SELECTION OF VALUABLE POTATO GENOTYPES WITH INTRODUCED RESISTANCE GENES DERIVED FROM WILD SPECIES

SELEKCIA CENNÝCH GENOTYPOV ĽUĽKA ZEMIAKOVÉHO S INTRODUKOVANÝMI GÉNMI REZISTENCIE Z DIVORASTÚCICH DRUHOV

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Introduction of wild species into cultivated potato genotypes allowed obtaining new sources of resistance providing long-term effect against viruses and they are useful in breeding of new varieties. Wild species, *Solanum acaule, Solanum tuberosum* subsp. *andigena* and *Solanum vernei*, were used to breed varieties and clones with extreme resistance to potato virus X (PVX). There were selected 90 genotypes from the gene bank and 166 genotypes from crossings of 4 resistant genotypes in simplex form (*Rxrxrxrx*) and 1 susceptible variety Svella (*rxrxrxrx*) for marker selection. Two molecular markers detected extreme resistance to PVX in two Polish, four German, one Hungarian, three Dutch, twelve Slovak genotypes maintained in the gene bank and sixty three resistant genotypes from four crossings of resistant and susceptible genotypes. The allele − specific marker 221R is a reliable tool for marker assisted selection. The results indicate that the Rx gene for extreme resistance to PVX could be derived from one genetic source which was the ancestor of *Solanum tuberosum* subsp. *andigena, Solanum acaule* and *Solanum vernei* or presence of three genes with the same markers could be a consequence of the fact that the primary source could be any of the mentioned wild species as well.

Key words: potato, wild species, extreme resistance, PVX

Potato virus X (PVX) causes the yield losses up to 30% (Bokx and Want 1987). PVX is spread among potato plants only by mechanical means, what significantly simplifies protection against its wide spreading. In spite of the fact that PVX does not represent a great risk for potato seed production and potato growing, there are several reasons to engage in resistance against this virus. Extreme resistance to PVX represents a suitable model for the study of host − pathogen interactions in accordance with the model of gene against gene and mutual relationships may be relevant also for resis-tance to fungi, bacteria and other pathogens. The analysis of resistance to PVX in protoplasts indicates that multispectral resistance to viruses can be induced by manipulation with resistance, based on the Rx gene, as well.

Resistance in potato (*Solanum tuberosum* L.) induced by the Rx gene is specific on recognition level and potato plant reaction may suppress not only PVX, but also the other viruses infecting potato (Bendahmane et al. 1995; Bendahmane et al. 1999).

Extreme resistance to PVX was detected in wild species *Solanum acaule*, *Solanum sucrense*, *Solanum vernei* and *Solanum tuberosum* subsp. *andigena*. The gene of extreme resistance to PVX derived from *Solanum acaule* – Rx_{acl} was used in German breeding programme, and several potato varieties with extreme resistance to PVX possess the *Rx* gene from it. On the other hand, American, Dutch and Polish varieties with extreme resistance to PVX possess the Rx_{adg} gene,

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originated from *Solanum tuberosum* subsp. *andigena* (Świeżyński 1994).

Extreme resistance to PVX is controlled by the dominant gene *Rx1* resp. *Rx2*. The gene *Rx1* was located on chromosome XII and the gene *Rx2* on chromosome V. On the base of analysis of pedigree, originating from diploid materials, it was assumed that *Rx1* corresponded with the Rx_{adg} gene derived from *Solanum tuberosum* subsp. *andigena* and the *Rx2* gene from *Solanum acaule* (Ritter et al. 1991).

In this work, genotypes from the gene bank and F_1 generations originated from crossing of genotypes with extreme resistance to PVX were analysed. Parental genotypes with the *Rx* gene were selected from several European breeding programmes and were crossed with genotypes susceptible to PVX infection. Seedlings from these populations were used in bulk segregant analysis (BSA) (Michelmore et al. 1991) for the study of genetic relationship between potato varieties derived from different breeding programmes and origins as well as for screening of markers suitable for marker assisted selection (MAS).

MATERIAL AND METHODS

Plant material

Seedling populations consisting of 166 tetraploid genotypes from four different cross combinations (Bobr × Svella, Boda × Svella, Fanal × Svella, Solara × Svella), the group of cultivars with extreme resistance to PVX (Agria, Boda, Fanal, Santé, Solara, White Lady) and the group of susceptible cultivars (Cicero, Desirée, Monalisa, Svella) together with another genotypes from the gene bank of Potato Research and Breeding institute, a.s., Veľká Lomnica, were used for markers verification.

Inoculation of plants with PVX suspension

Seedlings from the four combinations and ten virus-free plants of each parental variety and clone were mechanically inoculated with PVX suspension. Potato leaves were mechanically inoculated using a mixture consisting of the sap of PVX infected potato leaves and 0.1 M phosphate buffer pH 7.2. Totally 0.05 ml of infective solution was applied on carborundum scattered potato leaves, mechanically rubbed into the leave tissue and rinsed by water. Infections were evaluated at weekly intervals. Development of disease symptoms on individual plants induced by PVX was evaluated visually 4 weeks after inoculation.

Detection of PVX in inoculated plants

PVX was detected in infected plants by ELISA method (Clark and Adams 1977). Antibodies for ELISA were purchased from BIOREBA AG (Switzerland).

Bulk segregant analysis

Resistant and susceptible pools were created on the basis of visual symptoms and ELISA readings to two groups (Michelmore et al. 1991). The resistant DNA bulk (resistant bulk – BR) originated from 5 uninfected, symptom-free plants from each of the tested cross combinations (Bobr × Svella, Boda × Svella, Fanal × Svella, Solara × Svella) and in the same way the susceptible bulk (susceptible bulk – BS) were prepared using infected plants. Individual plants of both resistant and susceptible genotypes were also used in all combinations for verification of selected markers.

DNA extraction

Genomic DNA was extracted from potato leaves according to the CTAB method (Rogers and Bendich 1994). The DNA concentration was adjusted to 30 ng μl^{-1}.

DNA amplification with specific primers

PCR analyses were performed in 25 µl reaction volume which consisted of 1 × PCR buffer (20 mM Tris-HCl, 50 mM KCl), 0.2 mM of each dNTPs, 3.0 mM $MgCl_2$, 300 nM primer, 1 U Taq DNA polymerase (Invitrogen) and 30 ng genomic DNA. The DNA was amplified at 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 20 s, annealing for 25 s and extension at 72°C for 1 min, with final extension for 5 min. Primer sequences and annealing temperatures are shown in Table 1. MJ Research PTC-200 was used for DNA amplification.

Electrophoretic separation and visualization of PCR products

PCR products were separated by electrophoresis in horizontal 1.5% agarose gel in TBE buffer (Tris-Borate-EDTA, 90 mmol dm^{-3} Tris-HCl pH=8.0; 90 mmol dm^{-3} boric acid; 10 mmol dm^{-3} EDTA) and visualised with ethidium bromide staining (0.5 µg cm^{-3}).

Evaluation of DNA profiles

Presence or absence of PCR products in electrophoretic patterns of individual genotypes was recorded

and compared with phenotypic data and ELISA readings for resistant and susceptible genotypes. Expected segregation ratio was compared with infection test results and results from PCR analysis.

RESULTS AND DISCUSSION

Virus-free plants of resistant and susceptible potato varieties and clones together with 166 F_1 genotypes derived from four crossing combinations were mechanically inoculated using PVX suspension at 4–6 leaf stage. Symptoms of infection – very mild mosaic − were very slightly visible. Non-inoculated newly developed leaves from each plant were analysed for the presence of virus antigen. ELISA was used for selection of PVX-free and PVX-infected plants in each crossing population. The presence of PVX was proved in all susceptible genotypes (Table 2) and there was no evidence of PVX accumulation in newly developed leaves of genotypes with declared extreme resistance to PVX.

There were no symptoms developed on the leaves of inoculated plants of resistant genotypes from all four crossings and the presence of virus antigen was not found by ELISA.

Ratio of resistant and susceptible genotypes did not significantly differ from ratio for simple gene presented in heterozygotic stage (*Rxrxrxrxr*) in all parental genotypes with extreme resistance to PVX (Table 3).

The virus did not replicate and did not spread into the uninfected tissues and tubers of resistant genotypes. Thus all resistant genotypes met the requirements for extreme resistance to PVX (Solomon-Blackburn and Barker 2001; Valkonen 1994). On the other hand, plants of the susceptible varieties Cicero, Desirée, and Monalisa did not exhibit clear disease symptoms, but virus presence was clearly detected by ELISA.

Genotypes with the known resistance to PVX (Boda, Fanal, San, Santé, Solara, White Lady) were used for marker selection associated with extreme resistance to PVX, genotypes originating from breeding programmes, which had declared extreme resistance to PVX derived from three Solanum species − *Solanum acaule*, *Solanum vernei* and *Solanum tuberosum* subsp. *andigena* as well as varieties and clones, which resistance was detected on the basis of PVX infection tests.

Table 1

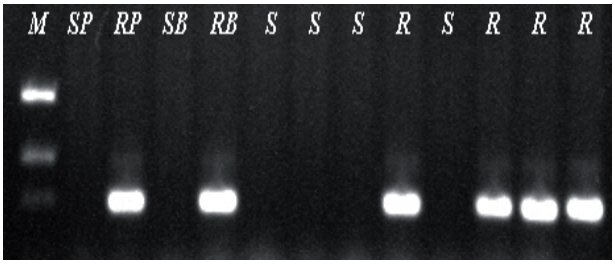
Primer sequences used for PCR amplification of markers associated with *Rx*. Restriction enzymes used to detect polymorphism are shown in brackets below marker if required

Marker name	Forward and reverse primer sequence (5′−3′)	Annealing temperature [°C]
221R	GCT TAC ATT TGC TCG AAG AAG CCAC CCT TAA TAA TCA ATA GAT TCA ACT CG	60
218R (*Alu*I)	GAT TAC AGT TGT GAA TTA GTT CGG TA GCA ACA GAT ATA TTC CAC TTA CCA TTC	60
GP21	AGT GAG CCA GCA TAG CAT TAC TTG GGT TGG CCT ATT AGC CAT GC	56
STM0003	GGA GAA TCA TAA CAA CCA G AAT TGT AAC TCT GTG TGT GTG	53
STM0007	GGA CAA GCT GTG AAG TTT AT AAT TGA GAA AGA GTG TGT GTG	54
STM0030	AGA GAT CGA TGT AAA ACA CGT GTG GCA TTT TGA TGG ATT	55
STM0032	GGC TGC AGG AAT TAT GTG TTC GAT GTA AAA CAC GTG TGC GTG	55

Only one PCR product was detected in resistant genotype using specific marker 221R and no PCR product was observed in susceptible genotypes. Profile of PCR products of resistant and susceptible genotypes from the crossing Boda × Svella is on Figure 1. The application of CAPS marker 218R and digestion of PCR product with restriction endonuclease *Alu*I revealed polymorphism between resistant and susceptible varieties, clones as well as resistant and susceptible genotypes from the crossings Bobr × Svella, Boda × Svella, Fanal × Svella, Solara × Svella (Table 4).

Presence of marker GP21 did not correlate with phenotypic data, ELISA readings and results obtained by the markers 221R and 218R. These results indicate that none of the analysed genotypes carried the *Rx2* gene for extreme resistance to PVX, derived from *Solanum acaule* (Ritter et al. 1991).

The gene *Rx* was mapped on chromosome XII. Four microsatellites located on the same chromosome (STM0003, STM0007, STM0030, STM0032) were used to analyse potato genotypes with extreme resistance to PVX. None of microsatellite markers segregated with ELISA readings and phenotypic data. On the other hand, CAPS marker 218R and allele- specific marker 221R were associated with the *Rx* allele.



M – DNA ladder (1200 bp, 800 bp, 400 bp), SP – susceptible parent variety Svella, RP – resistant parent variety Boda, RB – resistant bulk, R – resistant genotype, SB – susceptible bulk, S – susceptible genotype. The arrow points to the marker product characteristic for a resistant genotype.

Fig. 1. Patterns of amplified DNA of PVX resistant and susceptible genotypes from crossing Boda × Svella indicating allele − specific marker 221R

Totally 90 genotypes from different breeding programmes were tested. Two markers, 221R and 218R, were found in Polish varieties Bobr, Boda, German varieties Fanal, Roeslau, Saphir, Solara, in Hungarian variety White Lady, in American variety Atlantic, Canadian variety Jemseg, in Dutch varieties Darwina, Santé, in Slovak varieties Vila, Erídia and other 10

Table 2

Results of ELISA test after infection using PVX suspension

Genotype	Country of origin	Type of resistance	Source of resistance	ELISA (A_{405}) after infection
Agria	D	ER	*Rx*	0.08
Bobr	PL	ER	*Rx*	0.04
Boda	PL	ER	*Rx*	0.04
Fanal	D	ER	*Rx*	0.03
Santé	NL	ER	*Rx*	0.04
Solara	D	ER	*Rx*	0.07
White Lady	HU	ER	*Rx*	0.07
Cicero	NL	−	−	1.05
Desirée	NL	−	−	1.44
Monalisa	NL	−	−	1.62
Svella	SK	−	−	1.58

Value of ELISA reading greater than 0.1 was considered positive for presence of PVX in tested sample

Slovak clones (Y01/87, VL 143/99, Y02/ 55, Y02/67, Y02/69, Y02/87, Y02/150, VrSt Y/93-102, VrSt Y/93-109, VrSt Y/99-4/14) and confirmed the association of extreme resistance to PVX and markers 221R and 218R. These results are in accordance with previous findings concerning identification of genotypes with extreme resistance to PVX (Kanyuka et al. 1999) and imply that both markers have diagnostic value and are suitable for MAS.

Extreme resistance to PVX was introduced into varieties of North American breeding programme (Atlantic, Jemseg, Saco) from *Solanum tuberosum* subsp. *andigena*. The *Rx* gene from *Solanum acaule* was introduced into the varieties of German breeding programme (Saphir, Roeslau). Extreme resistance to PVX from *Solanum vernei* was introgressed into Dutch varieties Darwina, Produzent and Santé (Ross 1986; Świeżyński, 1994). Extreme resistance to PVX was reliably detected using CAPS marker 218R and allele-specific marker 221R in all tested varieties with the known extreme resistance to PVX as well as in genotypes with unknown origin of extreme resistance to PVX. Thus all genotypes with extreme resistance to PVX, irrespective of their wild species ancestors, possessed the same marker associated with the *Rx* gene of extreme resistance to PVX. Both markers allowed selection of genotypes with extreme resistance to PVX in populations of crossings where one of the parents possesses the gene of extreme resistance to PVX in simplex form (*Rxrxrxrx*).

The gene *Rx* was located on chromosome XII between RFLP markers CT99 and CT129. Analysis of

Table 3

Segregation of resistant and susceptible genotypes to PVX after mechanical infection of seedlings of four crossing combinations

Crossing	Observed number of plants after PVX infection (n)		Theoretical sesgregation ratio	Chi-square value
	resistant	susceptible		
Bobr × Svella	24	26	1:1	0.04
Boda × Svella	5	5	1:1	0.00
Fanal × Svella	6	14	1:1	1.60
Solara × Svella	28	12	1:1	3.20

Critical value of chi-square test is 3.84 for $P_{0.05}$ and 6.635 for $P_{0.01}$

Table 4

Segregation of resistant and susceptible genotypes to PVX determined by PCR using specific primers 221R and 218R in seedlings of four crossings

Crossing	Observed number of plants after PCR (n)		Theoretical segregation ratio	Chi - square value
	resistant	susceptible		
Bobr × Svella	24	26	1:1	0.04
Boda × Svella	5	5	1:1	0.00
Fanal × Svella	6	14	1:1	1.60
Solara × Svella	28	12	1:1	3.20

Critical value of chi-square test is 3.84 for $P_{0.05}$ and 6.635 for $P_{0.01}$

BAC libraries of genotypes carrying genes *Rx* and *rx* affirmed position of the *Rx* gene between markers 77L and 77R. Both markers, 221R and 218R, were found out on chromosome region near marker 77R (Kanyuka et al. 1999). The PCR analysis of 166 genotypes from four crossing combinations confirmed that the *Rx* gene, irrespective of origin, was inherited into progeny together with markers 221R and 218R as a simple dominant gene in segregation ratio 1:1. Ritter et al. (1991) identified two different genes of resistance to PVX – *Rx1* and *Rx2*. The *Rx1* gene was introgressed from *Solanum tuberosum* subsp. *andigena* into the clone CPC1673 and was located on chromosome XII. On the other hand, the *Rx2* gene, introgressed from *Solanum acaule* into the clone MPI 44.1016/10, was localized on chromosome V (Ritter et al. 1991). The results of this study are in accordance with hypothesis that all analysed genotypes possessing the *Rx* gene for extreme resistance to PVX, irrespective of their declared origin, could be derived from one genetic source, which was an ancestor of three species − *Solanum tuberosum* subsp. *andigena*, *Solanum acaule* and *Solanum vernei*, or one of them could be a primary source of the *Rx* gene for extreme resistance to PVX. The genetic studies of relationships between wild species of *Solanum* using different types of molecular markers found *Solanum tuberosum* subsp. *andigena*, *Solanum acaule* and *Solanum vernei* in different clusters (Hawkes 1994; Jacobs et al. 2008; Solomon-Blackburn and Barker 2001; Spooner et al. 2007) and these findings to a certain extent prefer the idea that the source of resistance could be a mutual ancestor. However, the explanation of the origin of related molecular basis for extreme resistance to PVX derived from different wild species will require additional studies.

CONCLUSION

There were selected 2 molecular markers (221R, 218R), suitable for detection of genotypes with extreme resistance to PVX. Usefulness of both markers for marker assisted selection was confirmed by evaluation set of resistant and susceptible varieties, clones and seedling populations from four crossings. New genotypes with extreme resistance to PVX were identified among genotypes maintained in the gene bank.

The gene *Rx* for extreme resistance to PVX was inherited into F1 generation together with markers 221R and 218R and no recombinations were found among 166 genotypes from four crossings.

Extreme resistance to PVX derived from three different wild species *Solanum tuberosum* subsp. *andigena*, *Solanum acaule* and *Solanum vernei* was detected using two markers − 221R and 218R.

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SÚHRN

Introdukcia divorastúcich druhov do kultúrnych genotypov ľuľka zemiakového umožnila získať nové zdroje rezistencie, ktoré zabezpečujú dlhodobý účinok proti vírusom a sú využiteľné v šľachtení nových odrôd. Potomstvo z divorastúcich druhov, *Solanum acaule*, *Solanum tuberosum* subsp. *andigena* a *Solanum vernei*, bolo použité pri získavaní odrôd a klonov s extrémnou rezistenciou proti vírusu zemiaka X (PVX). Pre selekciu vhodných markerov bolo vybratých 90 genotypov z génovej banky a 166 genotypov z kríženia 4 rezistentných genotypov v simplexnej forme (*Rxrxrxrx*) a 1 náchylnej odrody Svella (*rxrxrxrx*). Dva molekulové markery detegovali extrémnu rezistenciu proti PVX v dvoch poľských, štyroch nemeckých, jednom maďarskom, troch holandských, dvanástich slovenských genotypoch uchovávaných v génovej banke a šesťdesiatich troch rezistentných genotypoch z potomstva kríženia rezistentných a náchylných genotypov. Marker 221R je vhodným nástrojom pre markerom asistovanú selekciu v šľachtení ľuľka zemiakového. Výsledky naznačujú, že gén *Rx* pre extrémnu rezistenciu proti PVX mohol pochádzať z jedného genetického zdroja, ktorý bol predchodcom *Solanum tuberosum* subsp. *andigena*, *Solanum acaule* a *Solanum vernei* alebo prítomnosť troch génov s rovnakým markerom mohla vyplynúť zo skutočnosti, že primárnym zdrojom mohol byť aj niektorý z uvedených divorastúcich druhov.

Kľúčové slová: *Solanum tuberosum*, divorastúce druhy, extrémna rezistencia, PVX