Characterisation of resistance genes resources against late blight available for Czech potato breeding by means of selected DNA markers

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ABSTRACT

Marker assisted selection (MAS) in potato breeding is the most developing area at present time. Methods of DNA markers are developed in all top world potato research institutes and universities oriented on plant production. This paper presents results obtained from the testing of gene resources encompassing different resistance genes against *Phytophthora infestans* potentially exploitable for Czech potato breeding. Three different DNA markers were studied that are linked to *R1* locus in the potato chromosome V operating as a resistance against some races of *P. infestans*. Markering capability and frequencies of these markers was evaluated with respect to their usage in practical Czech plant breeding that has vital importance in finding the tools as a prerequisite for creating new varieties efficiently.

Keywords: *Solanum tuberosum* L.; potato resistance breeding; gene resources; *Phytophthora infestans*; marker-assisted selection; DNA markers

The linkage groups on chromosome V (Leonards-Schippers et al. 1992) were very closely studied because of the presence of many important resistance genes at this region. Gebhardt et al. (1991) introduced the most interesting region surrounded by two RFLP markers, GP21 and GP179. This is a group of loci for the resistance gene controlling among other things the potato resistance against most viruses (PVX, PVY, PVS) and potato cysts nematodes (*Globodera rostochiensis* and *G. pallida*) obtained by potato hybridisation of *Solanum tuberosum* ssp. *tuberosum* L. and *S. tuberosum* ssp. *andigena* L. (Meksem et al. 1995). However, at this region there is placed also the most prevalent resistant gene controlling the hypersensitive response to race 1 of *Phytophthora infestans* (Mont.) de Bary, *R1* gene originated from *S. demissum* Lindl. (Bradshaw and Mackay 1994, Chloupek 2000). De Jong et al. (1997) studied this region by means of AFLP markers and his hybridisation experiments demonstrated a very tight linkage of the *R1* gene and marker SPUD237. This marker was converted in CAPS (Cleaved Amplified Polymorphic Sequence) marker more usable for a wider research public. Nevertheless, research of this region has continued and Ballvora et al. (2002) cloned *R1* gene and transformed successfully in susceptible potato cultivar Desiree. To identify of transformed plants they used the allele specific marker constructed on the basis of a known sequence of the transgene. These three different markers linked to nearest neighbourhood of *R1* gene were applied on a large collection of different potato varieties and wild *Solanum* plants in an effort to obtain a better and more objective view on the use of these markers in the breeding of tetraploid plants.

MATERIAL AND METHODS

Biological material. Biological material (plants *in vitro*) was obtained in cooperation with the Gene Bank of Potato Research Institute in Havlíčkův Brod. Table 1 shows a collection of genotypes with the known presence of different R genes (A set). Each clone was sorted in view of the phenotypic membership to the group according to its resistance to different pathogen races. Those groups were R1, R3, R4, R10 and groups with a larger amount of accumulated R alleles. Apart from these varieties were studied in total 196 different varieties of Czech and of worldwide assortment and 90 different clones belonging to the species *S. demissum*, *S. tuberosum* ssp. *andigena*, *S. phureja*, *S. bulbocastanum* and *S. stoloniferum*.

Supported by the Ministry of Agriculture of the Czech Republic, Projects Nos. QF 4107 and MSM 412100002.

DNA isolation. DNA was isolated from 100 mg of plant material by GeneElute Plant genomic DNA Kit (Sigma, Germany). We obtained a sufficient amount of high quality DNA. The DNA was then quantified by UV spectrophotometer (Eppendorf, Gemany) and its quality was tested by means of separation through a 1% agarose gel.

CAPS analyse. Markers GP21 and SPUD237 according to De Jong et al. (1997) were optimised and used for CAPS analyses. Marker SPUD237 (SPUD237 F 5′ TTC CTG CTG ATA CTG ACT AGA AAA CC 3′, SPUD237 R 5′ AGC CAA GGA AAA GCT AGC ATC CAA G 3′) was amplified under these conditions: 50 ng of total genomic DNA, 2.5mM of $MgCl_2$, 0.4mM of each dNTP, 0.5μM of each primer and 0.5 unit of *Taq*I recombinant DNA polymerase (Fermentas, Lithuania) in 25 μl reaction. Optimised time-temperature scheme was 1 × 94°C for 180 s, 40 × 94°C for 15 s annealing 55°C for 15 s and extension 72°C for 40 s. The final extension was 72°C for 120 s. Marker GP21 (GP21R 5′ AGT GAG CCA GCA TAG CAT TAC TTG 3′ GP21F 5′ GGT TGG TGG CCT ATT AGC CAT GC 3′) was realised in similar conditions: 150 ng of total genomic DNA, 2.5mM of $MgCl_2$, 0.4mM of each dNTP, 0.5μM of each primer and 0.7 unit of *Taq*I in 25 μl reaction. The optimised time-temperature scheme was 1 × 94°C for 180 s, 40 × 94°C for 15 s annealing 60°C for 20 s and extension 72°C for 60 s. The final extension was 72°C for 360 s.

To distinguish the dominant and recessive genotypes in the marker, the amplification products of both markers were digested by *Alu*I restriction endonuclease (Fermentas, Lithuania) for 2 hours. The conditions of digestion were kept according to manufacturer.

Amplification of R1 allele specific marker. For the amplification R1 allele the specific primers according to Ballvora et al. (2002) (R1F – 5′ CAC TCG TGA CAT ATC CTC ACT A 3′, R1R – 5′ CAA CCC TGG CAT GCC ACG 3′) were used. The optimised protocol was: 60 ng of total genomic DNA, 2.5mM of $MgCl_2$, 0.4mM of dNTP, 0.5μM of each primer and 1 unit of *Taq*I polymerase in 25 μl reaction. The optimised time-temperature scheme was 1 × 94°C for 180 s, 35 × denaturation 94°C for 35 s, annealing 62°C for 45 s and extension 72°C for 90 s. The final extension was 72°C for 180 s. Results of the amplification are shown in Figure 1.

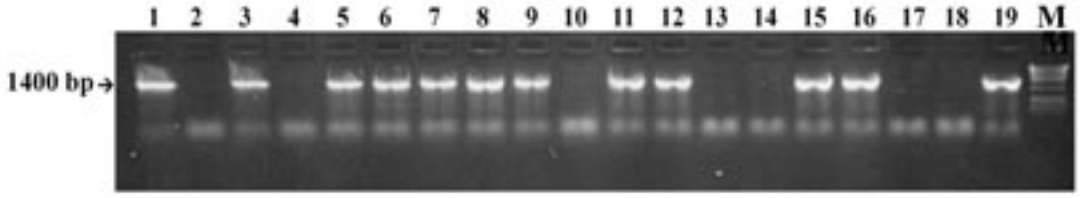
All PCR analyses were realised in a thermocycler T-gradient (Biometra, Germany).

Electrophoretic analyses. The PCR products were analysed by horizontal agarose electrophoresis (Bio-Rad, USA) through a 1.5% gel stained by ethidium bromide. The length of obtained PCR product was detected by ladder Lambda DNA Eco47I/AvaII (Fermentas, Lithuania).

RESULTS AND DISCUSSION

All methods of analyse were optimised. Described by way of DNA isolation was absolutely relative sufficient to the quantity and quality of DNA in high molecular conditions. Although De Jong et al. (1997) and Ballvora et al. (2002) described the use of more complicated isolation methods, our methods offered identical results. In comparison to cited authors we had other genotypes for disposal and we did not make any hybridisation experiments. From this standpoint our work only covered population characteristics, because it was not our goal to verify the segregation ratios and co-segregation of the marker and *R1* gene. Table 1 shows a situation in a set of varieties with declared presence or absence of single genes (A set). The other clones were for us unknown relative to genotypic configuration in *R1* allele. We have analysed mutually also phenotype and presence of part of the wild potato genome in the genome of single varieties in the A set in context to the presence of individual markers. Nevertheless, there was not found any relationship between marker presence and the level of resistance in leafs or in tubers.

In evaluating the collection of the remaining clones (data not shown) there was detected



1. Szignal, 2. Vivax, 3. Resy, 4. Ilona, 5. Troll, 6. Aguti, 7. Tondra, 8. Kristalla, 9. Mariella, 10. Rotkelchen, 11. Kennebec, 12. Charlotte, 13. Baraka, 14. Corine, 15. Provita, 16. Escort, 17. Isola, 18. Juliver, 19. Draga

Figure 1. Amplified R1 allele specific marker represented by fragment 1400 bp in selected potato varieties was detected also in cultivars carrying distinct allele than *R1*

Table 1. A set of studied potato genotypes; the stars represent presence of marker in the genotype

Variety	Declared gene of resistance	Resistance against *P. infestans* (tubers/leaves)	Identified marker GP21	Identified marker SPUD237	Identified marker R1	Wild species in origin
Maris Peer	*R1*	h/h	*	*	*	dms
Clivia		m/m	–	*	*	adg, dms
Kennebec		m/m	*	*	*	?
Troll		m/l	*	*	*	?
Prior		m/m	*	*	*	?
Mariella		m/m	*	*	*	adg, dms
Charlotte	*R3*	h/m	–	–	*	?
Christa		m/m	–	*	*	adg, dms
Dorisa		h/h	–	*	*	?
Eba		h/m	–	*	*	dms
Korrigane		h/m	–	*	–	dms
Lipsi		h/h	*	*	–	?
Ukama		m/l	*	*	*	adg, dms
Fina		m/l	*	*	*	dms
Baraka		h/m	*	*	*	dms
Atlas		m/h	*	–	*	dms
Aguti		h/h	–	*	*	dms, adg, acl
Forelle		m/m	–	*	*	dms, adg, sto
Ilona		h/h	*	*	*	adg, dms
Fatima		h/m	*	*	*	adg, dms
Isola	*R4*	h/h	–	–	–	adg, dms
Jaerla	*R10*	h/m	–	–	*	adg, dms
Premiere		l/m	–	*	–	adg, dms
Draga		h/m	*	*	*	adg, dms
Kristalla		h/m	*	*	*	dms, adg
Provita		m/m	*	*	*	dms, adg
Corine		h/m	–	*	*	?
Anosta		h/m	*	*	*	dms, adg
Vivaks		h/m	*	*	–	adg, dms
Marijke	*R1, R3*	m/m	*	*	–	adg, dms
Resy		h/m	*	*	*	adg, dms
Rotkehlchen		m/m	*	*	–	?
Olev	*R1, R4*	l/h	–	*	*	?
Magyar Rozsa	*R1, R2, R3*	h/h	*	*	*	dms, sto, acl
Szignal		v/v	*	*	*	acl, adg, dms, sto
Fresco		h/m	–	*	–	?
Tondra	*R1, R2, R3, R4*	m/m	–	*	*	dms, adg
Escort	*R1, R2, R3, R10*	h/m	*	–	*	dms

Level of resistance: l = low, m = medium, h = high, v = very high

Wild species: dms = *S. demissum*, adg = *S. tuberosum* ssp. *andigenum*, acl = *S. acaule*, sto = *S. stoloniferum*

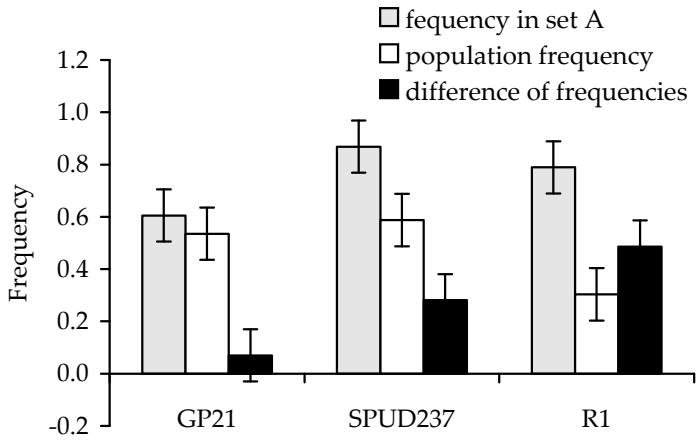


Figure 2. Markers frequencies in collections of potato genotypes and difference can indicate level of markers reliability; marker R1 according Ballvora et al. (2002) is in comparison to SPUD237 and GP21 lesser spread out in potato population and because of more reliable as marker of *R1* gene

a high polymorphism in the presence or absence of individual marker names in conformation with the studied gene. Except for the R1 allele specific marker, markers GP21 and SPUD237 were spread out with high population frequencies. This phenomenon was above all obvious in the results from the wild potato clones, where the frequencies of both CAPS markers were higher then 0.33 but the R1 allele specific marker was detected only in a single clone of *Solanum demissum* (PI160220) and the frequency of the marker was only 0.014. In the A set, the frequency of R1 allele specific marker was 0.789 and practically all of the genotypes with declared allele R1 have proven to have this marker. A similar situation was observed in the case of SPUD237 (frequency 0.868). With a relatively tight gene linkage to *R1* (0.4cM in cis) and ease of realisation, we presume that the presence of SPUD237 could be a perfect marker for the *R1* gene, but it is essential to perfectly know the genotypic constitution and segregation ratios of the marker in the potential parents. The situation is more complicated because of the tetraploid character of the potato. In Figure 2 is presented a comparison of individual evaluated marker frequencies and their differences. The graph shows that the R1 marker has far lower population frequency than SPUD237 or GP21, and the difference between population frequency and the frequency in set A is the highest. From evaluating the phenotypic level of resistance against late blight and presence of *S. demissum* in the origin of the studied varieties (Potato database 2004), it is obvious that all genotypes in the A set are originating from the clone of *S. demissum*. It could be argument grounding the high frequency of R1 marker in the A set, although its presence was not detected only in *R1* gene donors. This was a very unpleasant situation for us. For this reason we performed restriction digestion of the amplified DNA fragment. But the analyses by an elected set of restriction endonucleases confirmed the identity of the fragment in all described varieties. This fact is advanced by two conjectures. At first, the *R1* gene is presented in all varieties showing the R1 marker and it is necessary to reappraise the phenotypic membership of the disputable varieties. Secondly, in the disputable varieties is presented only a non-functional part of the gene represented by the marker as the result of recombination event.

These questions could be restudied for a better understanding of the R1 markers role in the future, because it is a highly perspective marker in Czech potato breeding.

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Received on September 30, 2004

ABSTRAKT

Charakterizace genových zdrojů rezistence bramboru k plísni bramborové pomocí vybraných DNA markerů

Selekce s využitím markerů (MAS) je v současnosti nejrychleji se rozvíjejícím oborem v oblasti šlechtění bramboru. Metody DNA markerů jsou vyvíjeny ve všech hlavních světových výzkumných centrech a univerzitách zaměřených na rostlinnou produkci. Tento příspěvek přináší výsledky testování některých genových zdrojů rezistence k plísni bramborové (*Phytophthora infestans*), dostupných pro české šlechtění. Byly studovány tři různé DNA markery spojené s *R1* lokusem na pátém chromozomu, který odpovídá za lokalizaci dominantního genu rezistence k rase 4 plísně bramborové. Byla sledována markerovací schopnost jednotlivých markerů s ohledem na jejich využitelnost v českém šlechtitelství, které má eminentní zájem získat některé moderní nástroje, u nichž se předpokládá pozitivní vliv na urychlení a zefektivnění selekčního procesu.

Klíčová slova: *Solanum tuberosum*; šlechtění bramboru na rezistenci; genové zdroje; *Phytophthora infestans*; markery asistovaná selekce; DNA markery

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