Selection of DNA Markers for Detection of Extreme Resistance to Potato Virus *Y* in Tetraploid Potato (*Solanum tuberosum* L.) F_1 Progenies

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Abstract: Marker-assisted selection is progressively introduced into potato breeding especially in the case of monogenically inherited traits. Varieties and clones with extreme resistance to potato virus *Y* (PVY) from German, Hungarian, Polish, Dutch and Slovak breeding programmes were used as female parents in 6 crosses. F_1 progenies were subjected to the bulk segregant analysis. Polymerase chain reaction (PCR) based markers for Ry_{sto} and Ry_{adg} viral resistance alleles were used for the evaluation of varieties, clones and seedlings of F_1 progenies. All resistant F_1 genotypes were reliably identified using three molecular markers, STM0003, $GP122_{718}$ and $GP122_{406}$, associated with the Ry_{sto} allele. No genotype corresponded with phenotypic data and Ry_{adg} markers. Ry_{adg} and three selected Ry_{sto} markers were applied to 40 genotypes from an *in vitro* gene bank and 14 genotypes were recognized to have Ry_{sto} and none of them possessed Ry_{adg}. Indirect selection is based on DNA polymorphism linked to the extreme resistance to PVY resulting in error when polymorphism is not conserved in all genetic backgrounds. A failure to detect extreme resistance to PVY using the Ry_{sto} markers in variety Santé, resistant progeny of variety Santé and clone Y01-30 may be linked to recombination events or different *Solanum stoloniferum* background.

Keywords: potato; potato virus *Y* (PVY); extreme resistance; DNA markers; Ry_{sto}

Potato virus *Y* (PVY) belongs to a group of the most important potato viruses infecting the potato (*Solanum tuberosum*, L.) and being transmitted from infected to healthy potato by aphids in a non-persistent manner. Depending on the genotype, PVY may reduce the yield from 10 to 80% (De Bokx & Huttinga 1981). The strain PVY^{NTN} affects the tuber quality negatively by forming necrotic ring (Leromancer & Nedellec 1997). In order to prevent the spread of PVY from one vegetative generation to the next one, many countries have implemented seed certification schemes that guarantee virus-free seed potatoes (Hinrich *et al.* 1998). The control of the virus spread is based on different methods of prevention including chemical protection from virus vectors, elimination of infection sources and breeding of new varieties with extreme resistance (Kang *et al.* 2005). However, the use of varieties with extreme resistance to PVY is considered to be the most effective and environment friendly way of potato protection from PVY.

Natural genes for resistance to PVY were detected in wild potato species and were introgressed into the genome of cultivated potato (Valkonen 1994). Basically the resistances to PVY are grouped into

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two main classes. Hypersensitivity, expressed as a necrotic reaction to infection, is conferred by *N* genes. Plants with hypersensitive resistance (HR) show a local necrotic reaction or systemic necrosis, which inhibits the virus spread in spite of the virus presence in affected leaves. Extreme resistance (ER) is considered to be durable since *Ry* is effective against a wide range of known PVY strains. Plants with ER to the virus show no symptoms or limited necrosis when inoculated with PVY. Only an extremely low amount of the virus, if any, can be detected by sensitive techniques (Barker 1996; Solomon-Blackburn & Barker 2001).

ER genes introduced into the potato gene pool have originated from relatively few sources. The genes conferring extreme resistance to PVY are known in *Solanum tuberosum* subsp. *andigena*, *Solanum hougasii* and *Solanum stoloniferum* and were used in different breeding programmes (Swieżynski 1994). The Ry_{adg} gene was introduced into various breeding lines, however it has not been used widely (Galvez *et al.* 1992). Resistance from *Solanum chacoense* is inherited in a dominant, monogenic way. Two varieties with resistance to PVY from *Solanum chacoense* were released from a Japanese breeding programme (Sato *et al.* 2006). Also, the extreme resistance to PVY from *Solanum stoloniferum* was introgressed into *Solanum tuberosum* and about 20 varieties in Europe were listed to carry the Ry_{sto} gene (Ross 1986).

The extreme resistance gene Ry_{adg} from *Solanum tuberosum* subsp. *andigena* was mapped on chromosome XI (Hämälainen *et al.* 1997). The sequence characterized amplified region (SCAR) marker RYSC3 developed for the detection of Ry_{adg} (Kasai *et al.* 2000) was used in breeding for resistance to PVY (Ruiz de Arcaute *et al.* 2002). Using map location marker 38-530 in RAPD along with segregating diploid F_2 population from the cross of *Solanum chacoense* and *Solanum phureja* Juz. et Buk., the Ry_{chc} gene was deduced to be located on chromosome IX (Hosaka *et al.* 2001). The location of this gene is different from the locations of other genes of extreme resistance to PVY, possibly in one of the resistance gene clusters (Sato *et al.* 2006). The Ry_{sto} gene from *Solanum stoloniferum* was originally mapped on chromosome XI at the same position as Ry_{adg} (Brigneti *et al.* 1997), two additional mapping attempts proved that it resides on chromosome XII (Flis *et al.* 2005; Song *et al.* 2005).

Genetic analyses of potato were usually carried out at a diploid level to avoid problems with the heterozygous constitution of the cultivated potato. In this study tetraploid F_1 populations were obtained from the crosses of genotypes with extreme resistance to PVY. Female Ry_{sto} parents were selected from different European breeding programmes and hybridized with PVY susceptible male parents. The hybrid populations obtained so far were used for the bulk segregant analysis, for the study of genetic relationships between Ry_{sto} varieties and for the screening of valuable markers for marker-assisted selection.

MATERIAL AND METHODS

The tetraploid F_1 populations consisting of 370 genotypes from 6 different cross combinations (San × C2264, White Lady × C2264, Santé × C2264, Fanal × C2264, Y 02-05 × Cicero, Y 02-90 × Cicero) were used for a virus resistance assay and for marker evaluation. These populations were obtained from *Solanum stoloniferum* varieties and clones with ER to PVY which had originated from breeding programmes in Poland, Hungary, the Netherlands, Germany and Slovakia. The groups of resistant (Bettina, Bobr, Boda, Fanal, Forelle, San, Santé, White Lady, Alva, Y 02-05, Y 02-14, Y 02-90, Y 02-112, Y 01-30) and susceptible (Agria, C2264, Desirée, Impala, Viola, Cicero) varieties and clones along with additional 20 genotypes from the gene bank of Potato Research and Breeding Institute, Veľká Lomnica, Slovakia were used for marker verification (Table 3).

Seedlings from the six combinations and ten virus-free plants of each variety and clone were mechanically inoculated with PVY suspension. Leaves were inoculated mechanically using a mixture which consisted of the sap of infected potato leaves with PVY-3 isolate (NTN strain) and 0.1M phosphate buffer pH 7.2.

The course of infection in individual plants was inspected visually for disease symptoms and tested by ELISA four weeks after inoculation. Antibodies for ELISA were purchased from Bioreba AG (Reinach, Switzerland). Plants were considered positive when the value of A_{405} was higher than 0.1.

After vegetation three tubers were collected from each plant and subsequently planted to examine the PVY presence in tuber progeny plants. Visual inspection and ELISA were performed four weeks after emergence. It was taken for sure that a genotype is resistant if it failed to become infected with PVY after mechanical inoculation and if no

infected tubers in the progeny were found by visual inspection and ELISA.

Resistant and susceptible pools were created on the basis of visual symptoms and ELISA readings. The resistant DNA bulk originated from 2 uninfected plants of 5 combinations (San × C2264, White Lady × C2264, Fanal × C2264, Y 02-05 × Cicero, Y 02-90 × Cicero) and the susceptible bulk using infected plants was prepared in the same way. Individual plants of both the resistant and the susceptible pool were also used in all combinations for verification of selected markers.

DNA was extracted from leaves according to the CTAB method (Rogers & Bendich 1994) with minor modifications. Young leaves (500 mg) frozen in liquid nitrogen were ground in a mortar and transferred into glass tubes with 3 ml of 2× CTAB buffer. The mixture was incubated for 1 hour at 60°C. An equal volume of chloroform/isoamyl alcohol (24:1) was added and the tube was inverted for 5 min. After centrifugation (20 min, 3000 g) the supernatant was treated with RNase (10 mg/ml) for 1 hour at room temperature and repeatedly extracted with chloroform/isoamyl alcohol and centrifuged. DNA

Table 1. Primer sequences used for the PCR amplification of markers associated with Ry_{sto}; restriction enzymes used to detect polymorphism are shown in brackets below the marker if required

Marker name	Forward and reverse primer sequences (5'–3')	Annealing temperature (°C)
M33[a] (AluI)	GGC GAA GAA TTG TCA TCG CCG TTG CCA GTG TAA TCC CAT AGG TCC GGG	56
M39[a] (DdeI)	AAT CTT GAG GAG GTT TTC AAA CTC AAT TAG GTG ACA ACC AAT ATG AG	56
M35[a]	GAC TGC GTA CAT GCA GAG TTA G CGT ATT TGG AGA CTT AGA CCC AGT CC	56
GP163[a]	CTG CAG TTT TGA AAT TAC CAT CT CTG CAG CCA ACT GAT AAC TCT CA	56
CT182[a]	GGG AGG GAA CAA GTT ACT CTA GCC AAC TTC TTA GGC CGT TTC	56
$GP122_{718}$[b] (EcoRV)	TAT TTT AGG GGT ACT TCT TTC TTA GAT ACT TCC AAC CGC TTC AC	53
$GP122_{406}$[c] (EcoRV)	CAA TTG GCT CCC GAC TAT CTA CAG ACA ATT GCA CCA CCT TCT CTT CAG	53
GP81[b]	GCA GCG TTT CCT ACA AT AGA GAC TAA TGC TGA AAA T	50
GP204[b]	CAT AGA TGG CTC AAA CAA CTC GTG GAA ACA TGG CTT ACC	56
GP269[b]	TCG CAA TGA AAG ATA AGC TGT GAT AAA GAG TGT AGC AGT C	53
UBC857[b]	ACA CAC ACA CAC ACA CG	42
STM0003[d]	GGA GAA TCA TAA CAA CCA G AAT TGT AAC TCT GTG TGT GTG	53
RYSC3[e]	ATA CAC TCA TCT AAA TTT GAT GG AGG ATA TAC GGC ATC ATT TTT CCG A	60

[a]Brigneti *et al.* (1997), [b]Flis *et al.* (2005), [c]Marczewski (personal communication), [d]Song *et al.* (2005), [e]Kasai *et al.* (2000)

was precipitated from the supernatant by adding 2/3 of the isopropanol volume and collected by 10 sec. spinning. The DNA pellet was rehydrated in high salt TE buffer, precipitated by two volumes of 100% ethanol and centrifuged. Purified DNA was washed in 80% ethanol, dried at room temperature and rehydrated in TE buffer. The final concentration of DNA was adjusted at 30 ng/μl.

PCR analysis was performed in 25 μl reaction volume which consisted of 1× PCR buffer (20mM Tris-HCl, 50mM KCl), 0.2mM of each dNTPs, 3.0mM $MgCl_2$, 300nM 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. PCR products were separated by electrophoresis in 1.5% agarose gel in Tris-Borate-EDTA (1× TBE) buffer and visualised with ethidium bromide staining. Alternatively PCR products were separated in 6% polyacrylamide gels and visualised with silver staining. Polymorphic bands were scored as present or absent and their goodness-of-fit to the expected 1:1 segregation ratio was checked by chi-square test.

RESULTS

Virus-free plants of resistant and susceptible varieties along with clones and 370 F_1 plantlets from 6 crosses were mechanically inoculated using a PVY suspension at 4–6 leaf stage. Visible symptoms of PVY infection were recorded on plants of

Table 2. Results of ELISA after infection with PVY in tuber progeny

Genotype	Country of origin	Type of resistance	Source of resistance	ELISA (A_{405}) after infection/in progeny
Bettina	D	ER	Ry_{sto}	0.01/0.02
Fanal	D	ER	Ry_{sto}	0.02/0.02
Forelle	D	ER	Ry_{sto}	0.02/0.03
Bobr	PL	ER	Ry_{sto}	0.02/0.02
Boda	PL	ER	Ry_{sto}	0.02/0.02
San	PL	ER	Ry_{sto}	0.02/0.03
Santé	NL	ER	Ry_{sto}	0.01/0.02
White Lady	HU	ER	Ry_{sto}	0.01/0.02
Alva	SK	ER	Ry_{sto}	0.02/0.02
Y 01-30	SK	ER	unknown	0.02/0.02
Y 02-05	SK	ER	Ry_{sto}	0.04/0.04
Y 02-14	SK	ER	Ry_{sto}	0.03/0.03
Y 02-90	SK	ER	Ry_{sto}	0.02/0.02
Y 02-112	SK	ER	Ry_{sto}	0.03/0.03
Agria	D	S	–	2.00/2.00
Desirée	NL	S	–	2.00/2.00
Impala	NL	S	–	2.00/2.00
Cicero	NL	S	–	2.00/2.00
C2264	SK	S	–	2.00/2.00
Viola	SK	S	–	2.00/2.00

The value of ELISA reading higher than 0.1 was considered positive for the presence of PVY in the tested sample
ER – extreme resistance; S – susceptibility

Table 3. The presence of markers STM0003, $GP122_{718}$, $GP122_{406}$ and RYSC3

Genotype	Marker STM0003	Marker $GP122_{718}$	Marker $GP122_{406}$	Marker RYSC3
Bettina	+	+	+	–
Bobr	+	+	+	–
Boda	+	+	+	–
Fanal	+	+	+	–
Forelle	+	+	+	–
San	+	+	+	–
Santé	–	–	–	–
White Lady	+	+	+	–
Alva	+	+	+	–
Y 01-30	–	–	–	–
Y 02-05	+	+	+	–
Y 02-14	+	+	+	–
Y 02-90	+	+	+	–
Y 02-112	+	+	+	–
Agria	–	–	–	–
C2264	–	–	–	–
Desirée	–	–	–	–
Impala	–	–	–	–
Viola	–	–	–	–
Cicero	–	–	–	–
Albina	–	–	–	–
Breza	–	–	–	–
Ciklamen	+	+	+	–
Čajka	–	–	–	–
Eta	–	–	–	–
Iva	–	–	–	–
Jarabina	–	–	–	–
King Edward	–	–	–	–
Lipa	–	–	–	–
Lipta	–	–	–	–
Livera	–	–	–	–
Lomnica	–	–	–	–
Lúčnica	–	–	–	–
Nela	–	–	–	–
Patria	–	–	–	–
Rema	–	–	–	–
Solara	+	+	+	–
Sosna	–	–	–	–
Tatranka	–	–	–	–
Vila	–	–	–	–

+ = presence of marker, – = absence of marker

susceptible genotypes 3–5 weeks after inoculation. The symptoms of PVY infection on plants were exhibited as mosaic, rugosity, and vein necrosis on leaves in susceptible genotypes. Young uninoculated leaves from each plant were analysed for the presence of virus antigen. The presence of PVY was proved in each plant by visual symptoms of PVY infection and confirmed by ELISA (Table 2). There was no evidence of PVY accumulation in newly developed leaves of resistant genotypes.

Three tubers were taken from each inoculated plant and planted in the glasshouse. Infected plants of susceptible genotypes showed clear visual symptoms on leaves and the presence of PVY was confirmed by ELISA four weeks after emergence (Table 2). Plants grown from infected tubers of susceptible genotypes exhibited more enhanced symptoms than primarily infected plants.

No symptoms were developed on plants of resistant genotypes. ELISA did not reveal PVY in plants without symptoms and without visible necrotic reaction. The ratio of resistant to susceptible genotypes was near the 1:1 ratio and did not differ significantly from this ratio for a single gene present in heterozygous stage (Ryryryry) in all parental genotypes with extreme resistance to PVY (Table 4).

Using the cleavage amplified polymorphic sequence (CAPS) markers $GP122_{718}$ and $GP122_{406}$ PCR amplicons were obtained which after digestion with EcoRV showed polymorphism between resistant and susceptible varieties, clones (Table 3) and genotypes from the crosses San × C2264, White Lady × C2264, Fanal × C2264, Y 02-05 × Cicero and Y 02-90 × Cicero (Table 5). Restriction profiles of resistant and susceptible genotypes from the cross San × C2264 are shown in Figure 1. The use of marker STM0003 resulted in the amplification of a small about 110 bp product found in PAGE as a clear band present in all resistant genotypes. The segregation ratios of phenotypic data did not differ from segregation ratios based on three PCR markers (Table 5).

Neither variety Santé nor resistant genotypes from the cross Santé × C2264 displayed the resistant associated bands of 718 bp ($GP122_{718}$), 406 bp ($GP122_{406}$) and 110 bp (STM0003) in size. Additionally, the utility of RYCS3 marker for detection of Ry_{adg} was not proved in PCR.

The markers GP81, GP204, GP269 and UBS857 did not show full congruence with the results of $GP122_{718}$, $GP122_{406}$ and STM0003 markers in 5 combinations. The markers M33, M35, M39, GP163 and CT182 did not correlate with phenotypic data and ELISA readings.

Totally 80 genotypes from different breeding programmes were tested. All the three markers were found in Polish varieties San, Bobr, Boda, German varieties Forelle, Fanal, Bettina, Solara, in Hungarian varieties White Lady, Ciklamen, in Slovak variety Alva and in clones Y02-05, Y02-14, Y02-90, Y02-112. The extreme resistance was confirmed in the clone Y01-30 using infection tests, but no adequate PCR products were found with Ry_{sto} and Ry_{adg} markers.

DISCUSSION

In our experiments varieties with known resistance to PVY (Bettina, Fanal, Forelle, Santé, White

Table 4. Segregation of PVY resistant and susceptible genotypes after mechanical infection of seedlings of six crosses

Cross	Observed segregation ratio in the number of plants after infection (n)		Theoretical segregation ratio	χ^2 value
	resistant	susceptible		
San × C2264	28	32	1:1	0.13
Santé × C2264	21	29	1:1	0.64
White Lady × C2264	45	33	1:1	0.92
Fanal × C2264	24	29	1:1	0.24
Y 02-05 × Cicero	22	24	1:1	0.04
Y 02-90 × Cicero	40	43	1:1	0.05

The critical value of χ^2 test is 3.84 for $P_{0.05}$ and 6.635 for $P_{0.01}$

Table 5. Segregation of PVY resistant and susceptible genotypes determined by PCR using specific primers in seedlings of six crosses

Cross	Observed segregation ratio in the number of plants after infection (*n*)		Theoretical segregation ratio	χ^2 value
	resistant	susceptible		
(a) STM0003				
San × C2264	28	32	1:1	0.13
Santé × C2264	0	50	1:1	25.0*
White Lady × C2264	45	33	1:1	0.92
Fanal × C2264	24	29	1:1	0.24
Y 02-05 × Cicero	22	24	1:1	0.04
Y 02-90 × Cicero	40	43	1:1	0.05
(b) $GP122_{718}$				
San × C2264	28	32	1:1	0.13
Santé × C2264	0	50	1:1	25.0*
White Lady × C2264	45	33	1:1	0.92
Fanal × C2264	24	29	1:1	0.24
Y 02-05 × Cicero	22	24	1:1	0.04
Y 02-90 × Cicero	40	43	1:1	0.05
(c) $GP122_{406}$				
San × C2264	28	32	1:1	0.13
Santé × C2264	0	50	1:1	25.0*
White Lady × C2264	45	33	1:1	0.92
Fanal × C2264	24	29	1:1	0.24
Y 02-05 × Cicero	22	24	1:1	0.04
Y 02-90 × Cicero	40	43	1:1	0.05

The critical value of chi-square test is 3.84 for $P_{0.05}$ and 6.635 for $P_{0.01}$
*significant difference ($P \leq 0.01$)

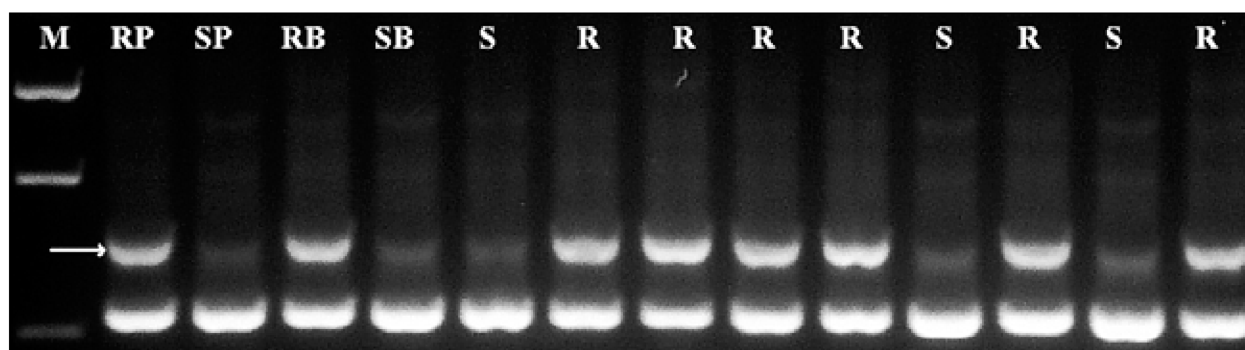

Figure 1. Patterns of the amplified DNA of PVY resistant and susceptible genotypes from the cross San × C2264 indicating CAPS marker $GP122_{718}$ after digestion with *EcoRV*

M – DNA ladder as molecular size marker (1200 bp, 800 bp, 400 bp), RP – resistant parent San, SP – susceptible parent C2264, RB – resistant bulk, SB – susceptible bulk, R – resistant individual, S – susceptible individual; the arrow points to the marker product

Lady) were used along with the varieties and clones the resistance of which was determined on the basis of PVY infection tests. Resistant varieties and clones exhibited no symptoms after infection. We did not observe any limited necrosis at the place of infection. The virus did not replicate and did not spread into the uninfected tissues and tubers of resistant genotypes. Additionally, no plants grown from the tubers of inoculated resistant material showed any symptoms and no virus was detected by ELISA. Thus all resistant genotypes originating from Polish, Hungarian, German, Dutch and Slovak breeding programmes met the requirements for extreme resistance to PVY (Valkonen 1994; Solomon-Blackburn & Barker 2001). On the other hand, plants of the varieties Desirée, Impala, Viola, Cicero and clone C2264 showed mosaic symptoms, mottling and vein necrosis with the virus detected by ELISA.

The SCAR marker RYSC3 (Kasai *et al.* 2000) for the selection of genotypes with resistance from *Solanum tuberosum* subsp. *andigena* was not detected in any PVY resistant varieties tested in this study. The same is true also of the German (Song *et al.* 2005) and Polish varieties (Flis *et al.* 2005). These results are in accordance with the conclusion of Galves *et al.* (1992) that *Solanum tuberosum* subsp. *andigena* was used as a source of extreme resistance to PVY mainly in breeding programmes in North and South America.

The use of one F_1 generation, where one of the parents was of Ry_{sto} genotype with different pedigree history, resulted in the selection of different markers. Applications of such markers for the selection of PVY resistant genotypes may lead to inconsistent results. Therefore in this study the resistant bulk was assembled from DNA samples isolated from 10 resistant plants, two DNA samples from 5 crosses where Ry_{sto} parents originated from different breeding programmes. So it was possible to select quickly and reliably 3 markers associated with Ry_{sto} – STM0003, $GP122_{718}$ and $GP122_{406}$ (Flis *et al.* 2005; Song *et al.* 2005; Marczewski, personal communication). Only these three markers co-segregated with all resistant genotypes of five F_1 populations. None of the markers identified by Brigneti *et al.* (1997) showed polymorphism between resistant and susceptible DNA pools. The failure to use these markers could be connected with insufficient pedigree information (Gebhardt & Valkonen 2001) or the markers could be associated with recombination events in crosses and could participate in dissociation between the marker and the phenotype.

The allele $Ry\text{-}f_{sto}$ was mapped on chromosome XII and marker $GP122_{718}$ was successfully used for the detection of this allele in German and Polish varieties which possess extreme resistance from *Solanum stoloniferum* (Flis *et al.* 2005). On the contrary, Song *et al.* (2005) failed to select the extremely resistant Polish varieties using the same marker $GP122_{718}$ concluding that $Ry\text{-}f_{sto}$ and Ry_{sto} represent two different genes for extreme resistance to PVY. The analysis of 370 F_1 individuals from crosses of German, Polish, Hungarian and Slovak varieties and clones with three markers (STM0003, $GP122_{718}$ and $GP122_{406}$) provided the unambiguous identification of resistant genotypes of 5 combinations supporting the idea that Ry_{sto} and $Ry\text{-}f_{sto}$ may represent the same allele.

On the basis of segregation ratios of phenotypic data in the progenies of the varieties San, Santé, Fanal, White Lady and clones Y 02-05 and Y02-90 it was confirmed that extreme resistance to PVY is inherited by a single dominant gene. The results of the analysis obtained with molecular markers were in accordance with phenotypic data except for the variety Santé, progeny of Santé and one clone Y01-30. Clone CPC 2093(sto) found in the pedigree of variety Santé (http://www.dpw.wau.nl/pv/) possessed extreme resistance to PVY from *Solanum stoloniferum*. The failure to detect extreme resistance in the variety Santé, in its progeny and in clone Y01-30 using markers for Ry_{sto} and Ry_{adg} detection allows us to speculate about the more unlike alleles derived from different sources of *Solanum stoloniferum* that were introgressed into cultivated potato.

Molecular markers are used in genetic studies (Kardolus *et al.* 1998), variety differentiation (Kormuťák *et al.* 1999; Braun & Wenzel 2004/5; Braun *et al.* 2004/5; Moisan-Thiery *et al.* 2005), identification of genes conferring resistance to diseases and pests (Niewöhner *et al.* 1995; Celebi-Toprak *et al.* 2002) and in marker-assisted selection (MAS). MAS in the breeding context involves scoring indirectly for the presence or absence of a desired phenotype based on banding patterns of molecular markers located in or near the genes controlling resistance to PVS (Marczewski *et al.* 2001, 2002), resistance to PVX (Ritter *et al.* 1991; Kanyuka *et al.* 1999), resistance to PVM (Marczewski *et al.* 2006) and resistance to PVY. Banding patterns of STM003,

$GP122_{718}$ a $GP122_{406}$ are indicative of the presence or absence of a Ry_{sto} and can be used for selection purposes. There is still a lack of evidence on the nature of extreme resistance in variety Santé and on its relationship to different sources of extreme resistance from *Solanum stoloniferum*.

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