Theor Appl Genet (2005) 111: 879–887
DOI 10.1007/s00122-005-0010-7

ORIGINAL PAPER

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Mapping of extreme resistance to PVY (Ry_{sto}) on chromosome XII using anther-culture-derived primary dihaploid potato lines

Received: 24 February 2005 / Accepted: 31 May 2005 / Published online: 26 July 2005

Abstract The inheritance of extreme resistance to PVY (Ry_{sto}) by a single dominant locus was confirmed by obtaining a 1:1 segregation ratio in a virus inoculation test with 28 resistant (*Ryry*) to 29 susceptible (*ryry*) anther culture-derived dihaploid lines ($2n = 2x = 24$) from cv. "Assia" ($2n = 4x = 48$) having extreme resistance derived from *Solanum stoloniferum* in simplex constitution (*Ryryryry*). Twelve Ry_{sto} markers selected in AFLP assays using bulked segregant analysis were applied to 106 tested potato cultivars from Germany, The Netherlands and Poland and 19 potato cultivars were identified by these markers as extremely resistant to PVY in alignment with phenotypic data. The locus for extreme resistance (Ry_{sto}) to PVY was mapped on chromosome XII co-segregating with the SSR marker STM0003. The utility of anther-culture derived dihaploid potatoes for genetic marker development was demonstrated. Marker transferability from diploids to tetraploids provides an optimistic potential for marker-assisted selection in potato breeding programs.

Introduction

Potato virus Y (PVY) belongs to *Potyvirus*, the largest genus of plant viruses (Hooker 1981; Shukla et al. 1994), and is one of the most important viruses in *Solanaceae*. PVY infection can reduce potato production up to 80% (De Bokx and Huttinga 1981). Quick accumulation of virus concentration in tubers caused by the vegetative propagation requires an intensive seed production management. Breeding of resistant cultivars becomes more important, since a new strain, PVY^{NTN}, causes large necrotic ring blemishes on the tubers (Weidemann 1993; Le Romancer et al. 1994) and affects also the ware potato market, in contrast to the well-described PVY strains PVY^{O}, PVY^{N} and PVY^{C} (Jones 1990; Świeżyński 1994).

Different resistances to PVY are already known in potato. The extreme resistance mechanism is characterized by a strong reduction of virus replication in infected cells, whereas the hypersensitive resistance mechanism inhibits virus spread from cell to cell and through the vascular system (Ponz and Bruening 1986; Valkonen and Somersalo 1996; Bendahmane et al. 1999). For hypersensitivity, the Ny_{tbr} locus from *S. tuberosum* was mapped on chromosome IV (Celebi-Toprak et al. 2002). Extreme resistance to PVY was found in *S. tuberosum* ssp. *andigena* (Muñoz et al. 1975; Galvez and Brown 1980), *S. hougasii* (Cockerham 1970) and *Solanum stoloniferum* (Cockerham 1943). The extreme resistance from *S. stoloniferum* was defined as immunity and combines extreme resistance to virus Y and virus A (Cockerham 1943; Ross 1986). In most European cultivars, extreme resistance to PVY originates in *S. stoloniferum* owing to the introgression of the single dominant resistance gene Ry_{sto} into *S. tuberosum* by Ross (1952, 1958). Approximately 20 cultivars were listed as carrying the gene *Ry* from *S. stoloniferum* in Europe (Ross 1986). The locus Ry_{chc} from *S. chacoense* was mapped on chromosome IX (Hosaka et al. 2001). The resistance genes Ry_{adg} from *S. tuberosum* ssp. *andigena* (Hämäläinen et al. 1997) and Ry_{sto} from *S. stoloniferum* (Brigneti et al. 1997) were mapped on the same region of chromosome XI. However, marker utility was only given for Ry_{adg} (Hämäläinen 1999; Ruiz de Arcaute et al. 2002). Unreliable pedigree information for the population used by Brigneti

Communicated by C. Möllers

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et al. (1997) might be the reason (Gebhardt and Valkonen 2001; Flis et al. 2005).

Most genetic analysis in potato has been performed at diploid ($2n = 2x = 24$) level to reduce the complexity of the genome due to the highly heterozygous autotetraploid constitution of the cultivated potato ($2n = 4x = 48$). For ploidy reduction, parthenogenic (Hougas and Peloquin 1958; Hermsen and Verdenius 1973; van Breukelen 1975, 1977) and androgenic processes (Irikura and Sakaguchi 1972; Foroughi-Wehr et al. 1977; Jacobsen and Sopory 1978; Sopory et al. 1978; Wenzel and Uhrig 1981; Rokka et al. 1996) were used, whereby anther culture was only applicable for a few cultivars and breeding lines because of strong genotype specific influences of the donor plant on regeneration (Schwarzfischer et al. 2002). Primary dihaploid lines obtained by parthenogenesis were, for example, used for localization of the nematode resistance gene H1 on chromosome V (Pineda et al. 1993) and for QTL analysis (Douches and Freyre 1994).

In this study, anther-culture-derived dihaploid lines were used successfully for marker development in potato by combining AFLP analysis (Vos et al. 1995) and bulked segregant analysis (Michelmore et al. 1991). The applicability of the resulting markers for tagging Ry_{sto} at the tetraploid level was demonstrated, and marker-assisted breeding is now feasible.

Materials and methods

Plant material

A primary dihaploid ($2n = 2x = 24$) potato (*Solanum tuberosum* ssp. *tuberosum*) population with 57 individuals was used for virus resistance assay and for localization of the resistance locus. This mapping population was produced by anther culture from the German cultivar "Assia" ($2n = 4x = 48$) in 1996 and 1997 (Schwarzfischer et al. 2002). The cultivar "Assia" (*acl*, *adg*, *dms*, *sto*, *vrn*) from the cross MPI 65.346/19 and UP 5.332/10 was registered with extreme resistance to PVY, PVA and PVX by the Bundessortenamt (Beschreibende Sortenliste 1980). According to pedigree data, extreme resistance of cv. "Assia" was derived from *S. stoloniferum* (Ross 1986; Świeżyński et al. 1997). Detailed pedigree information is compiled in the potato pedigree database (http://www.dpw.wau.nl/pv). In addition, 10 resistant and susceptible potato cultivars ("Artis", "Bettina", "Forelle", "Kuras", "Laura", "Petra", "Sempra", "Sibu", "Tomba" and "Ute") were used for marker verification. Furthermore, 106 different potato cultivars from different European nations were analyzed for identification of cultivars with extreme resistance to PVY.

PVY inoculation

Before proceeding with the virus inoculation test, in vitro plant material of the mapping population was analyzed by ELISA (Enzyme-Linked Immunosorbent Assay) to exclude any prior PVY contamination. Each line was propagated in vitro. Twenty repeats were pricked out into 96-cell planting trays containing potting soil (Ø 3 cm) and hardened under a net in the greenhouse (13 h, 20°C day/11 h, 15°C night). Additionally, cultivars "Assia" and "Walli" were prepared as negative and positive controls. After 2 weeks, these lines were planted in pots (Ø 9 cm) in May 2000. Potato virus Y inoculation was performed after 3 weeks using mechanical inoculation methods with a mixture of four different strains (PVY^{o}, PVY^{c}, PVY^{N} and PVY^{NTN}). Two weeks after PVY inoculation, plants were observed for symptoms of primary infection and minitubers were harvested 4 months after inoculation. Tuber dormancy was artificially broken by treatment with Rindite and 20 tubers per line were planted separately in pots in the greenhouse on December 2000. When the plants had grown up to 15–20 cm 3 weeks after replanting, a few leaves of each pot were analyzed by ELISA (visual color reaction) and the plants were evaluated for visual symptoms. Six lines died before evaluation was completed. Resistant lines were defined as completely free of PVY in ELISA and without visible mosaic, wrinkle or necrotic symptoms in 20 repeats. Based on the results of the PVY-ELISA test and symptom evaluation, twelve selected resistant and susceptible lines with unequivocal test results in all repeats were composed in pools. Bulked segregant analysis was performed with pools of mixed DNA from eight lines and individual DNA from four lines.

DNA preparation

Genomic DNA was extracted from in vitro cultured plants according to CTAB methods (Saghai-Maroof et al. 1984) with modifications. Fresh plant material (6 g) was finely ground in a pre-chilled (−20°C) mortar using liquid nitrogen and transferred into a 50-ml Falcon tube containing 24 ml 2x-CTAB buffer. After vortex, the mixtures were incubated in a water bath at 60°C for 1 h. A 25-ml mixture of chloroform/isoamylalcohol (49:1) was added and the tube was inverted for 30 min at 60 rpm in an invert shaker (Reax2, Germany). After centrifugation at 5410 g for 15 min, the supernatants (24 ml) were transferred into new tubes containing 1 µl of RN*ase* (10 mg/l) and incubated at room temperature for at least 30 min or overnight. The chloroform extraction was repeated again for purification. DNA was precipitated by adding two-thirds volume of iso-propanol. The resulting DNA pellet was hooked with a glass rod and washed first with 0.2 M sodium acetate in 76% ethanol, then with 10 mM ammonium acetate in 76% ethanol. The DNA pellet was dried at room temperature and dissolved in TE buffer (pH 8.0). DNA concentration was adjusted to 500 ng/µl.

Molecular marker analysis

The non-radioactive AFLP analysis was performed with small modifications according to Hartl and Seefelder (1998) based on the "AFLP-Protocol for Public Release" version 2.0 from KeyGene Company, The Netherlands (Vos et al. 1995). Two restriction enzyme combinations were used to prepare template DNA: *Eco*RI/*Mse*I and *Pst*I/*Mse*I (New England Biolabs, England). Genomic DNA (250 ng) was first digested with 2.5 U of *Eco*RI or *Pst*I and 1.0 U of *Mse*I in 20-µl reaction volume at 37°C for 3 h. The adapters corresponding to each enzyme cleavage site were ligated to the restricted DNA at 37°C for 1 h. Preamplification of the restricted-ligated fragments was performed using primers complementary to the adapter sequence plus an 1-bp extension at their 3′end: *Eco*RI + A/*Mse*I + C and *Pst*I + G/*Mse*I + C. For selective amplification, the primers had the adapter sequence plus a 3-bp extension at the 3′end. *Eco*RI and *Pst*I primers were 5′end labeled with fluorescein. In total, 480 selective primer combinations were screened. The amplified DNA fragments were separated on pre-heated 5% polyacrylamide (Acrylamide:Bisacrylamide 19:1, Roth, Karlsruhe), 7 M Urea, 1X TBE denaturing sequence gel at constant power (50 W, 1,600 V and 250 mA) for 2 h. The amplified DNA fragments were visualized by a laser scanner (Typhoon 9200, Amersham-Pharmacia, Freiburg) under highly sensitive conditions (excitement wavelength: 488 nm, band-pass filter: 530 ± 15 nm) and documented with the software package: FRAGMENT ANALYSIS 1.1 (Amersham-Pharmacia, Freiburg). Polymorphic bands were scored by using the program AFLP-Quantar 1.0 (Keygene, The Netherlands). The published sequence characterized amplified region (SCAR) markers from Kasai et al. (2000) were used for marker verification in ten German potato cultivars. Simple sequenced repeats (SSRs) from Milbourne et al. (1998) were used for identifying linkage groups in the primary dihaploid population of 57 individuals to assign AFLP linkage groups to chromosomes: STM0001 (chromosome VI), STM0003 (XII), STM0004 (VII), STM0007 (XII), STM0010 (IX), STM0013 (V), STM0014 (VII, XII), STM0017 (IX), STM0019 (VI), STM0024 (VIII), STM0025 (XI), STM0028 (VII), STM0030 (XII), STM0031 (VII), STM0032 (XII), STM0038 (II), STM0051 (X), STM1021 (IX), STM1049 (I), STM1100 (VI), STM2005 (XI), STM2012 (X), STM3016 (IV). The amplified DNA fragments were separated under the same conditions as described above and stained with 1:10,000 diluted Vistra Green (Amersham Pharmacia, Freiburg) for 5 min. Bands were detected by laser scanner (Typhoon 9200).

The PCR marker GP 81 and CAPs markers GP 122 (*Eco*RV), GP 204 (*Taq*I) and GP 269 (*Dde*I) were tested in the dihaploid Assia population and in 8 resistant ("Assia", "Barbara", "Hinga", "Klepa", "Meduza", "Nimfy", "Pirola", "Ute") and 8 susceptible cultivars ("Amingo", "Christa", "Gloria", "Hansa", "Producent", "Sarturna", "Ukama", "Ulla") to compare the results with the mapped *Ry-f*$_{sto}$ gene of Flis et al. (2005).

Polymorphic bands from SSRs and AFLPs were tested for segregation distortion considering an X^2 test at a given significance level of $P = 0.05$ for one-to-one segregation. Linkage groups were constructed with one-to-one segregating markers, which were converted to F_2 backcross population mode under Kosambi mapping function using an LOD threshold of 5.0 and maximum distance of 25 cM in the Mapmaker program (Lander et al. 1987).

Results

Evaluation of PVY resistance

Potato virus Y was not detected in any in vitro material by ELISA before virus inoculation. The phenotypic response of susceptible lines was clearly visible mainly in the form of mosaic symptoms 2 weeks after mechanical inoculation of the seedlings in the greenhouse, however, many of the seedlings were too small to select the resistant lines precisely. The phenotypic symptoms caused by PVY were more clearly seen in plants grown from tubers (secondary infection) rather than in the infected seedlings. A phenotypic segregation of 23 resistant and 28 susceptible lines was obtained by PVY-ELISA and visual evaluation of symptoms. In the plants P 72 and P 76c, PVY was not detected by ELISA, but they showed some mild mosaic symptoms and weak growth (Table 1). The segregation of resistance showed a 1:1 ratio by an X^2 test at a significance level of $P = 0.05$. The genetic constitution of extreme resistance to PVY of "Assia" is inferred as simplex (*Ryryryry*) according to the segregation results.

Bulked segregant analysis

Out of 480 primer combinations, 27 AFLP marker candidates were identified by analysis of resistant (R) and susceptible (S) pools composed respectively of 12 rigorously selected resistant and susceptible lines. These marker candidates were tested again with individual DNA samples from each line of the R and S pool (Fig. 1). Finally, 15 *Ry*$_{sto}$ AFLP markers from the 27 AFLP marker candidates were differentiated clearly as resistant and susceptible dihaploid lines in the bulked segregant analysis.

The 27 selected *Ry*$_{sto}$ AFLP marker candidates from bulked segregant analysis were tested; in the six lines, which could not be analyzed in the inoculation test and in ten potato cultivars having reliable data for PVY-resistance as control for marker selection. Among the six lines, five showed the resistance-associated bands. Cultivars "Bettina", "Forelle" and "Ute" were registered to have extreme resistance to PVY (Beschreibende Sortenliste Kartoffeln 1979; 1982; 1988), "Kuras", "Sibu", "Petra" and "Tomba" were classified as highly resistant

Table 1 Results of the potato virus Y inoculation test for 51 dihaploid lines

Line	ELISA[a]	Phenotypic evaluation[b]	Pool[c]		Line	ELISA[a]	Phenotypic evaluation[b]	Pool[c]	
			R	S				R	S
P 1	+	S		X	P 69	–	R	X	
P 2	+	S			P 72	–	S		
P 3	+	S			P 81	–	R	X	
P 3.1	+	S			P 85	+	S		X
P 4	+	S			P 86	–	R	X	
P 5	–	R			P 87	+	S		X
P 9.1.1	+	S		X	P 98	–	R	X	
P 11	–	R			P 99	–	R		
P 12.2	+	S		X	P 102	–	R	X	
P 15	–	R	X		P 103	+	S		X
P 19	+	S		X	P 104	+	S		
P 26	+	S			P 109	+	S		
P 27	–	R	X		P 110	–	R		
P 30	+	S			P 113	–	R		
P 33	–	R			P 115	–	R		
P 34	–	R			P 118	+	S		
P 35	+	S		X	P 120	–	R	X	
P 36	+	S			P 123	+	S		
P 39	–	R	X		P 125	–	R		
P 41	–	R	X		P 63a	–	R	X	
P 49	–	R	X		P 76a	+	S		X
P 50	+	S			P 76b	+	S		X
P 51	+	S			P 76c	–	S		
P 60	+	S		X	P 76d	–	R		
P 61	–	R			P 78a	+	S		X
P 66	+	S							

[a]Reaction to PVY by ELISA: "-" no reaction (resistant), "+" positive reaction (susceptible)
[b]R stands for resistance and S for susceptibility

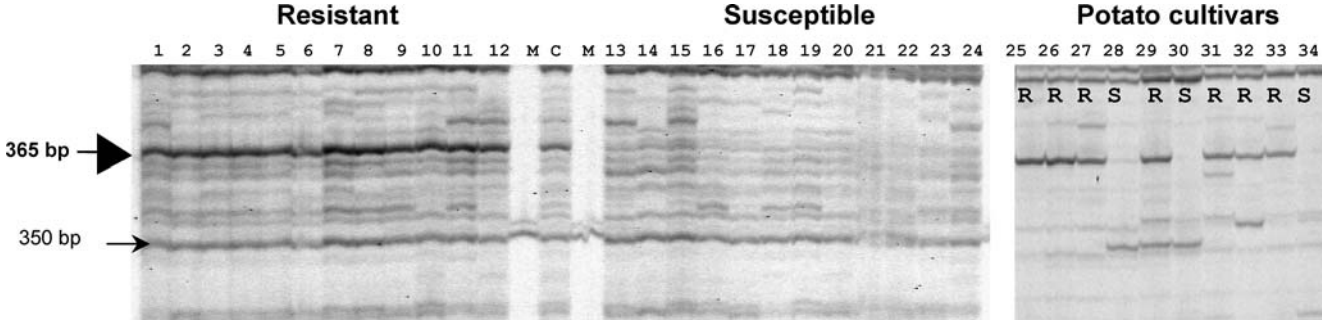

Fig. 1 Ry_{sto} AFLP marker (E + ACC/M + CTC-365) in dihaploid lines and ten potato cultivars. *Lanes 1–12* resistant dihaploid lines, *lanes 13–24* susceptible dihaploid lines, *lanes 25–34* potato cultivars ("Bettina", "Forelle", "Kuras", "Laura", "Petra", "Artis", "Sibu", "Tomba", "Ute" and "Sempra"), *M* 50–500 bp size marker, *C* "Assia" (control)

to potato virus Y while "Artis" and "Sempra" were recorded with low resistance to PVY (Beschreibende Sortenliste 2001). Cultivar "Laura" is recorded as highly resistant, whereas to our experience the resistance level is medium. The Ry_{sto} AFLP markers selected in ten potato varieties agreed with the selected markers in dihaploid lines from bulked segregant analysis (Fig. 1) except marker E + ACT/M + CGA-163.

By contrast, when the SCAR markers published by Kasai et al. (2000) were tested in these ten potato cultivars, no resistant cultivar was detected. Simultaneously the CAPs marker GP 122 (*Eco*RV) was tested, however, no polymorphism was observed.

Mapping of Ry_{sto} with AFLP and SSR markers

One locus (111 bp), amplified by the SSR primer STM0003, was detected as a Ry_{sto} marker by co-segregating with Ry_{sto} AFLP markers. For linkage analysis, 306 one-to-one segregating AFLP markers were selected from, in total, 620 polymorphic bands produced by 27 marker candidates and 7 additional AFLP primer combinations. The Ry_{sto} markers were tightly linked with LOD grouping thresholds over 3.0. According to linkage analysis, the Ry_{sto} resistance markers are linked to chromosome XII (Fig. 2) based on the reference maps from Milbourne et al. (1998) and Gebhardt et al. (1991).

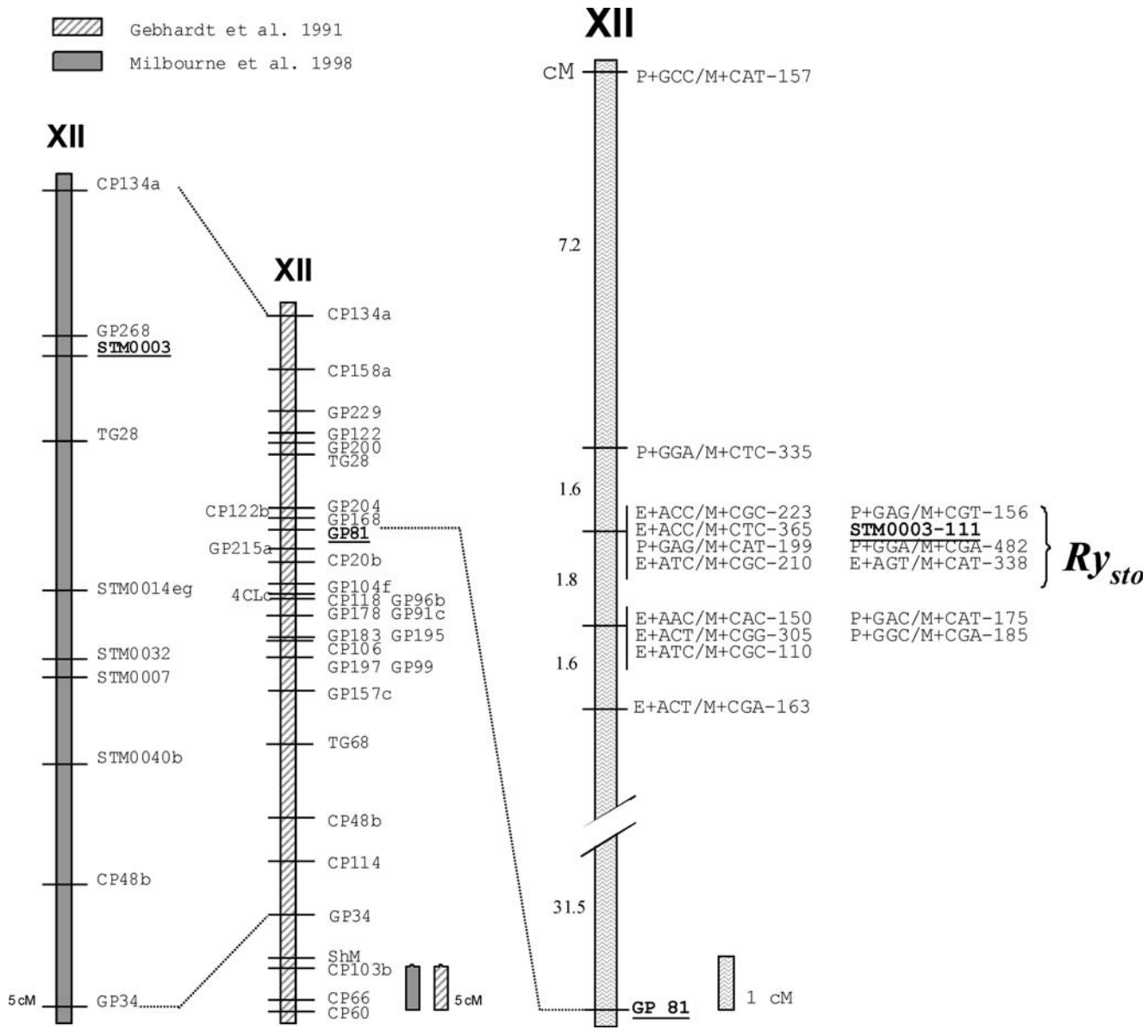

Fig. 2 Potato linkage group XII with the Ry_{sto} locus for extreme resistance to PVY. Map distances are given in centimorgans (cM)

In addition, the marker GP 81 was linked with a map distance of 34.9 cM. If one line (P3) was removed in our linkage analysis, five more markers (E + AAC/M + CAC-150, E + ACT/M + CGG-305, E + ATC/M + CGC-110, P + GAC/M + CAT-175, P + GGC/M + CGA-185) with a distance of 1.8 cM also co-segregated with STM0003-111. So, P3 was detected as a recombinant plant.

Identification of potato cultivars with extreme resistance to PVY

Selected Ry_{sto} markers were applied to 106 potato cultivars (Table 2). Two different AFLP marker types (A, B) could be distinguished according to their cultivar profile (Fig. 3). Nineteen cultivars were detected as extremely resistant to PVY by observing nine AFLP markers from Type A (P + GAC/M + CAT-175, P + GAG/M + CAT-199, P + GAG/M + CGT-156, P + GGA/M + CGA-482, P + GGA/M + CTC-335, P + GGC/M + CGA-185, E + AAC/M + CAC-150, E + ACC/M + CTC-365, E + AGT/M + CAT-338) and the SSR marker STM0003-111 in agreement with phenotypic reports (Table 2): "Arosa", "Alwara", "Assia", "Bettina", "Dania"(= Rania), "Forelle", "Franzi" (1977), "Hinga", "Jumbo", "Klepa", "Kuras", "Meduza", "Nimfy", "Oktan", "Petra", "Sibu", "Solara", "Tomba" and "Ute". Marker type B (E + ACC/M + CGC-223, E + ACT/M + CGG-305, E + ATC/M + CGC-110) did not detect "Alwara", "Arosa", "Hinga", "Meduza" and "Nimfy" out of the 19 selected

Table 2 Analysis of 106 potato varieties with Ry_{sto}markers

Cultivar	Resistance level to PVY[a]			Presence of the Ry_{sto} marker[d]			Cultivar	Resistance level to PVY[a]			Presence of the Ry_{sto} marker[d]		
				AFLP		SSR					AFLP		SSR
	D[b]	NL[b]	Po[b]	A	B	$STM0003_{111}$		D[b]	NL[b]	Po[b]	A	B	$STM0003_{111}$
Acapella	2			–	–	–	Klepa			E.R.[c]	+	+	+
Adora		6.5		–	–	–	Kuras		9.5		+	+	+
Agria	2			–	–	–	Laura	1			–	–	–
Alwara	1			+	–	+	Likaria	5			–	–	–
Amigo	7			–	–	–	Liseta		9.5		–	–	–
Angela	5			–	–	–	Marabel	1			–	–	–
Arosa	1			+	–	+	Marella	1			–	–	–
Artana	6			–	–	–	Maxilla	2			–	–	–
Artis	4			–	–	–	Meduza			E.R.[c]	+	–	+
Assia	1*			+	+	+	Miriam	3			–	–	–
Astarte		8.5		–	–	–	Mondial		7.5		–	–	–
Astoria	2			–	–	–	Moni	2			–	–	–
Atica	3			–	–	–	Monza	1			–	–	–
Baronesse	2			–	–	–	Natalie	2			–	–	–
Bettina	1*			+	+	+	Nimfy			E.R.[c]	+/–	–	+
Brigit	2			–	–	–	Nora	1			–	–	–
Bolero	1			–	–	–	Oktan	1			+	+	+
Bonanza	1			–	–	–	Pallina	4			–	–	–
Calla	2			–	–	–	Panda	3			–	–	–
Camilla	1			–	–	–	Patrona	5			–	–	–
Carmona	2			–	–	–	Petra	1			+	+	+
Christa	6			–	–	–	Ponto	3			–	–	–
Colette	1			–	–	–	Power	3			–	–	–
Combi	1			–	–	–	Presto	1			–	–	–
Dania (=Rania)	1			+	+	+	Producent	6			–	–	–
Debora	4			–	–	–	Quarta	4			–	–	–
Desiree	2			–	–	–	Rebecca	2			–	–	–
Ditta	2			–	–	–	Renate	3			–	–	–
Elkana		7.5		–	–	–	Rikea	2			–	–	–
Exempla	3			–	–	–	Rita	2			–	–	–
Exquisa	2			–	–	–	Rincona	2			–	–	–
Fausta	3			–	–	–	Salome	1			–	–	–
Festien	1			–	–	–	Satina	1			–	–	–
Flora	2			–	–	–	Saturna	6			–	–	–
Forelle	1*			+	+	+	Scala	2			–	–	–
Fox	1			–	–	–	Selma	3			–	–	–
Franca	3			–	–	–	Sempra	2			–	–	–
Franzi	1*			+	+	+	Seresta		7.0		–	–	–
Franzi	2			–	–	–	Sibu	1			+	+	+
Frieda	3			–	–	–	Sieglinde	3			–	–	–
Gloria	6			–	–	–	Sjamero	1			–	–	–
Granola	3			–	–	–	Solara	1			+	+	+
Gunda	5			–	–	–	Sommergold	1			–	–	–
Hansa	8			–	–	–	Spunta		7.5		–	–	–
Hinga			E.R.[c]	+	–	+	Terra	1			–	–	–
Indira	2			–	–	–	Tessi	2			–	–	–
Impala	4			–	–	–	Tomba	1			+	+	+
Irmgard	4			–	–	–	Ukama	6			–	–	–
Jetta	4			–	–	–	Ulla	8			–	–	–
Juliane	2			–	–	–	Ulme	1			–	–	–
Jumbo	1*			+	+	+	Ute	1*			+	+	+
Kardent		7.5		–	–	–	Velox	4			–	–	–
Kartel		4.5		–	–	–	Walli	3			–	–	–

1*: Registered extremely resistant cultivar to potato virus Y by Bundessortenamt

[a]Resistance level to PVY: one (highly resistant) to nine (highly susceptible) in Germany, nine (highly resistant) to one (highly susceptible) in The Netherlands

[b]Nation: *D* Germany, *NL* The Netherlands, *Po* Poland

E.R.[c] Extremely resistant cultivar in Poland referenced by Flis et al. (2005)

[d]Ry_{sto} AFLP and SSR markers: + = present, – = absent, +/– = unclear to score, A and B indicate two different Ry_{sto} AFLP marker types

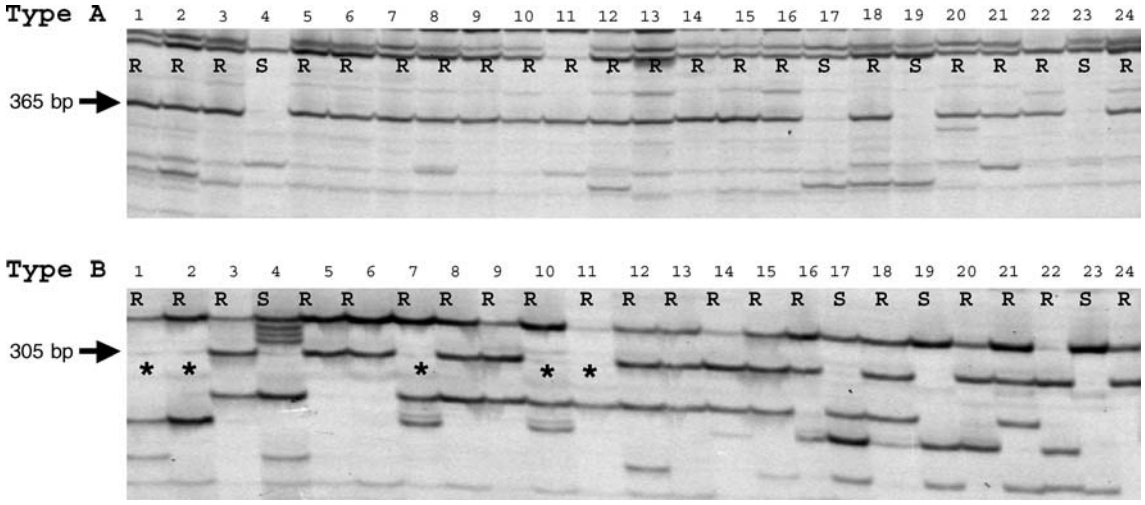

Fig. 3 Ry_{sto} AFLP marker types in 24 cultivated potatoes having a high level of PVY resistance. Type A: Ry_{sto}AFLP marker(-E + ACC/M + CTC-365), Type B: Ry_{sto}AFLP marker (E + ACT/M + CGG-305). *Lane 1* "Alwara", *lane 2* "Arosa", *lane 3* "Dania"(= Rania), *lane 4* "Fox", *lane 5, 6* "Franzi", *lane 7* "Hinga", *lane 8* "Jumbo", *lane 9* "Klepa", *lane 10* "Meduza", *lane 11* "Nimfy", *lane 12* "Oktan", *lane 13* "Solara", *lane 14* "Bettina", *lane 15* "Forelle", *lane 16* "Kuras", *lane 17* "Laura", *lane 18* "Petra", *lane 19* "Artis", *lane 20* "Sibu", *lane 21* "Tomba", *lane 22* "Ute", *lane 23* "Sempra" and *lane 24* "Assia", *Asterisk* (*) indicates that these cultivars were not detected by marker type B

cultivars (Fig 3, Table 2). Three markers (P + GCC/M + CAT-157, E + ACT/M + CGA-163, E + ATC/M + CGC-210) with a far distance (8.8 cM, 3.4 cM and 1.8 cM) to Ry_{sto}, were not selectable in 106 potato varieties. From two markers (E + ATC/M + CGC-210, E + ATC/M + CGC-110) derived from the same selective primer combination and with a map distance of 1.8 cM, just the marker E + ATC/M + CGC-110 identified the extreme resistant potato cultivars. The Polish cultivar "Nimfy" showed additional differences in marker pattern because the target band was unclear with the marker P + GAC/M + CAT-175 and the band of marker P + GGA/M + CGA-482 was absent.

Discussion

In this work, molecular markers for potato were established by the use of anther culture-derived plants. Anther culture of potatoes is difficult and there are some limitations in obtaining a large population because of strong genotype specific influences of the donor plant on regeneration (Jacobsen and Sopory 1978; Wenzel and Uhrig 1981; Uhrig 1985; Rokka et al. 1996; Schwarzfischer et al. 2002). Nevertheless, this unusual mapping strategy was very successful for marker development. Recently, anther-culture-derived monoploids have also been the basic material for linkage analysis (Tai et al. 2000; Varrieur 2002).

Our population size with 57 individuals was relatively small for localizing the Ry_{sto} locus. However, often small diploid populations with a size of 67 individuals (Gebhardt et al. 1991) or 68 individuals (Van Eck et al. 1995) were used for map construction. Furthermore, Hämäläinen et al. (1997) also succeeded in localizing the Ry_{adg} locus on chromosome XI with a dihaploid population of 54 individuals.

The male sterile cultivar "Assia" was the only one in our laboratory which showed good regeneration results in anther culture. Furthermore, also a few lines could be regenerated from the cultivars "Petra" and "Ute", which are also male sterile. Dihaploids from the cultivar "Petra" were also induced by Rokka et al. (1996). In conclusion, male sterility does not influence the androgeneric process.

All examined cultivars with known extreme resistance to PVY were detected by our markers. The resistance level of Polish cultivars "Hinga", Klepa", "Meduza" and "Nimfy" was kindly confirmed by Dr. Zimnoch-Guzowska at the Plant Breeding and Acclimatization Institute (IHAR) in Poland and was recently reported by Flis et al. (2005). Further cultivars were selected which were registered with very high resistance to PVY but not with extreme resistance, for example, "Alwara", "Arosa", "Dania"(="Rania"), "Oktan", "Petra", "Solara" and "Tomba". These cultivars presumably had never been tested for extreme resistance to PVY because this trait will only be examined by the Bundessortenamt by breeders' request. The cultivar "Fox" has a high resistance level to PVY and is derived from the same cross parents as "Assia". However, the loss of extreme resistance was confirmed by the graft inoculation test at the breeding station Uniplanta-Saatzucht KG (personal communication).

Previous studies on mapping of PVY resistance genes (Ry_{sto} and Ry_{adg}) revealed the region between the probes GP 125 and CT 182 on chromosome XI (Brigneti et al. 1997; Hämäläinen et al. 1997; Solomon-Blackburn and Barker 2001). In agreement with the results of Ruiz de Arcaute et al. (2002), we could not apply the Ry_{adg}

SCAR markers (Sorri et al. 1999; Kasai et al. 2000) for selection of PVY resistant cultivars in the spectrum of German potato cultivars and we also failed to locate the Ry_{sto} gene locus on chromosome XI. In contradiction with the earlier hypothesis of Brigneti et al. (1997), Ry_{adg} and Ry_{sto} represent different genes on different chromosomes. Therefore, marker utility must be considered dependent on the genetic background of the test material.

Our results indicate a localization of Ry_{sto} on chromosome XII according to the co-segregation with a locus amplified by the STM0003 primer, mapped to the region between the probes GP268 and TG28 on chromosome XII by Milbourne et al. (1998). They obtained a PCR product with 141 bp by STM0003, whereas in our population, an additional band (111 bp) was amplified. Interestingly, this band co-segregates with all resistant lines in the dihaploid Assia population and was only observed in the extreme resistant potato cultivars derived from *S. stoloniferum*. Additionally, the probe GP 81 confirmed the Ry_{sto} localization on chromosome XII based on the reference map of Gebhardt et al. (1991).

Recently, Flis et al. (2005) mapped the locus *Ry-f*$_{sto}$ on the chromosome XII. In comparison, the closely linked marker GP 122_{718} did not deliver polymorphism in our dihaploid Assia population. In contrast to Flis et al. (2005), we also failed to select the extremely resistant cultivars "Assia", "Barbara", "Hinga", "Klepa", "Meduza", "Nimfy", "Pirola" and "Ute". Only marker GP 81 could be linked to our Ry_{sto} with a map distance of 34.9 cM. This result indicates that the loci *Ry-f*$_{sto}$ and Ry_{sto} could represent two different genes for extreme resistance to PVY, both situated on chromosome XII.

There were two different Ry_{sto}AFLP marker types (A, B) according to their response to the cultivars "Alwara", "Arosa", "Meduza", "Hinga" and "Nimfy". We argue that recombination events occurred in this marker-specific region in earlier pedigrees. The polish cultivar "Nimfy" showed in addition differences in reaction profiles to AFLP marker type A, this maybe caused by methylation. The used restriction enzyme *Pst*I is reported to be much more sensitive to cytosine methylation than *Eco*RI and *Sac*I according to segregation data from a heterozygous diploid potato population in AFLPs (Isidore et al. 2003).

As extreme resistance to PVY (Ry_{sto}) is inherited by one single dominant gene, marker transfer from diploid to tetraploid level was possible. With our results based on good phenotypic evaluation data, exact pedigree information and well-established molecular methods, we can start a very precise resistance breeding strategy based on marker-assisted selection for the important breeding trait extreme resistance to PVY (Ry_{sto}). Ry_{sto} AFLP markers (Type A) provide reliable information to be converted to Site Target Sequence (STS) markers. Therefore selection of PVY resistant genotypes could be done very exactly in early stages of the breeding process, for example, in the seedling stage with small leaf samples by application of simple analytical methods independent of environmental influences. The additional costs for MAS and man power will be saved by reduction of greenhouse and field area and by minimization of laborious resistance tests for several years.

Acknowledgments The authors thank Heike Scholz for producing the dihaploid population by anther culture and Josef Schwarzfischer for his information and technical support. Especially, we want to thank the DAAD (Deutscher Akademischer Austauschdienst) for financial support.

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