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Marker-assisted combination of major genes for pathogen resistance in potato

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Abstract Closely linked PCR-based markers facilitate the tracing and combining of resistance factors that have been introgressed previously into cultivated potato from different sources. Crosses were performed to combine the Ry_{adg} gene for extreme resistance to *Potato virus Y* (PVY) with the *Gro1* gene for resistance to the root cyst nematode *Globodera rostochiensis* and the *Rx1* gene for extreme resistance to *Potato virus X* (PVX), or with resistance to potato wart (*Synchytrium endobioticum*). Marker-assisted selection (MAS) using four PCR-based diagnostic assays was applied to 110 F1 hybrids resulting from four 2× by 4× cross-combinations. Thirty tetraploid plants having the appropriate marker combinations were selected and tested for presence of the corresponding resistance traits. All plants tested showed the expected resistant phenotype. Unexpectedly, the plants segregated for additional resistance to pathotypes 1, 2 and 6 of *S. endobioticum*, which was subsequently shown to be inherited from the PVY resistant parents of the crosses. The selected plants can be used as sources of multiple resistance traits in pedigree breeding and are available from a potato germplasm bank.

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Introduction

Pests and diseases are the major threat to potato cultivation worldwide, particularly on smallholder farms in less developed countries, where certified seed potatoes and chemical protection are not generally accessible, but also in organic farming in industrialized countries. Classical breeding for resistance to pests and pathogens involves the identification of resistance sources, which are often found in wild and unadapted germplasm, the introgression of resistance factors into cultivars by repeated backcrossing to different *S. tuberosum* ssp. *tuberosum* breeding clones and phenotypic selection of resistant progeny (Ross 1986). Over the past 15 years, a number of these resistance factors introgressed in previous decades has been located on the potato molecular linkage map using DNA-based markers. They were mapped either as major genes (*R* genes) or as quantitative resistance loci (QRL; reviewed in Gebhardt and Valkonen 2001). The knowledge of map position and closely linked DNA-based markers now facilitates to trace and combine resistance factors from different sources, using as parents resistant genotypes from the corresponding mapping populations.

Potato virus Y (PVY, genus *Potyvirus*) is one of the most important viral pathogens of the cultivated potato. A single, dominant gene for extreme resistance (ER) to PVY, Ry_{adg}, has been identified in *S. tuberosum* ssp. *andigena* (Muñoz et al. 1975), and was mapped to a distal position on potato chromosome XI (Hämäläinen et al. 1997). The Ry_{adg} gene is closely linked to a gene family with high-sequence homology to the tobacco *N* gene for resistance to *Tobacco mosaic virus* (Leister et al. 1996; Vidal et al. 2002). *N* is the prototype for resistance genes of the Solanaceae family that have in common a nucleotide binding (NB) domain and a leucine-rich

repeat (LRR) domain (Whitham et al. 1994; Leister et al. 1996; Hämäläinen et al. 1998; Hehl et al. 1999; Vidal et al. 2002). Based on polymorphisms in resistance-gene-like (RGL) sequences linked to the Ry_{adg} locus, a diagnostic PCR-based marker has been developed for the Ry_{adg} gene (Sorri et al. 1999; Kasai et al. 2000). It simultaneously functions as a marker for the gene Na_{adg} that is tightly linked to Ry_{adg} in the same gene cluster (Hämäläinen et al. 1998). Na_{adg} controls efficient hypersensitive, non strain-specific resistance (HR) to *Potato virus A* (PVA) by blocking phloem loading and systemic movement of the virus from the inoculated leaves (Hämäläinen et al. 2000).

Potato wart is a quarantine disease caused by the soilborne fungus *Synchytrium endobioticum* (Hampson 1996). A single dominant gene for resistance to *S. endobioticum* pathotype 1, *Sen1* of unknown origin, was detected in diploid germplasm of the Max-Planck Institute (MPI) for Plant Breeding Research and mapped to a similar position on potato chromosome XI as the Ry_{adg} gene (Hehl et al. 1999). Like Ry_{adg}, *Sen1* is linked to *N* homologous genes.

The root cyst nematodes *Globodera rostochiensis* and *Globodera pallida* are important soil pathogens in middle Europe, and the incorporation of resistance to *G. rostochiensis* is compulsory for the release of new potato varieties. Several resistance factors have been introgressed in the past into breeding clones and cultivars from *S. tuberosum* spp. *andigena*, *S. vernei* and *S. spegazzinii* (Ross 1986). The dominant gene *Gro1* for resistance to all known pathotypes of *G. rostochiensis* most likely originated from *S. spegazzinii* and was mapped to potato chromosome VII (Barone et al. 1990). *Gro1* is possibly identical with the *Fb* gene first described by Ross (1962). At the molecular level, the *Gro1* locus consists of a clustered family of NB-LRR type genes. One member of this family, *Gro1–4*, was shown to confer resistance to *G. rostochiensis* pathotype Ro1 (Paal et al. 2004).

Major genes for ER to *Potato virus X* (PVX, genus *Potexvirus*) were also identified in diploid breeding clones of the MPI for Plant Breeding Research, and the dominant genes *Rx1* and *Rx2* were mapped to potato chromosomes XII and V, respectively (Ritter et al. 1991). The origin of *Rx1* and *Rx2* is unclear from pedigree information. ER to PVX has been introgressed from *S. tuberosum* spp. *andigena* and *S. acaule* (Ross 1986). Most likely, *Rx1* corresponds to Ry_{adg} from *S. tuberosum* spp. *andigena*, whereas *Rx2* corresponds to Rx_{acl} from *S. acaule* (Ritter et al. 1991).

In this paper we describe the use of closely linked, PCR-based markers for combining the Ry_{adg} gene for extreme resistance to PVY with *Gro1* for nematode resistance and with *Rx1* for extreme resistance to PVX, or with *Sen1* for wart resistance. The resulting potato clones were shown to have multiple pathogen resistance traits. They are available from the IPK (Institut für Pflanzengenetik und Kulturpflanzenforschung) potato germplasm bank maintained at 18190 Groß-Lüsewitz, Germany.

Materials and methods

Plant material

Parental genotypes Ry126 (v2-126) and Ry62 (v2-62) are F1 hybrids of the cross between the dihaploid ($2n = 2\times = 24$) clone 2×(V-2)7 containing the Ry_{adg} gene for ER to *Potato Virus Y* (PVY), and the susceptible diploid clone 84.194.30 (Valkonen et al. 1994; Hämäläinen et al. 1997). Genotypes Ry126 and Ry62 both carried the Ry_{adg} resistance gene. Parental genotypes F1840/52 and F1840/67 were F1 hybrids of the cross between the diploid clones P18 carrying the *Rx1* gene for ER to *Potato Virus X* (PVX), and P40 having the *Gro1* gene for resistance to *Globodera rostochiensis* (Ritter et al. 1991; Barone et al. 1990). Plants F1840/52 and F1840/67 were selected from the F1840 family (Gebhardt et al. 1991) for carrying both the *Rx1* and *Gro1* resistance genes (*Rx1*/*rx1*, *Gro1*/*gro1*). The diploid clone P3 (H80.577/1) was heterozygous for the *Sen1* gene for resistance to *Synchytrium endobioticum* pathotype 1 (Hehl et al. 1999).

Determination of ploidy level

The average number of chloroplasts per guard cell was determined by counting the chloroplasts in four guard cells each of three different leaves (Frandsen 1968; Rothacker et al. 1966). Tetraploid standards were the cvs Desiree and Linda.

DNA extraction

Total genomic DNA was extracted from frozen leaflets of seedlings using the DNeasy plant mini kit (Qiagen, Hilden, Germany) following the supplier's instructions.

Diagnostic PCR marker assays

The Ry_{adg} gene was selected using the SCAR marker RYSC3 as described by Kasai et al. (2000). The *Rx1* gene was selected with CAPS marker CP60 after restriction with *DdeI* according to Bendahmane et al. (1997). A 350 base pair *DdeI* fragment was diagnostic for *Rx1* in progeny of the resistant parent P18. *Sen1* was selected based on the PCR assay for cDNA clone Nl25 (Hehl et al. 1999) as described in Bormann et al. (2004). Nl25 primers amplified a 1,400 bp fragment in the wart resistant clone P3, which was diagnostic for *Sen1* in progeny of P3. The *Gro1* locus was selected by using specific primers for the *Gro1–4* resistance gene (Paal et al. 2004). The forward primer was 5′-TCTTTGGA GATACTGATTCTCA-3′ and the reverse primer was 5′-CGACCTAAAATGAAAAGCATCT-3′. PCR was performed in a total volume of 25 µl containing 0.2 mM

dNTPs, 2 mM $MgCl_2$, 0.4μM of each primer and 1.25 U Taq DNA polymerase in the reaction buffer provided by the manufacturer (Invitrogen, Carlsbad, CA, USA). PCR conditions were as follows: 3 min at 94°C, followed by 35 cycles of 45 s at 92°C, 45 s at 58°C and 1 min at 72°C and finally 10 min at 72°C.

Resistance tests

Tests for resistance to PVY, PVA and PVX

Plants were grown in an insect-proof greenhouse at the Genetics Centre, SLU, Uppsala. The virus isolates (PVY^N-RUS, PVA-U and PVX-UK) used have been described (Valkonen et al. 1996; Valkonen 1997). They were maintained in potato cv. Pito. Plants were multiplied by taking stem cuttings and rooting them in soil. Inoculation with viruses was carried out by side-grafting a scion from the virus-infected potato cv. Pito onto a 4-week old test potato plant, as described (Valkonen et al. 1994, 1996). A total of four plants of each genotype were tested with each virus in two experiments during January and February. Daylight was extended to 18 h using illumination from fluorescent lamps. Night and day temperatures were 18–20 and 20–25°C, respectively. Inoculated plants were observed for symptoms and the upper fully expanded leaves were tested for viruses by double antibody sandwich ELISA (DAS-ELISA) at 3 and 5 weeks post-inoculation. PVY and PVX were tested with polyclonal antibodies (Boehringer, Mannheim, Germany), whereas PVA was tested with monoclonal antibodies (Adgen, Ayr, Scotland), as previously described (Hämäläinen et al. 2000). Virus-infected cv. Pito and healthy potato plants were included as positive and negative controls, respectively. Absorbances (A_{405}) were recorded with a Benchmark microtitre plate reader (Bio-Rad Laboartories, Hercules, CA, USA) when the positive controls gave the value of 2.50.

Test for nematode resistance

Three shoot cuttings of each clone were inoculated with *G. rostochiensis* pathotype Ro1 as described (Paal et al. 2004). Newly formed cysts were extracted from dried soil through the Fenwick can and counted.

Test for wart resistance

Tubers of plants grown in pots in the greenhouse were tested for resistance to *S. endobioticum* pathotypes 1, 2 and 6 at the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau (Freising-Weihenstephan, Germany) based on the methods of Glynne (1925) and Lemmerzahl (1930). Up to 10 tubers per clone were inoculated with each pathotype. When less than 30 tubers were available per clone, the test for resistance to pathotype 1 was prioritized.

Field test

In the first week of May 2001, five tubers per clone were planted in the field at the MPI for Plant Breeding Research in one row without replication. Seed tubers were from plants grown in pots in the greenhouse in the previous year. The plot was not treated with chemicals other than once with Sprucid (Neudorf) against an infestation with the Colorado potato beetle. Tubers were harvested in the second week of October. Tuber weight and tuber starch content were determined after combining the tubers of all plants per plot.

Results

The PVY resistant, full sib clones Ry126 and Ry62, were crossed as pollen parent either with the full sib clones F1840/52 and F1840/67, both being resistant to PVX and *G. rostochiensis*, or the wart resistant clone P3 as seed parent (Table 1). Seed set was very low in all four cross-combinations. Seed germination rates were between 75 and 92%, and 110 seedling plants were obtained from the four crosses (Table 1). Of 110 plants screened with marker RYSC3 (Fig. 1) for presence of the Ry_{adg} gene, 50 plants (45%) were positive. This segregation ratio fitted the model of a single dominant gene present in the heterozygous state in the Ry parents ($\chi^2 = 0.909$, $P > 0.5$). All 68 plants of families H98A and H98B were then screened for presence of *Gro1* using the *Gro1–4* specific marker assay (Fig. 1). Forty-five positive plants (66%) were found, more than expected for the 1:1 segregation ratio of a dominant heterozygous gene ($\chi^2 = 7.117$, $P < 0.01$). Twenty-one plants (31%) were positive for both markers, RYSC3 and Gro1–4. These 21 plants were subsequently screened for presence of the *Rx1* gene with the diagnostic restriction fragment *Dde*I-350 of marker CP60 (Fig. 1), and 16 positive plants were identified. The 16 of 68 plants (23%) had therefore all three markers, twice as many as expected for three independent dominant genes, each heterozygous present in the parent ($\chi^2 = 13.235$, $P < 0.005$). The 17 plants of families H98C and H98D, which were RYSC3 positive, were further screened for presence of the PCR fragment Nl25-1400, which was diagnostic for the *Sen1* gene (Fig. 1), and 14 positive plants were identified. The 14 of 42 plants (33%) of families H98C and H98D had therefore both markers RYSC3 and Nl25-1400. This number did not significantly deviate from the number expected for two dominant, independent genes, both present in heterozygous state in the parents ($\chi^2 = 2.333$, $P > 0.5$).

Based on pedigree information, 2× by 2× crosses had been performed between all parental clones. However, the selected F1 hybrids exhibited a growth phenotype typical for tetraploid potato plants having a more vigorous growth habit and broader leaflets when compared to diploid plants. Chloroplast counts were therefore conducted in guard cells of parental and progeny plants,

Table 1 Summary of crosses made and plants selected with PCR markers RYSC3, Gro1–4, CP60 and Nl25

F1 family	Seed parent	Pollen parent	No. of berries/total no. of seeds	No. of plants	RYSC3 present in no. of plants	Gro1–4 present in no. of plants	RYSC3 and Gro1–4 present in no. of plants	RYSC3, Gro1–4 and CP60 present in no. of plants	RYSC3 and Nl25 present in no. of plants
H98A	F1840/52	Ry126	19/50	46	22	28	12	7	NT
H98B	F1840/67	Ry126	27/26	22	11	17	9	9	NT
H98C	P3	Ry62	27/12	9	5	NT	NT	NT	4
H98D	P3	Ry126	76/37	33	12	NT	NT	NT	10
				$\Sigma = 110$	$\Sigma = 50$	$\Sigma = 45$	$\Sigma = 21$	$\Sigma = 16$	$\Sigma = 14$

NT Not tested

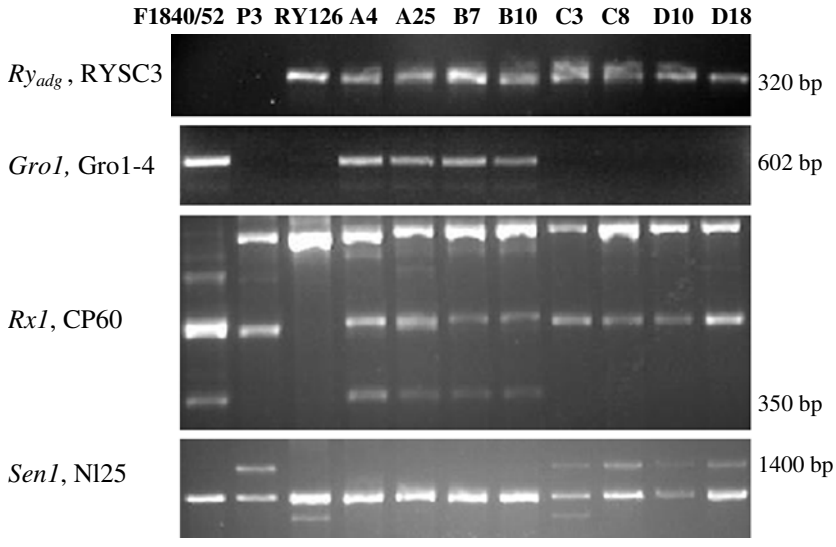

Fig. 1 PCR-based marker phenotypes used for MAS. The resistance locus and the linked marker are shown on the *left of each panel*. The diagnostic DNA fragment for each resistance locus is indicated by its approximate size in base pairs shown on the *right*. The *first three lanes from the left in each panel* show the parental marker phenotypes. The *remaining lanes* show the marker phenotypes of two individuals, each of the H98A, H98B, H98C and H98D family

which showed that the pollen parent Ry126 and all F1 hybrids were, in fact, tetraploid (19–27 chloroplasts per guard cell), whereas the seed parents F1840/52, F1840/67 and P3 were diploid (14–15 chloroplasts per guard cell). The crosses performed were in fact 2× by 4×, and the few seeds obtained resulted from unreduced gametes of the seed parents.

Marker-selected plants of the families H98A, H98B, H98C and H98D were tested for ER to PVY and PVX. All plants selected for having marker RYSC3 were extremely resistant to PVY (Table 2), as indicated by no detectable amounts of PVY and no symptoms observed in the graft-inoculated plants. Eight of these plants (H98A/4, H98A/25, H98A/35, H98B/1, H98B/7, H98B/15, H98D/5 and H98D/12) were also tested with PVA. All of them developed necrotic lesions in the top leaves following graft-inoculation, as expected for plants that carry Na_{adg} and express HR to PVA (Hämäläinen et al. 2000). All plants selected for having the diagnostic restriction fragment *Dde*I-350 of marker CP60 (families H98A and H98B) were extremely resistant to PVX, whereas H98C and H98D plants lacking the *Rx1* gene were susceptible. When plants selected from the families H98A and H98B for having the Gro1–4 marker were tested for resistance to *G. rostochiensis* pathotype Ro1, all plants were resistant (Table 2). Finally, plants of all four families were evaluated for resistance to wart. All H98C and H98D plants selected for having the diagnostic PCR fragment Nl25-1400, which were tested, were resistant to *S. endobioticum* pathotype 1. Some, but

Table 2 Resistance tests of marker-selected plants

F1 family	PVY^N resistant no. of plants (plants tested)	PVX resistant no. of plants (plants tested)	Ro1 resistant no. of plants (plants tested)	Wart pathotype 1 resistant no. of plants (plants tested)	Wart pathotype 2 resistant no. of plants (plants tested)	Wart pathotype 6 resistant no. of plants (plants tested)
H98A	8 (8)	8 (8)	7 (7)	6 (7)	3 (7)	5 (7)
H98B	8 (8)	9 (9)	9 (9)	2 (8)	4 (8)	5 (8)
H98C	5 (5)	0 (5)	NT	4 (4)	1 (8)	1 (4)
H98D	7 (7)	0 (8)	NT	7 (7)	5 (7)	4 (6)

NT Not tested

not all of these plants were also resistant to pathotype 2 and/or pathotype 6 (Tables 2, 3). All plants of the families H98A and H98B were expected to be susceptible to wart because none of the parents had the *Sen1* resistance gene. On the contrary, resistance to each pathotype of *S. endobioticum* was observed in families H98A and H98B (Table 2). Only 3 of the 15 tested genotypes were fully susceptible to *S. endobioticum*. Four genotypes, H98A/11, H98A/25, H98A/41 and H98B/7 (Table 3) were resistant to all three pathotypes in 2 years of testing. When the parents were tested for wart resistance, F1840/52 and F1840/67 were susceptible, as expected, P3 having the *Sen1* gene was resistant to pathotype 1, as expected, but susceptible to pathotypes 2 and 6 (Table 3). Unexpectedly, Ry126 and Ry62 were both resistant to all three pathotypes (Table 3), thereby explaining the additional wart resistance that segregated in all four families.

The 30 marker-selected clones were propagated in the field without fungicide treatments during one growing season for an initial assessment of agronomic performance. Tubers were harvested from 23 clones. Their tuber weight, tuber starch content, resistance to PVY, PVX, *G. rostochiensis* pathotype Ro1 and *S. endobioticum* pathotypes 1, 2 and 6 are reported in Table 3. Nineteen genotypes (indicated in Table 3) were submitted to the IPK potato germplasm bank.

Discussion

To the best of our knowledge, the experiment described in this paper is the first example for using PCR-based markers to select potato clones with multiple, monogenic resistance traits. The results show that marker-assisted selection for major gene-mediated pathogen resistance in potato is an efficient approach for combining important resistance traits in breeding lines and cultivars. Markers linked to the four independent, major resistance loci Ry_{adg}, *Rx1*, *Gro1* and *Sen1* were diagnostic for the presence of the corresponding resistance phenotype in F1 progeny of the resistant parents. No recombination event was observed between the diagnostic marker fragment and phenotypic resistance in the four families. However, the size of the families was too small to reliably assess the frequency of 'false positives' due to recombination. The marker RYSC3 has been shown to be diagnostic for the Ry_{adg} gene conferring extreme resistance to PVY when tested in different genetic backgrounds (Kasai et al. 2000). Markers Gro1–4, CP60 and Nl25 have so far not been tested for association with the linked resistance traits in wider germplasm pools. Their diagnostic value is therefore restricted at present to progeny of the parental clones F1840/52, F1840/67 and P3.

Table 3 Summary of trait evaluation of parents and F1 progeny of families H98A, B, C and D

MPI clone	Tuber weight (kg/plot)	Tuber starch content (%)	PVX	PVY^N	*G. rostochiensis* pathotype Ro1	*S. endobioticum* pathotype 1	*S. endobioticum* pathotype 2	*S. endobioticum* pathotype 6
H98A/4[a]	3.48	19.0	ER	ER	R	S	S	S
H98A/11[a]	4.47	13.7	ER	ER	R	R	R	R
H98A/18[a]	5.01	15.7	ER	ER	R	R	S	S
H98A/25[a]	5.48	19.9	ER	ER	R	R	R	R
H98A/33[a]	1.58	21.9	ER	ER	R	R	S	R
H98A/35[a]	1.77	22.8	ER	ER	R	R	S	R
H98A/41	1.41	14.7	ER	ER	R	R	R	R
H98B/1[a]	1.68	15.2	ER	ER	R	S	R	R
H98B/2[a]	4.19	16.4	ER	ER	R	R	S	S
H98B/4	1.07	18.0	ER	NT	R	S	S	R
H98B/7[a]	1.65	15.7	ER	ER	R	R	R	R
H98B/10[a]	3.08	18.5	ER	ER	R	S	R	R
H98B/14[a]	4.12	19.0	ER	ER	R	S	R	R
H98B/15[a]	2.38	17.7	ER	ER	R	S	S	S
H98B/19	1.20	16.4	ER	ER	R	S	S	S
H98C/4[a]	2.19	16.7	S	ER	NT	R	S	S
H98C/8[a]	1.69	20.4	S	ER	NT	R	S	S
H98D/2	1.10	> 23	S	ER	NT	R	R	S
H98D/5[a]	2.75	15.2	S	ER	NT	R	S	NT
H98D/10[a]	2.04	< 13	S	ER	NT	R	R	R
H98D/12[a]	1.72	15.9	S	ER	NT	R	R	R
H98D/16[a]	3.13	15.7	S	ER	NT	R	R	S
H98D/30[a]	1.71	17.7	S	ER	NT	R	R	R
Ry126	3.35	14.2	S	ER	S	R	R	R
Ry62	1.14	16.4	S	ER	S	R	R	R
F1840/52	2.17	16.7	ER	NT.	R	S	S	S
F1840/67	0.53	18.2	ER	NT	R	S	S	S
P3	3.11	17.5	S	S	S	R	S	S

ER Extreme resistant, *R* Resistant, *S* Susceptible, *NT* Not tested
[a]These genotypes have been submitted to the IPK potato germplasm bank

Genes for ER to viruses in potato are effective against different virus strains and therefore preferred to the genes for HR. The gene Ry_{adg} is used in breeding programs (Muñoz et al. 1975; Ross 1986; Galvez et al. 1992) and effectively protects the plants against PVY^{O} and PVY^{N}, the two main strains of PVY (Valkonen et al. 1994; this study). The interspecific mapping population involving *S. tuberosum* subsp. *andigena* as the progenitor for virus resistance, from which the clones Ry62 and Ry126 originated, segregates for ER and HR to PVY (Valkonen et al. 1994), HR and a non-necrotic, novel type of resistance to PVA (Hämäläinen et al. 2000) and HR to *Potato virus V* (genus *Potyvirus*) (J. Valkonen, unpublished data). Resistance to PVA was also detected in all plants tested of the H98A, H98B and H98D families. Similarly, *Rx* genes protect potato plants very efficiently against different strains of PVX (Cockerham 1970). Only isolates of PVX strain group 4 are known to overcome *Rx* (Moreira et al. 1980; Tozzini et al. 1994; Querci et al. 1995) due to a specific mutation in the viral coat protein (Kavanagh et al. 1992), and such isolates have been found only twice outside South America (Ross 1986). The *Gro1* locus protects against all known pathotypes of *G. rostochiensis* (Barone et al. 1990), whereas *Sen1* for wart resistance is effective only against pathotype 1 of *S. endobioticum*.

Two rather fortunate 'accidents' resulted in additional positive properties of the marker-selected plants. First, all selected plants of the families H98A, B, C and D were tetraploid instead of diploid as expected; and second, resistance to wart was not only inherited from parent P3 carrying the *Sen1* gene but also from parents Ry126 and Ry62. The pollen parents Ry126 and Ry62 were found to be tetraploid, although they descended from diploid parents that were used to generate the population for mapping the Ry_{adg} locus (Hämäläinen et al. 1997). Duplication may have occurred during in vitro propagation of these clones. However, according to the observed 1:1 segregation ratio of the diagnostic marker RYCS3, Ry126 and Ry62 carried the PVY resistance allele in simplex condition. The few seeds obtained from the crosses with Ry126 and Ry62 resulted then from zygote formation of 2n pollen with unreduced 2n gametes of the seed parents. Unreduced 2n gametes originate from meiotic first (FDR) or second (SDR) division restitution. FDR transmits more than 80% of the maternal genotype intact to the progeny, whereas SDR transmits less, depending on the distance of the locus considered from the centromere (Hermsen 1984; Ross 1986). The observed segregation ratios of markers Gro1–4 and Nl25 suggest SDR rather than FDR as mechanism for unreduced gamete formation in the seed parents. The *Gro1* locus maps to a proximal position on potato chromosome VII, and may be close to the centromere (Barone et al. 1990, 1995). This could explain the higher than expected transmission rate of nematode resistance (66%) to the families H98A and H98B. The marker Nl25 diagnostic for *Sen1* segregated as expected, probably due to the distal position of the loci on the long arm of potato chromosome XI, which diminishes the effect of SDR.

As the MAS clones are tetraploid, they can be directly used as parents in further crosses with tetraploid cultivars. The best entry point for the application of MAS for resistance in a potato breeding program will depend on the cost benefit analysis for any particular breeding scheme. Pathogen resistance has to be combined with superior agronomic performance. MAS in the seedling generation may be too costly due to the large number of DNA extractions and PCR assays required. F1 hybrid families could therefore be preselected for 1 or 2 years in the field for general agronomic performance before the number of clones is drastically reduced further by MAS for resistance. The costs for the PCR assays can be optimized by consecutive screening as performed in the MAS experiment reported here or by developing multiplex PCR assays. Assuming that (1) each known resistance locus is present in the simplex state in the parental MAS clones, (2) the crossing parent is fully susceptible and (3) agronomic performance is independent from resistance phenotype, the expected frequency of F1 hybrids with combinations of two and three resistance loci is 25 and 12.5%, respectively. MAS for two or three resistance factors would then reduce the number of preselected clones by 75–90%, thereby reducing the costs for extended field evaluation and phenotypic resistance testing.

Besides being extremely resistant to PVY, seed parents Ry126 and Ry62 were also resistant to wart pathotypes 1, 2 and 6, whereas P3 carrying *Sen1* was only resistant to wart pathotype 1 (Table 3). The Nl25 marker fragment diagnostic for *Sen1* in P3 was absent in Ry126 and Ry62 (Fig. 1). This suggests that the wart resistance genes present in Ry126 and Ry62 were different from *Sen1* present in P3. Moreover, resistance to wart pathotypes 1, 2 and 6 did not co-segregate in the families H98A, B, C and D (Table 3) and could not result, therefore, from a single gene with multiple pathotype specificities. Whether the different wart resistance genes are linked could not be assessed due to the small number of plants in each family. A second, independent locus *Sen1–4* has been identified on potato chromosome IV (Brugmans et al. 2005), which may be responsible for the wart resistance alleles originating from Ry126 and Ry62. *Sen1* and Ry_{adg} are both located in the same 'hot spot' for pathogen resistance on the distal end of the long arm of potato chromosome XI, which is syntenic with the *N* locus in tobacco for resistance to tobacco mosaic virus (Gebhardt and Valkonen 2001). Resistance to PVY and wart also did not co-segregate in the families H98A, B, C and D (Table 3), indicating that different, non-allelic genes encode the two resistance traits. However, both may be members of a complex family of clustered *N* homologous genes that is closely linked to *Sen1* and Ry_{adg} (Leister et al. 1996; Hehl et al. 1999; Vidal et al. 2002). *N* homologous sequences were the basis for marker development for several resistance traits in this region of the potato genome (Sorri et al. 1999; Kasai et al.2000; Marczewski et al. 2001; Bormann

et al. 2004), demonstrating the value of *R* gene sequence information for MAS.

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