ progenies Ornella × Mira and Tábor × Mira. Cultivar Mira has resistance against Ro1 pathotype G. rostochiensis. Cultivars Ornella and Tábor are susceptible to Ro1. Seedlings of F$_1$ generations were used for analyses. Plants were cultivated in greenhouse. DNA was isolated from tissue discs by GenElute Plant Genomic DNA Kit (Sigma, SRN). PCR marker of major gene $H1$ was used for bulked analyses, according to (Niewöhner et al. 1995). Size of this marker was 760 bp. Standard infection tests with Ro1 pathotype G. rostochiensis according to Potoček (1987) in all of the analysed genotypes were made. Segregation ratios of F$_1$ progenies were determined. These ratios have described segregation of resistance markers and segregation of traits in the biological test. The both methods of evaluation of potato’s resistance were compared by correlation analyse. High correlations were found between occurrence of PCR marker for $H1$ and resistance to Ro1 in biological test. Coefficient of correlation $r = 0.962$ in F$_1$ progeny Ornella × Mira and $r = 0.964$ in hybrids Tábor × Mira. Statistical evaluation of real ratios of segregation by infection tests and DNA markers with theoretical ratios of segregation in simplex and duplex $H1$ gen qualitative determined resistance was made as well. Resistant cultivar Mira as donor of simplex determined resistance was confirmed.

**Keywords:** potato; Solanum tuberosum ssp. tuberosum L.; DNA markers; PCR; Ro1 pathotype Globodera rostochiensis; resistance; major gene $H1$; bulked segregation analysis

Nematodes of Globodera genus can be considered as a significant quarantine pest in all of the Temperate Zone. The highest incidence of G. rostochiensis has been during the last 40 years because the sale of potatoes has increased rapidly. In the affected areas G. rostochiensis causes big loss in quantity and quality of potato tubers, which can be more than 300 millions EUR per year (Benduz et al. 1998). The possibility of complete elimination of this pathogen is very problematic. Both of Globodera species are included on the list of quarantine pathogens worldwide. Both species are on list A2 (EPPO – Europe Plant Protection Organization) (Smith et al. 1992). Plant breeding for resistance is considered as very important and effective strategy of plant protection at present (Bartoš 1991, Smith et al. 1992). Benduz et al. 1998).

Genetics determination of resistance Solanum genus against G. rostochiensis

The resistance against this parasitical nematode is monogenic respectively oligogenic or polygenic. No genes are localised in the basic genome of S. tuberosum ssp. tuberosum L. The origin of resistant genotype is based on interspecies hybridisation; somatic hybridisation or it can be some result of genetics manipulations. From the historic view, the first was discovered dominant monogenic determined resistance against Ro1 pathotype of G. rostochiensis (Huijsman 1955). The gene, which controls this resistance, was called $H1$. The gene source of this resistance was S. tuberosum ssp. andigena Hawkes CPC11673. The gene $H1$ is inherited tetrasomically and the dominant control resistance is reflected already manifested in simplex assemblage ($H1h1h1h1$). This situation was discussed among others by Huijsman (1955), Bartoš (1991), Pineda et al. (1993). By crossing, this gene was incorporated in many cultivars of S. tuberosum ssp. tuberosum L. This gene is localised on chromosome V (Gebhardt 1994). The mechanism of resistance, which is controlled by gene $H1$, is based on nutrition deficiency and female G. rostochiensis ontogenesis (Piegat and Wilski 1965, Hoopes et al. 1978). The result of female larvae nutrition deficiency is their smaller size and the limited ovum production (Ross 1986). Mullin and Brodie (1988) stated that the degree of second stage juvenile’s infection is markedly lower in plants with this type of resistance. Forasmuch as Ro1 pathotype G. rostochiensis is considered as the most expanded, this resistance can be regarded as the great part of integrated potato protection. This resistance can be found in large number of potato cultivars. S. tuberosum ssp. andigena Hawkes was found later as a donor of resistance against...
Ro4 pathotype G. rostochiensis (Ross 1986). Among other dominant major genes controlled resistance against pathotypes G. rostochiensis belong gene H2 (Dunnet 1962), genes A and B (Huijsman 1960) and genes Fa and Fb (Ross 1986). Dominant major gene Fb brings resistance against Ro1, Ro2, and gene Fb against Ro2 and Ro5. Ross (1962) described other donor of genes resistance against Ro1 pathotype G. rostochiensis. The source of resistance was S. spegazzinii Bitt. EBS510. Barone et al. (1990) supposed that it is only one major gene. This major gene was called Gro1 and in his experiments, he localised it to the chromosome VII. Monogenic dominant controlled resistance corresponded to the segregation of backcross (1:1). This finding was repeatedly confirmed in his experiments. In the next experiments based on different DNA markers application was discovered that the resistance obtained by alloplodid with genom S. spegazzinii is probably determined as polygenic. Currently, this type of resistance is considered as a quantitative trait and for mapping of single loci is used strategy QTL mapping (Kreike et al. 1993, 1994, Leister et al. 1997). The next possibility of polygenic determined potato resistance against Ro1 pathotype G. rostochiensis source is botanic species S. vernei Bitt. et Wittm. The resistance S. tuberosum ssp. tuberosum L. obtained from this genetic source is markered as first described quantitative – polygene determined resistance against Ro1 pathotype G. rostochiensis (Pleistet al. 1962).

Detection of the gene of resistance against G. rostochiensis in Solanum genus using DNA markers

Currently, the problem of detection genes of resistance is studied by using all types of genetics markers, which are based on polymorphism of nucleic acids. Pineda et al. (1993) used the RFLP (Restriction Fragment Length Polymorphism) method for studying and localisation of gene H1 – gene of resistance against Ro1 pathotype G. rostochiensis in Solanum genus. As primary material, he has used diploid and tetraploide genotypes. By means of genomic probe, he also has studied different combination of alleles H1 and h1 in the tetraploide genotypes. He has statistically summarized his results to the constricted chromosome V map, which he has saturated by 21 RFLP markers. Using RFLP, the gene H1 was also mapped method by Gebhard (1994). She has used several RFLP cDNA type probes. Niewöhner et al. (1995) have made converse of RFLP marker to specific PCR (Polymerase Chain Reaction) marker. Kreike et al. (1993) was one of the firsts, who have used the RFLP method for gene Gro1 markering and mapping. In his analysis, he has used S. spegazzinii Bitt as a diploid botany donor of resistance and their hybrids with diploid S. tuberosum L. By means of molecular genetics markers mapping systems, he has proved that this type of resistance is not determined by one locus, which is localised in chromosome VII (Barone et al. 1990), but it is quantitative polygenic controlled type of resistant. In his experiments, he has obtained two RFLP markers, which statistic cogently proved localisation of two locus of Gro gene – locus Gro 1.2 in chromosome X and locus Gro 1.3 in chromosome XI. Kreike et al. (1996) present in RFLP map locus Gro 1.4 localised in chromosome III. Problems of polygenic determined resistance, which was brought from S. spegazzinii Bitt., Gebhardt (1994) studied by using RFLP markers. By means of cDNA probes, she has localised in chromosome VII the same locus as Barone et al. (1990).

Leister et al. (1997) have studied problems of locus Gro gene. Object of his experiments was cloning of single loci of this polygen by using YAC (Yeast Artificial Chromosome) libraries. In his work, he has made a brief of individual alleles inserted to gene group Gro1. In his experiments, he has evaluated 384 YAC colonos. With using methods RFLP and PCR-SPLAT he has detected individual alleles. He also presents some combination of primers, which are necessary for amplification of these allele’s markers. Results of crossing with S. spegazzinii have introduced in S. tuberosum some genes with resistance against: Ro1 pathotype G. rostochiensis, G. pallida, late blight and some viruses. Leister et al. (1997) in there results found two DNA markers covered chromosome II, one marker in chromosome III, five markers in chromosome V, four markers in chromosome VII, two markers in chromosome XI and one marker in chromosome XII.

MATERIAL AND METHODS

Plant material

Methods of detection of H1 gene based on PCR were used on the collection of potato cultivars with declared resistance against the Ro1 pathotype of G. rostochiensis (Accent, Arnika, Berber, Bettina, Darwina, Granola, Gusta, Hitta, Ilse, Indra, Lyra, Mira, Olga, Ponto, Tanja, Ute) and declared susceptibility to Ro1 (Ornella, Tábor). Those genotypes were cultivated in vitro, on the modified medium according to Murashige and Skoog (1962). The segregation of the specific PCR marker was studied on the F1 progeny of crosses Ornella × Mira and Tábor × Mira. The seedlings of hybrid progenies were cultivated in the greenhouse.

Detection of seedling resistance against the Ro1 pathotype of G. rostochiensis by infec- tion test

For the detection of resistance (susceptibility respectively) of seedlings against the Ro1 the method according to Potoček (1987) was used. The test is based on the artificial infection of plants by the standard pathogen (Ro1 pathotype) and by quantification of abundance of nematodes after the cultivation.

DNA extraction

DNA was obtained from stems in vitro plants and from foliar pieces of greenhouse cultivated seedlings. For ex-
traction of DNA the GenElute Plant Genomic DNA Kit (Sigma, Germany) was used.

**PCR detection of dominant H1 allele**

The pair of primers according to Niewöhner et al. (1995) for amplification of dominant H1 allele marker was used. The sequences of the primers were: F 5’GCG TTA CAG TCG CCG TAT 3’ and R 5’GTT GAA GAA A TA TGG AAT CAA A 3’. The melting temperature (Tm) was fixed 56°C for both of primers according to Sambrook et al. (1998). The thermocycler T-Gradient (Biometra, Germany) was used for amplification of DNA. Composition of the 25 µl reaction was: 100 ng of genomic DNA, 2.5mM MgCl2, 0.3mM of both primers, 0.2mM dNTP and 0.7 U of the Red Taq polymerase (Sigma, Germany). Conditions of amplification were: 1 × (93°C, 180 s), 35 × (93°C 30 s, 51°C 45 s, 72°C 90 s) and 1 × (72°C 90 s). Amplified markers were analysed in 1.5% agarose electrophoretic gel in TBE buffer. Electrophoretic diagrams were visualized by ethidium bromide.

**RESULTS**

The optimisation of chemical composition and conditions of amplification to the successful obtaining of specific PCR markers was necessary for completion. Selection of the thermal gradient during amplification, that is one of the functions of the thermocycler, was used. The cultivars with declared resistance and susceptibility against the Ro1 pathotype of the *G. rostochiensis* to optimisation of the PCR detection of the dominant H1 allele were used. The optimised method has enabled amplification of the 760 bp fragment in the case of the resistant genotypes. No product of amplification of the susceptible cultivars was detected. This fact manifests the applicability of the described method of detection of dominant allele of H1 gene. This is demonstrated in Figure 1. F1 progeny genotypes from described assortment of cultivars were selected. Crossings of cultivars: Ornella × Mira, Tábor × Mira were made. Ornella and Tabor are susceptible and Mira has declared resistance against Ro1. In Ornella × Mira hybridisation 365 seedlings and in Tábor × Mira hybridisation 223 seedlings were acquired.
All seedlings that were in the model hybridisation were used in infection test. Ratios between resistant and susceptible genotypes are described in Table 1.

Linear regression and correlation analysis have characterised the closeness of dependence between presence of 760 bp PCR marker and resistance of seedlings against the Ro1 detected by infection test. In both cases, there was very high correlation between presence of marker of dominant allele of major gene H1 and resistance detected by infection test. The index of correlation in F1 progeny of Ornella × Mira was $r = 0.962$ and in Tábor × Mira was $r = 0.964$.

Described hybrid progenies were used for PCR detection of dominant allele of H1 as well. Segregation of optimised PCR marker is described in Figure 2. The ratio between presence and absence of PCR marker in studied F1 progenies is shown in Table 1.

The dominant H1 allele controlled resistance indicates in simplex configuration in the tetraploid potato genome. The chosen PCR marker is not able to concretise a number of dominant alleles in studied locus. For this reason the statistical analyse of segregation ratios by t-test according to Myslivec (1957) was completed. Obtained segregation ratios that describe segregation of resistance in infection test and segregation of PCR markers were compared with theoretical ratios of segregation of F1 progeny, when the resistant parent could prove the resistance determined in simplex (H1h1h1h1), duplex (H1h1h1h1), triplex (H1h1h1h1) and quadruplex (H1h1h1h1) composition. Only for the simplex determined resistance of Mira was detected significance of t-test on the value $\alpha = 0.05$. This test has confirmed that there it is possible to characterise the resistance of Mira as simplex determined in probability $P = 0.95$. Detailed statistical evaluation is described in Table 1.

**DISCUSSION**

The possibility of using PCR markers for detection of dominant allele H1, which controls the resistance against Ro1 pathotype *G. rostochiensis* was unequivocally confirmed by this experiment. Breeding of nematode resistant potatoes can be considered as effective strategy of protection. This fact is supported by the reality that there was 75% cultivars with declared resistant against Ro1 pathotype *G. rostochiensis* in the List of cultivars registered in the State Book of Cultivars in 2001.

This work presents results of experiments leading to application of DNA markers, which make the production of new resistant cultivars of potatoes more effective. The kit GenElute Plant Genomic DNA Kit (Sigma, SRN) was used for isolation of DNA, which allows performance of DNA isolation from different parts of plants.

Conditions of amplification according to Niewöhner et al. (1995) were not proved as suitable for conditions in laboratory of The Czech University of Agriculture in Prague. Parameters of PCR, which the author used, did not allow amplifications of any fragments. By means of backcross optimisation tests concentration of some components of reaction mixture was adapted. Concentration of genomic DNA was increased for 20 ng in volume 25 µl, concentration of dNTP was increased six times, and concentration of MgCl₂ was increased for 20 ng in volume 25 µl, concentration of Taq polymerase and other components of reaction mixture was adapted. Concentration of genomic DNA was increased for 20 ng in volume 25 µl, concentration of dNTP was increased six times, and concentration of MgCl₂ was increased for 20 ng in volume 25 µl, concentration of Taq polymerase was inversely decreased for 0.3 U in 25 µl reaction. The reason of these changes was probably other type of used Taq polymerase and other strategy of DNA isolation.

By using couple of specific primers value $T_m$ 56°C was determined. For calculation of $T_m$ method according to Sambrook et al. (1989) was used. Annealing temperature (60.6°C), which Niewöhner et al. (1995) recommended did not allow amplification of PCR fragments with size 760 bp. This fact was obviously induced by disharmony between values of $T_m$ and used annealing temperature. Decrease of annealing temperature to 51.0°C allowed specifics amplification of desiderative PCR marker. In set cultivars with declared resistance or sensibility against Ro1 pathotype *G. rostochiensis* was confirmed that the decrease of annealing temperature did not influence the specificity of used PCR marker. For example De Jong et al. (1997) in

### Table 1. Segregation of infection tests and PCR markers in evaluated F1 progenies and their statistical evaluation

<table>
<thead>
<tr>
<th>Crossing</th>
<th>Real ratio of segregation</th>
<th>Theoretical ratio of segregation</th>
<th>Critical value of $t$-test</th>
<th>Value of $t$-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>infection test resistant plants</td>
<td>infection test susceptible plants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ornella × Mira</td>
<td>198</td>
<td>167</td>
<td>1</td>
<td>1</td>
<td>1.96</td>
</tr>
<tr>
<td>Tábor × Mira</td>
<td>110</td>
<td>113</td>
<td>1</td>
<td>1</td>
<td>1.96</td>
</tr>
</tbody>
</table>

Presumptive genotypes: Ornella: $h1h1h1h1$, Tábor: $h1h1h1h1$, Mira: $H1h1h1h1$
studying of potato genome has used lower annealing temperature to value Tm of specifics PCR primers. Necessary changes of amplification profile were induced by modified composition of reaction and by technical parameters of used thermocycler.

In set of model genotypes, it was confirmed that electrophoresis has led to highly sensitive and reliable detection of dominant allele of H1 gene in tetraploid potato genome. This reality was confirmed by analyses of regression and correlation, which was undertaken F1 generations genotypes. For optimisation of experiments an analogous assortment of world potato cultivars was purposely chosen, the same as used by Niewöhner et al. (1995). It was proved, that amplification of 760 bp PCR fragment marking the resistance of potato against Ro1 pathotype G. rostochiensis has run in the same genotypes.

Based on these informations the model crossings of resistant cultivar Mira with susceptible cultivars Ornella and Tábor were carried out. The objective of this hybridisation was evaluation of the allele composition of H1 of resistant strain Mira. Parental components Ornella and Tábor declared susceptibility to Ro1 pathotype of G. rostochiensis and for this reason the allelic composition in H1 locus is (h1h1h1h1). The PCR marker described according to Niewöhner et al. (1995) is dominant and is able to detect only dominant H1 allele in tetraploid potato genome. The marker does not allow distinguishing the simplex, duplex, triplex and quadruplex configuration of dominant H1 allele. Forasmuch as that is very advantageous for breeders to know compositions of allele H1 gene, two bulked analyses were made. Standard bulked tests and their combination with infection tests were described by several authors (Huijsman 1955, Pineda et al. 1993). Parallel evaluation of F1 progeny segregation of described crossings by using of infection tests and using of modified PCR marker according to Niewöhner et al. (1995) were made in this study. The statistical analyses confirmed at the level α = 0.05 that the segregation of infection tests and PCR marker correspond with model H1h1h1h1 × h1h1h1h1. The donor of the dominant H1 allele was predominantly cultivar Mira. Small differences between ratios of segregation of infection tests and PCR marker that are shown in Table 1 can be explained by interaction of genotype of resistant plant and external conditions. These conditions could affect accuracy of appraisal of resistance (susceptibility respectively) of evaluated plant. The possible mistake of evaluation refers for sample Bartoš (1991), Becker (1993) and Odenbach and Sacristan (1997).

The molecular-genetic part of this work is the model of the Marker Assisted Selection’s (MAS) application in potato breeding to qualitative resistance against Ro1. The method allows view resistance only on the base of evaluation of genetic information, which is not encumbered by influences of external environs.

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ABSTRAKT

Segregace DNA markerů rezistence bramboru (Solanum tuberosum ssp. tuberosum L.) vůči Ro1 patotypu Globodera rostochiensis ve vybraných F1 generacích

Selekce s využitím DNA markerů je rychlou a objektivní metodou detekce konkrétních majorenž rezistence. Tuto metodu lze použít rovněž pro identifikaci některých kandidátních genů polygenně determinované rezistence. Genetické markery založené na polymerážové řetězové reakci byly použity pro hodnocení vybraných F1 generací křížení Ornella × Mira a Tábor × Mira, kde odrůdy Ornella a Tábor mají deklarovánu senzitivitu a odrůda Mira rezistencí vůči Ro1 patotypu G. rostochiensis. Izolace DNA byla provedena z listových terčíků semenáčků kultivovaných ve skleníku s využitím GenElute Plant Genomic DNA Kit (Sigma, SRN). Pro segregační analýzu byl použit PCR marker majorgenu H1 (Niewöhner et al. 1995) o velikosti 760 bp. U všech analyzovaných genotypů byly provedeny standardní biologické infekční testy s Ro1 patotypem G. rostochiensis dle Potočka (1987). U genotypů F1 generace byly stanoveny štěpné poměry charakterizující segregaci DNA markerů i segregaci v rámci biologických infekčních testů. Obě metody stanovení rezistence bramboru byly statisticky porovnány korelační analýzou. U obou křížení byly nalezeny těsné korelace mezi výsledkem PCR markeru dominantního majorgenu H1 a rezistencí detekovanou infekčním testem. U F1 generace kříženců Ornella × Mira byl zjištěn korelační koeficient r = 0,962 a u kříženců Tábor × Mira r = 0,964. Rovněž bylo provedeno statistické porovnání skutečných štěpných poměrů infekčních testů a DNA markerů s předpokládaným štěpným poměrem simplexně a duplexně determinované rezistence různě dominantní majorgenem H1. Statistickou analýzou bylo potvrzeno, že použitý rezistentní genotyp (odrůda Mira) je donorem simplexně založené rezistence.

Klíčová slova: brambor; Solanum tuberosum ssp. tuberosum L.; DNA markery; PCR; Ro1 patotyp Globodera rostochiensis; rezistence; majorgen H1; segregační analýza

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