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The *Ry-f_{sto}* gene from *Solanum stoloniferum* for extreme resistant to *Potato virus Y* maps to potato chromosome XII and is diagnosed by PCR marker $GP122_{718}$ in PVY resistant potato cultivars

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Abstract

A novel locus for extreme resistance to *Potato virus Y* (PVY), *Ry-f_{sto}*, was identified on potato chromosome XII. The gene *Ry-f_{sto}* has been introgressed from the wild potato species *Solanum stoloniferum*. Inheritance of *Ry-f_{sto}* in the tetraploid potato population Rysto was consistent with the model of a single, dominant gene. Bulked segregant analysis identified an ISSR (inter-simple sequence repeat) marker UBC 857_{980} linked to *Ry-f_{sto}*. This marker mapped to linkage group XII of a reference potato RFLP (restriction fragment length polymorphism) map. Chromosome XII specific RFLP markers were converted into PCR-based STS and CAPS markers and tested for linkage with *Ry-f_{sto}* in the population Rysto. CAPS marker $GP122_{718}$ was tightly linked to the resistance gene and was successfully used to identify Polish and German cultivars expressing extreme resistance to PVY. This indicates that the source of *Ry-f_{sto}* has been widely utilized in various potato breeding programs and can be monitored by a diagnostic marker in marker-assisted selection.

Introduction

Potato virus Y (PVY) is the most important member of the *Potyvirus* genus (*Potyviridae* family) infecting potatoes. PVY is easily transmitted mechanically and by many aphid species in a non-persistent manner (Brunt 2001). Various PVY strains can cause yield losses from 10 to 90% and decrease plant productivity or tuber quality (Brunt 2001; Novy et al. 2002). Breeding of resistant cultivars is one of the most effective strategy to achieve protection against PVY (Swieżyński 1994). In potato, there are two main types of resistance to PVY, the hypersensitive (HR) and extreme (ER) resistance (Valkonen et al. 1996). The hypersensitive response to PVY is strain specific and may result in a range of necrotic reactions both in locally and systematically infected leaves (Valkonen et al. 1998). Genes causing HR (*Ny* genes) are widely distributed in potato cultivars (Ruiz de Galarreta et al. 1998). In some cases, however, hypersensitivity may be ineffective for restriction of PVY in plants (Vidal et al. 2002). The first HR gene, *Ny_{tbr}*, from *Solanum tuberosum* has been mapped on potato chromosome IV (Celebi-Toprak et al. 2002).

Genes for extreme resistance to PVY (*Ry* genes) confer extremely high level of protection against all strains of PVY. Potato plants possessing *Ry* remain symptomless, except for very limited systemic necrosis which may develop after graft-inoculation (Jones 1990; Valkonen et al. 1996; Vidal et al. 2002). The gene Ry_{adg} derived from *Solanum tuberosum* ssp. *andigena* and Ry_{chc} from *S. chacoense* were mapped on chromosome XI (Hämäläinen et al. 1997) and IX (Hosaka et al. 2001), respectively. A second gene for extreme resistance to PVY, Ry_{sto} from *S. stoloniferum* has also been mapped to the same position as Ry_{adg} on chromosome XI (Brigneti et al. 1997). However, pedigree information might not be reliable in this case (Gebhardt and Valkonen 2001). The genes Ry_{adg}, Ry_{sto} and Ry_{chc} are known to be utilized in potato breeding programs as described by Hämäläinen et al. (1997); Chrzanowska et al. (1998) and Hosaka et al. (2001), respectively. A range of molecular techniques has been applied for the detection of DNA-based markers useful for marker-assisted selection in crop plants. However, only markers that are tightly linked with a desired trait and are detected reproducibly, easily and cost effectively, have the potential for increasing selection efficiency in plant breeding programs (Mohan et al. 1997). In this paper we report a novel locus $Ry\text{-}f_{sto}$ for extreme resistance to PVY derived from *S. stoloniferum* on potato chromosome XII. The tightly linked CAPS marker $GP122_{718}$ was diagnostic for the $Ry\text{-}f_{sto}$ gene in a range of potato cultivars having extreme resistance to PVY derived from *S. stoloniferum*.

Materials and methods

Plant material

A family of 169 tetraploid F_1 hybrids (population Rysto) was obtained from a cross between potato clone PW-363, which is extremely resistant to PVY and the susceptible cv. Cicero. PW-363 was generated within the parental line breeding program at the Plant Breeding and Acclimatization Institute (IHAR), Młochów. Seed parent of PW-363 was the susceptible clone PW 82-568 and pollen parent was the extremely resistant clone PW 83-241. PW 83-241 originated from the male fertile, PVY resistant clone PW-211. The ancestry of PW-211 contained a *S. stoloniferum* accession from the collection of the Vavilow Research Institute of Plant Industry (VIR), St. Petersburg, Russia. Leaf tissue of Polish and German potato cultivars was provided by M. Chrzanowska (IHAR Młochów field collection) and K. Schüler (IPK Genbank Außenstelle Nord, 18190 Groß Lüsewitz, Germany), respectively.

Evaluation of resistance

Resistance to PVY of F_1 hybrids and their parents was evaluated by mechanical and graft inoculations. Three plants of each clone were mechanically inoculated with isolate PVY^N Wi, which belongs to the tobacco necrosis strain group (Chrzanowska 1991). The plants were tested 4 weeks after inoculation by ELISA using monoclonal antiserum obtained from Bioreba AG (Reinach, Switzerland). Two tubers were collected from each non-infected plant and subsequently planted to examine PVY resistance of tuber progeny plants. The tests for presence of PVY were performed 5 weeks after planting the tubers. F_1 individuals, which were free of PVY after mechanical inoculation and the resistant parent PW-363 were additionally tested by graft inoculation. Four plants of each clone were grafted with tobacco scions carrying PVY^N Wi. The experiments were carried out in a greenhouse, under natural daylight, at temperatures between 17 and 25 °C. Plants of extremely resistant cv. Bzura and susceptible cv. Irys were used as controls.

DNA isolation, PCR, and electrophoresis

Genomic DNA was isolated from freeze-dried leaf tissue using a GenElute Plant Genomic DNA Miniprep kit (Sigma–Aldrich, St. Louis, MI, USA). Equal DNA quantities from 8 resistant and 8 susceptible F_1 individuals were pooled to form a resistant and a susceptible bulk, respectively. The DNA concentration in the two bulks and the parental DNAs were adjusted to 20 ng/μl. PCR analyses for detection of inter-simple sequence repeat (ISSR) markers were performed in 20 μl of 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 0.1% Triton X-100, 1.5% formamide, 0.2 mM of each deoxynucleotide, 2.5 mM $MgCl_2$ and 300 nM primer, adding 1 unit of *Taq* DNA polymerase (Invi-

trogen, Carlsbad, CA, USA) and 30 ng genomic DNA. The PCR parameters were: 94 °C for 1 min followed by 40 cycles of denaturation at 93 °C for 20 s, annealing at 42 °C for 25 s and extension at 72 °C for 75 s, with 5 min final extension at 72 °C. A GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) was used for DNA amplification. PCR products were visualized by electrophoresis in 1.4% agarose gels in 1 × tris–borate–EDTA (TBE) buffer and ethidium bromide staining. One hundred ISSR primers (UBC set ≠ 9) were received from the Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada. PCR analyses of potato markers GP269 (GenBank accession CG783202), GP204 (CG783144), GP122 (AJ487339) and GP81 (AJ487376) were performed in 20 μl of 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 0.1 mM of each deoxynucleotide, 1.5 mM $MgCl_2$ and 200 nM of each primer, adding 1 unit of *Taq* DNA polymerase and 30 ng genomic DNA. The primer sequences (Table 1) were designed according to the Primer Select Program (DNA STAR. Inc., Madison, WI, version for Windows, 3.10. 1998) based on the DNA sequences of the markers. The PCR parameters for GP269 and GP122 were: 94 °C for 1 min followed by 40 cycles of denaturation at 93 °C for 20 s, annealing at 53 °C for 25 s and extension at 72 °C for 1 min, with 5 min final extension at 72 °C. Annealing temperatures were 50 °C and 56 °C for the amplification of GP81 and GP204, respectively.

Mapping of the Ry-f_{sto} locus

DNA samples of parental clones P18 and P40 and 80 F_1 hybrids of the diploid mapping population F1840 (Gebhardt et al. 1991, 2003; Leister et al. 1996) were used to map the ISSR marker UBC 857_{980}. Segregation of UBC 857_{980} was scored as presence or absence of the marker fragment. The map position was identified relative to the RFLP map existing for this population using the software package MAPRF (E. Ritter, NEIKER, 01080 Victoria, Spain). The linkage group XII of parent PW-363 was constructed by scoring markers GP81, GP122, GP204 and GP269 in the population Rysto. Marker order was derived by minimizing the number of double crossovers between the markers. Genetic distance between marker loci was calculated as fraction of recombinants.

Table 1. Primer sequences used for PCR amplification of the RFLP loci *GP81*, *GP122*, *GP204* and *GP269*

Marker name	Forward and reverse primer sequences (5′ to 3′)
GP81	GCAGCGTTTCCTACAAT
	AGAGACTAATGCTGAAAAT
GP122	TATTTTAGGGTACTTCTTTCTTA
	GCACTCAATAGCCCTTCTT
GP204	CATAGATGGCTCAAACAACTC
	GTGGAAACATGGCTTACC
GP269	TCGCAATGAAAGATAAGC
	TGTGATAAAGAGTGTAGCAGTC

Results

Segregation of extreme resistance to PVY

Of 169 F_1 individuals examined for resistance to PVY, 87 were extreme resistant after mechanical and graft-inoculations and 82 were susceptible. The segregation ratio of 1:1 confirmed the presence of a single, dominant gene for extreme resistance to PVY, in simplex state in the tetraploid parental clone PW-363.

Identification of markers linked to Ry-f_{sto}

Of 100 ISSR primers tested, 78 primers gave 319 DNA fragments, ranging in size from 300 to 2000 bp. Sixty fragments were polymorphic between the parents of which 29 were observed in the resistant parent PW-363. One primer, UBC 857 (5′-ACACACACACACACAC(CT)G-3′), amplified a 980 bp DNA fragment (UBC 857_{980}) that was present in the resistant DNA bulk as a clear, bright band and was not detectable in the susceptible bulk (Figure 1, lanes 1 and 2). The whole Rysto population was scored for UBC 857_{980}. The genetic distance between the *Ry-f_{sto}* locus and marker UBC 857_{980} was estimated to be 13.7 cM. Since UBC 857_{980} was amplified in the parent P18 of the F1840 mapping population (Gebhardt et al. 2003; Leister et al. 1996), 80 F_1 progeny were scored for segregation of the marker UBC 857_{980}. The 980 bp ISSR fragment mapped to linkage group XII, close to the RFLP marker locus *GP91-c*. To confirm the position of UBC 857_{980} and

Ry-f_{sto} on potato chromosome XII and to map the resistance locus more precisely, RFLP markers located in map segment GP99–GP229 on chromosome XII (Gebhardt et al. 2001) were converted into STS (sequence tagged site) or CAPS (cleaved amplified polymorphic sequence) markers informative for the PVY resistant parent PW-363. PCR-based assays for GP122, GP204 and GP269 revealed CAPS bands of 718, 800 and 650 bp in PW-363 after digestion of the amplified products with restriction enzymes *Eco*RV, *Taq*I and *Dde*I, respectively. These markers segregated in population Rysto. In the case of STS marker GP81, a DNA fragment of 400 bp was amplified in PW-363 that segregated in the Rysto population. The restriction patterns of the resistant and susceptible parent after digestion of the GP122 amplicon with *Eco*RV are shown in Figure 1 (lanes 3 and 4). The order and distance of marker loci *GP122*, *GP204*, *GP81*, *GP269* and *UBC* 857_{980} relative to the *Ry-f_{sto}* locus are shown in Figure 2.

Testing the CAPS marker $GP122_{718}$ in potato cultivars

Sixty nine cultivars bred in Poland, Germany or The Netherlands were tested for the presence of the CAPS marker $GP122_{718}$. Among 28 cultivars

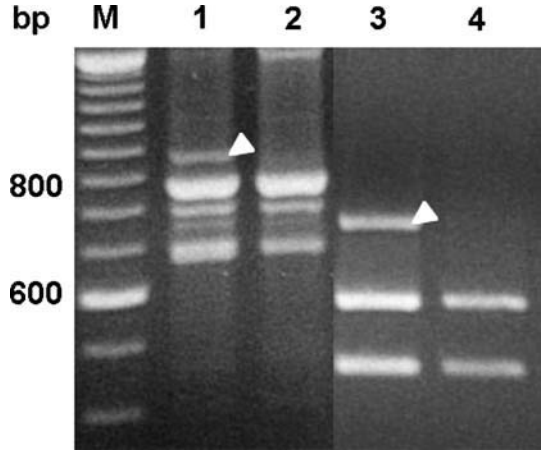

Figure 1. Patterns of amplified DNA of the resistant parent PW-363 (lanes 1 and 3) and the susceptible parent Cicero (lanes 2 and 4), indicating the ISSR marker UBC 857_{980} (lane 1) and the CAPS marker $GP122_{718}$ after digestion with *Eco*RV (lane 3). Arrows point to the marker products. Lane M contains the 100-bp DNA ladder as molecular size marker.

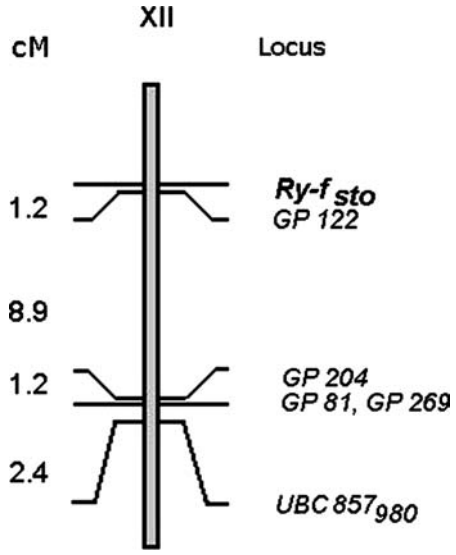

Figure 2. Potato linkage group XII of the parent PW-363, including the position of the *Ry-f_{sto}* locus for extreme resistance to PVY. Map distances are given in centimorgans (cM).

known to exhibit extreme resistant to PVY (Stegemann and Schnick 1985; Ross 1986; Zagórska et al. 2000), the 718 bp PCR product of GP122 was identified after *Eco*RV digestion in all 19 Polish and in 8 German cultivars tested, with the exception of the Dutch cv. Santé (Table 2). Cultivar Santé also lacked the marker allele RYSC3 specific for *Ry_{adg}* on chromosome XI (Kasai et al. 2000) (not shown). This indicates a recombination event that has separated the marker allele from the resistance gene or presence of another gene conferring extreme resistance against PVY in this cultivar. The *Ry-f_{sto}*-linked marker $GP122_{718}$ was not identified in any of 41 cultivars, which lack an *Ry* gene.

Discussion

Genetic mapping of a gene for extreme resistance to PVY present in the breeding clone PW-363 identified a novel locus, *Ry-f_{sto}* on potato chromosome XII. The marker UBC 857_{980} used for mapping *Ry-f_{sto}* is the next inter-microsatellite sequence linked with the resistance gene for disease described in potato. The other ISSR markers were found to be linked with loci against *Potato virus S* (Marczewski 2001; Marczewski et al. 2002b) and *Potato leafroll virus* (Marczewski et al. 2002a),

Table 2. Distribution of marker allele $GP122_{718}$ in Polish, German and Dutch cultivars

Cultivars bred in	Response to PVY	Potato cultivars	Presence of the marker $GP122_{718}$
Poland	ER	**Anielka**[a], **Barycz**, **Baszta**, **Beata**, **Bekas**, **Bzura**, **Dunajec**, **Fregata**, **Hinga**, **Klepa**, **Meduza**, **Nimfy**, **Omulew**, **Skawa**, **Umiak**, **Vistula**, Ania, Gabi, Maryna	+
	HR/S	**Arkadia**, **Grot**, **Ikar**, **Koga**, **Korona**, **Neptun**, **Triada**, Balbina, Cedron, Drop, Irga, Irys, Kolia, Lena, Mors, Oda, Orłan, Perkoz, Rybitwa, Salto, Sokół, Wolfram, Żagiel	–
Germany	ER	Assia, Barbara, Esta, Fanal, Heidrun, Pirola, Ute, Wega	+
	HR/S	Fortuna, Frühmölle, Granola, Hindenburg, Isola, Jubel, Karlena, Molli, Vineta, Nicola	–
The Netherlands	ER	Santé	–
	HR/S	Accent, Bintje, Felsina, Fresco, Gloria, Latona, Timate, Vital	–

[a] Boldface indicates origin of cultivar from parental lines bred at IHAR Młochów; ER, extreme resistance; HR/S, hypersensitivity or susceptibility; +, presence of marker; –, absence of marker.

mapped to potato chromosome-VIII and XI, respectively. The position of *Ry-f_{sto}* is different from the loci Ry_{adg} and Ry_{sto}, which both also confer extreme resistance to PVY but co-localize on potato chromosome XI (Brigneti et al. 1997; Hämäläinen et al. 1997). The source of extreme resistance to PVY used in our mapping experiment was therefore different from the sources used by Brigneti et al. (1997) and Hämäläinen et al. (1997). The *Ry-f_{sto}* resistance gene has been used in Polish and German cultivar breeding programs. This was shown by the closely linked CAPS marker allele $GP122_{718}$, which was diagnostic for all tested cultivars known to carry extreme resistance to PVY, with the exception of the Dutch cultivar Santé. The PVY resistant and male fertile parent PW-363 of the mapping population Rysto had in its pedigree *S. stoloniferum* from the VIR collection. Pedigree information for Polish and German cultivars with extreme resistance to PVY (Świeżyński et al. 1997; Stegemann and Schnick 1985) indicates various other sources of *S. stoloniferum* and breeding clones, for example clones Sto XII B from the IHAR collection, C.854 from the Scottish Crop Research Institute (Invergowrie, Scotland, received from G. Cockerham), and MPI 55.957/24, MPI 55.957/54, MPI 61.303/34, MPI 64.956/68, MPI 65.346/19 from the Max-Planck Institute for Plant Breeding Research (Cologne, Germany, received from H. Ross). *Ry* genes have been discovered in *S. stoloniferum* and other wild species by Stelzner (1950) and were characterized in a collection of *S. stoloniferum* clones (Ross 1958). Despite the different sources of *S. stoloniferum* used in the past for introgressing extreme resistance to PVY into *S. tuberosum* breeding clones, in most cases the same gene seems to have been transferred into cultivated potato.

Marker-assisted selection (MAS) is one of the most important applications of DNA-based markers in plant improvement programs. Using molecular markers in breeding may reduce costs and increase precision and efficiency of selection (Peleman and van der Voort 2003; Barone 2004). The essential requirement for MAS is cosegregation or tight linkage of a marker allele with a targeted trait allele. Moreover, the linkage should be stable across subsequent generations and in wide gene pools (Gebhardt and Valkonen 2001) and the marker assay should be easy to perform and cost effective. In potato, very few DNA-based markers are available to date, which fulfill these requirements. The sequence-characterized amplified region (SCAR) marker RYSC3 having sequence similarity to known plant genes for pathogen resistance, co-segregates with Ry_{adg} and is diagnostic in diploid and tetraploid potatoes for extreme resistance to PVY, which originates from *S. tuberosum* ssp. *andigena* (Sorri et al. 1999; Kasai et al. 2000). The genetic distance of 3.2 cM between the *Ns* locus and the dominant marker $SCG17_{321}$ was sufficient for selection resistant to *Potato virus S* diploid potatoes (Marczewski et al. 2001). PCR-based markers for the *R1* gene for resistance to late blight (*Phytophthora infestans)* and markers tightly linked to *R1* (within 0.3 cM) are associated with quantitative resistance to late blight in a large collection of potato cultivars (Gebhardt

et al. 2004). An SSR (simple sequence repeat) marker was found indicative for quantitative resistance to *Verticillium dahliae* in a collection of American potato cultivars (Simko et al. 2004). In other cases, marker alleles linked to a particular resistance allele were diagnostic only in progeny of a specific cross combination. For example, allele specific PCR assays for marker CP113, which is closely linked to the *H1* locus for resistance to the root cyst nematode *Globodera rostochiensis*, were indicative for resistance in only four of 136 cultivars tested. This limited predictive value might result either from using various sources of nematode resistance alleles in the particular breeding programs or from a recombination event that occurred early in the breeding history (Niewöhner et al. 1995). The CAPS marker $GP122_{718}$ described in this paper represents a new example for an easy to use marker that is diagnostic for a specific resistance distributed in a wide gene pool.

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