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EVALUATION OF A SCAR RYSC3 MARKER OF THE *Ry*$_{adg}$ GENE TO SELECT RESISTANT GENOTYPES TO *POTATO VIRUS Y* (PVY) IN THE INIA POTATO BREEDING PROGRAM

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ABSTRACT

The *Potato virus Y* (PVY) is distributed worldwide and is one of the most damaging viruses in terms of yield reduction in the potato (*Solanum tuberosum* L.) crop with losses sometimes reaching 80%. To reduce its impact, there is great interest in obtaining varieties carrying the *Ry*$_{adg}$ gene that provides extreme resistance to this virus. This could be facilitated with SCAR (sequence characterized amplified region) RYSC3 by molecular marker-assisted selection (MAS). This study compared the effectiveness of the RYSC3 marker in the detection of the *Ry*$_{adg}$ gene *vs.* biological tests on populations of the Potato Breeding Program of the Instituto de Investigaciones Agropecuarias (INIA), Chile. Within the group of 71 progenitors, 30 plants had some kind of resistance to the virus, of which 17 were carriers of the RYSC3 marker. These genotypes came from Cornell University, Centro Internacional de la Papa (CIP), Peru, and INIA. The analysis of 460 progenies which came from three different crosses showed that 299 individuals amplified the RYSC3 marker and had the resistant phenotype, with the exception of one plant. Within the group of non-RYSC3 carrier plants, a significant percentage (22.5%) showed a resistant phenotype, indicating that these progenies segregate other R genes (e.g., hypersensitivity) that reduce biological test effectiveness. This high effectiveness (99.7%) in the detection of the *Ry*$_{adg}$ gene in both parents and segregating progenies, showed that this marker is appropriate in assisting selection of genotypes with extreme PVY-resistance in the potato breeding programs.

Key words: *Solanum tuberosum*, MAS, extreme resistance.

INTRODUCTION

The *Potato virus Y* (PVY) is one of the most important in terms of yield reduction in the potato crop (*Solanum tuberosum* L.), also affecting other solanaceae species such as tomato (*Lycopersicum esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), and pepper (*Capsicum annuum* L.) (Brunt *et al*., 1996). PVY spreads through the use of infected tuber-seed, mechanical contact, and for more than 25 aphid species in a non-persistent way, producing losses of up to 80%.

The symptoms produced by PVY in potato foliage differ according to the virus strain and the cultivar, from a soft mosaic to severe necrosis and death of the affected plants (Hooker, 1980). Various strains of PVY have been identified in accordance with the symptoms caused in the potato and tobacco. PVY^{O} is the common strain and causes a symptom of soft to severe mosaics depending on the cultivar. PVY^{C} produces lines in the stipules of leaves in carrier cultivars of the resistance gene *Nc*. PVY^{N} causes soft mosaic symptoms in potato foliage, but produces necrosis in tobacco leaves (Hooker, 1980). A sub-group, serologically related with the necrotic breed PVY^{N}, known as PVY^{NTN}, produces necrotic rings in the tubers of the susceptible cultivars. This strain was first described in Europe in the 1970s and has been widely disseminated in the world being detected in the USA in 1993 (Piche *et al*., 2004). In addition, mixed infections of common and necrotic strains can occur, generating recombination of genetic material that has originated hybrid genotypes such as PVY$^{N:O}$ and PVY^{NTN} (Lorenzen *et al*., 2006).

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The apparition and propagation of new recombinants has caused enough damage in potato seed certification systems in North America (Lorenzen, J., 2006, University of Idaho, USA, personal communication). The PVY^N strains or its hybrid recombinants causing severe necrosis in tubers have not yet been detected in Chile. PVY strains can also interact with other viruses that attack the potato such as *Potato virus X* (PVX) and *Potato virus A* (PVA) that produce greater losses (Solomon-Blackburn and Barker, 2001a).

Plants have developed diverse mechanisms to overcome the attack of phytopathogens that can be used to develop resistant cultivars within an integrated control strategy. Genetic resistance consists in the general response of the plant in which the effect of the viral infection is reduced or totally eliminated. There are different types of resistance that go from tolerance, hypersensitive response to the most durable, extreme resistance or immunity to the virus (Solomon-Blackburn and Barker, 2001b). Various sources of resistance to PVY have been found in the potato and its wild relatives. The most studied are the genes from *S. tuberosum* L. subsp. *andigenum* (Juz. & Bukasov) Hawkes (Ry_{adg}), *S. chacoense* Bitter (Ry_{chs}), and *S. stoloniferum* Schltdl. & Bouché (Ry_{sto}) that presented extreme resistance to all PVY strains (Solomon-Blackburn and Barker, 2001a).

At present, breeding programs are quite interested in incorporating these genes in commercial potato varieties. In the genetic map, both genes Ry_{adg} and Ry_{sto} are located in the XI chromosome and the Ry_{chs} gene is found in the IX chromosome (Brigneti *et al*., 1997; Hämäläinen *et al*., 1997). Another extremely resistant gene, $Ry\text{-}f_{sto}$, also from *S. stoloniferum*, is located in the XII chromosome (Flis *et al*., 2005). Using an amplification strategy by PCR (polymerase chain reaction) for conserved regions of the resistance genes types LRR (leucine rich repeat), leucine zippers, and NBS (nucleotide binding site) Hämäläinen *et al*. (1998) cloned and sequenced two fragments of DNA, ADG1 and ADG2 that are narrowly linked to the Ry_{adg} gene. Then, by comparison of the allelic DNA sequence of these fragments between resistant and susceptible individuals, Kasai *et al*. (2000) designed SCAR (sequence characterized amplified region) type markers called RYSC3 and RYSC4 to selectively detect the presence of the Ry_{adg} gene with PCR. Evaluation of the 103 different potato cultivars in Europe, North America, and Japan indicated that the SCAR RYSC3 marker is the most effective to detect the presence of the Ry_{adg} gene (Kasai *et al*., 2000).

In order to implement molecular marker-assisted selection (MAS) methods that increase the effectiveness for selecting PVY-resistant genotypes, carriers of the Ry_{adg} gene in the INIA potato breeding program, this study evaluated the SCAR RYSC3 marker to identify carrier genotypes of the Ry_{adg} gene in different populations (progenitors and progenies). The effectiveness of the SCAR RYSC3 marker was evaluated by comparing the phenotype that was resistant or susceptible to PVY, determined by means of biological inoculation tests of the PVY virus versus the presence or absence of the SCAR RYSC3 marker in the different groups of analyzed plants.

MATERIALS AND METHODS

Plant material

Seventy-one potato genotypes corresponding to the most frequent germplasm used for the potato breeding program of the Instituto de Investigaciones Agropecuarias (INIA) were evaluated as progenitors. This material was maintained *in vitro* and/or in the field in the Centro Regional de Investigación Remehue of INIA, Osorno (23º38` S, 70º25` W), Chile. The segregant progenies of the Ry_{adg} gene were obtained from the seed of Eva x Pike (EP), T88-19 x NY123 (TN), and Eva x Chipeta (EC) crosses. The seeds of these progenies were sterilized, germinated, and maintained as *in vitro* seedlings in a growth chamber until used.

Obtaining PVY inoculums

The PVY inoculum for the biological resistance tests were obtained from potato plants, cv. Atlantic, only infected with PVY virus, in the following manner: 15 field plants were selected that had clear viral symptoms and underwent serological tests for the PVX, PVY viruses, *Potato leaf roll virus* (PLRV), and *Potato virus S* (PVS), with DAS-ELISA in accordance with Salazar (1986). A plant that was infected exclusively with PVY virus was chosen as inoculum and those that showed co-infection with one or more of the other three viruses were rejected. The plant PVY-infected was propagated and cultivated in a greenhouse under isolation conditions. It was assumed that the PVY inoculum corresponded to a common strain predominant in the South of Chile.

Determination of resistant/susceptible phenotype to PVY virus

Plant resistance/susceptibility to PVY was determined by means of biological inoculation tests of the virus through grafting with PVY-infected plants or mechanical transmission. Prior to inoculation with PVY, mechanically, or by grafting, all plants from the *in vitro* culture were acclimated and transplanted into soil (humus:sand 1:1) in pots, and were maintained in the greenhouse until they reached a minimum growth of five leaves. The germplasm genotypes (n = 71) were inoculated with PVY by grafting the leaves of an infected

plant in accordance with Jayasinghe and Salazar (1993). The graft region was covered with Parafilm (American National Can, Greenwich, Connecticut, USA) and the whole plant was covered with a nylon bag to maintain high relative humidity. Then, 7 days after grafting, plants were uncovered and managed in normal fertilization and irrigation conditions. All the tests were carried out in duplicate, and in the cases where the graft did not survive, the process was repeated on a new plant. In turn, plants of the EC, TN, and EP progenies were inoculated by the carborundum method using sap from a PVY-infected plant (Jayasinghe y Salazar, 1993).

Three weeks after mechanical or grafting inoculation, leaf samples were taken to evaluate the presence of the PVY virus by DAS-ELISA (Salazar, 1986). The specific antibodies used against the PVY virus, IgG and IgG-AP, were bought at the Centro Internacional de la Papa (CIP), Lima, Peru, and were diluted 1:200 as recommended. The positive detection of the virus in the sap from the leaves of the grafted plants was interpreted as susceptibility (+) and resistance (-) when absent. For those genotypes that were resistant in the first assay, a second plant replicate was inoculated again with PVY and re-evaluated by DAS-ELISA.

DNA extraction

For molecular characterization of the different plants, for both germplasm and progenies, DNA was obtained from fresh leaves of plants cultivated in a greenhouse with the protocol described by Fulton *et al*. (1995).

PCR conditions of SCAR RYSC3 resistant to PVY

Primers to amplify the RYSC3 marker with PCR were described by Kasai *et al*. (2000) and correspond to the oligonucleotoids 3.3.3s (5'-ATA CAC TCA TCT AAA TTT GAT GG-3') and ADG23R (5'-AGG ATA TAC GGC ATC ATT TTT CCG A-3').

A 20 µL reaction mixture containing 50 ng of template DNA, 2 mM $MgCl_2$, 0.125 mM dNTP, 0.25 µM of each primer, and 1 unit of Taq polymerase enzyme was used. The amplification program, carried out in a thermocycler (model PTC-100, MJ Research, Ramsey, Minnesota, USA) consisted of an initial denaturalization time of 2 min of the DNA at 94 ºC, then 35 cycles at 94 ºC for 15 s, 55 ºC for 15 s, and 72 ºC for 40 s. Subsequently, the PCR products were separated in agarose gels at 1% in TBE 1X for 30 min, then were dyed in a 5 µg mL^{-1} ethidium bromide solution for 10 min, and were photographed with a digital photo camera (Kodak, DC290) in a UV light transilluminator. Controls were DNA from the 2(v-2)7 (positive control) and 8 4.194.30 (negative control) genotypes kindly provided by Dr. Jari Valkonen of the Uppsala Genetic Center, University of Agricultural Sciences in Uppsala, Sweden.

Segregant progeny analysis

The segregation analyses of resistance to PVY and the RSYC3 marker were carried out in three progenies EP, TN, and EC. In an autotetraploid species like the potato ($2n = 4x = 48$), if random mating of each one of the four homologous chromosomes is assumed in meiosis, without considering double reduction, the expected ratios in the gametes of a dominant gene or marker are 1:1 and 5:1, in the case of a simplex (one copy) and duplex (two copies), respectively (Hackett *et al*., 1998). Triplex (three copies) and quadruplex (four copies) did not show segregation. Triplex could only show segregation in the double reduction case which is not considered in this study. The χ^2 test was used as the statistical indicator of the distinct segregation patterns studied.

RESULTS AND DISCUSSION

Standardization of PCR conditions for the SCAR RYSC3 marker

The effectiveness of a marker like SCAR RYSC3 to detect carrier genotypes of the resistance gene Ry_{adg} to PVY can be negatively affected by various causes such as inadequate PCR conditions, excessive distance between the marker and the gene *loci* that lead to its recombination, presence of other alleles of the same locus not previously identified and/or union of the PCR primers in other sequences of the genome that amplify products of similar size that do not correspond to the marker. Not very selective PCR conditions with low alignment temperature and/or high primer concentration and Mg^{+2} can generate unspecified PCR products. On the other hand, very stringent PCR conditions can diminish amplification efficiency of the product. Both situations could lead to an error with an increase of the number of false positives or negatives, respectively. Due to the fact that PCR conditions can vary between laboratories, marker reproducibility was evaluated under our work conditions. It was possible to achieve amplification of the expected fragment of 321 pb, amplified with the RYSC3 marker from the DNA of the 2(v-2)7 genotype used as positive control (Figure 1). On the other hand, DNA of the 84.194.30 genotype used as negative control did not generate a product. This result indicated that the PCR conditions described by Kasai *et al*. (2000) for the RYSC3 marker were adequate under the conditions of this study.

Presence of Ry_{adg} gene in INIA germplasm

The SCAR RYSC3 marker was used to determine the presence of the Ry_{adg} gene over a population of 71 genotypes of the INIA germplasm, corresponding to the progenitors most frequently used. Table 1 and Figure 2

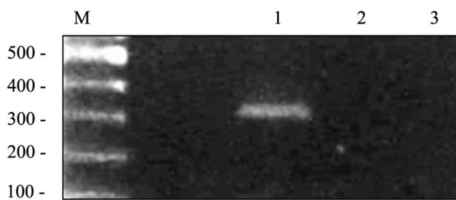

Figure 1. Amplification control of SCAR RYSC3 marker for the Ry_{adg} gene. (M) Molecular size marker100 bp Ladder. (1) 2(v-2)7 positive control; (2) 84.194.30 negative control; (3) control without DNA.

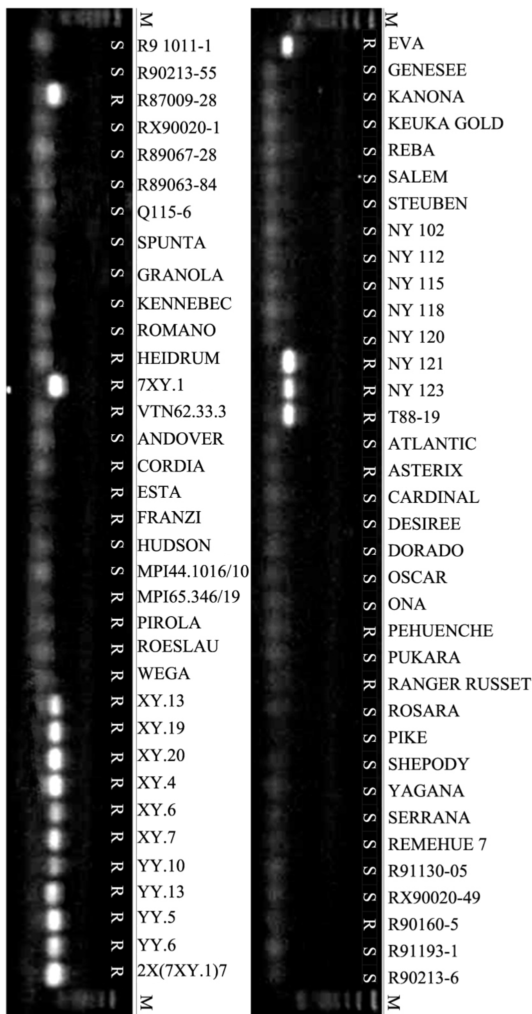

Figure 2. Evaluation of progenitors of the germplasm of the Instituto de Investigaciones Agropecuarias (INIA) breeding program with SCAR RYSC3 marker. (M) Molecular size marker 100 pb Ladder; (R) individual resistant to *Potato virus Y* (PVY); (S) susceptible to PVY.

show the results of the biological evaluation (phenotype) by inoculation of the PVY virus versus the presence or absence of the RYSC3 marker (genotype). The results of SCAR RYSC3 analysis show that 17 individuals (56%) of the germplasm resistant to PVY were carriers of the RYSC3 marker (Table 1). Genotypes that carry the marker and susceptible did not show up. Of the carrying genotypes, two groups stand out in accordance with their origin, var. Eva and the clones NY121, NY123, and T88-19 from the potato breeding program at Cornell University, and the clones 7XY.1, XY.13, XY.19, XY.20, XY.6, XY.7, YY.10, YY.13, YY.6, and 2X(7XY.1)7 from CIP.

Various genotypes included in this study were also evaluated by Kasai *et al*. (2000) for both carriers of the R_{adg} gene (XY.1, NY121, NY123) and non-carriers (‘Atlantic’, ‘Salem’, ‘Desiree’, ‘NY112’, ‘NY115’, ‘Pike’, ‘Serrana’, ‘Yagana’) which coincide with respect to the presence or absence of the RYSC3 marker. Exceptions were the cvs. Desiree and Serrana described by Kasai *et al*. (2000) as having a hypersensitive response to PVY, while in this study they behaved as susceptible. This difference is probably due to the use of distinct isolates of PVY in the bioassays. In this germplasm, other genotypes were also detected that resulted resistant to PVY and non-carriers of the RYSC3 marker corresponding to cvs. Asterix, Pehuenche, Ranger Russet, R91130-05, R87009-28, Heidrun, VTN 62.33.3, Cordia, Esta, Franzi, MPI 65.34619, Pirola, Roeslau, and Wega. Of these genotypes, the cv. Pehuenche and the R91130-05 and R87009-28 clones correspond to selections of the INIA breeding program; var. Ranger Russet was developed in Idaho, USA; Asterix and VTN 62.33.3 genotypes are from Holland; and Cordia, Esta, Franzi, MPI 65.34619, Pirola, Roeslau, and Wega correspond to materials of German origin. The observed resistance in these potato genotypes is probably due to the action of other genes such as Ry_{sto}^{na} (R^2), Nc_{tbr} (*Nc*), Nv_{tbr} (*Nv*), or Ny_{tbr} (*Ny*) that provide hypersensitive type (HR) resistance, or the extreme resistance $Ry\text{-}f_{sto}$ gene. Recently, Flis *et al*. (2005) found that the varieties of German origin Esta, Heidrum, Pirola, and Wega are carriers of the $GP122_{718}$ marker specific for the $Ry\text{-}f_{sto}$ gene.

SCAR-RYSC3 evaluation for Ry_{adg} in progenies

From the results of the phenotypic and genotypic evaluation of the germplasm, with regards to the resistance to PVY and the presence of the RYSC3 marker, three families were chosen to evaluate segregation of the Ry_{adg} gene and its marker. The objective was to obtain information about possible recombination cases between the gene and the marker, genetic donor conformation, and determine marker effectiveness in breeding populations in

Table 1. Presence of RYSC3 marker of Ry_{adg} gene in potato germplasm of the Instituto de Investigaciones Agropecuarias (INIA). R and S phenotypes are resistant and susceptible to *Potato virus* Y (PVY), respectively.

Nº	Clone or cultivar	Phenotype	Presence of RYSC3 marker	PVY resistance gene	Origin
1	Eva	R	+	Ry_{adg}	USA
2	Genesee	S	-		USA
3	Kanona	S	-		USA
4	Keuka Gold	S	-		USA
5	Reba	S	-		USA
6	Salem	S	-		USA
7	Steuben	S	-		USA
8	NY 102	S	-		USA
9	NY 112	S	-		USA
10	NY 115	S	-		USA
11	NY 118	S	-		USA
12	NY 120	S	-		USA
13	NY 121	R	+	Ry_{adg}	USA
14	NY 123	R	+	Ry_{adg}	USA
15	T88-19	R	+	Ry_{adg}	USA
16	Atlantic	S	-		USA
17	Asterix	R	-	nd	Holland
18	Cardinal	S	-		Holland
19	Desiree	S	-		Holland
20	Dorado	S	-		Holland
21	Oscar	S	-		Holland
22	Ona	S	-		Chile
23	Pehuenche	R	-	nd	Chile
24	Pukara	S	-		Chile
25	Ranger Russet	R	-	nd	USA
26	Rosara	S	-		Germany
27	Pike	S	-		USA
28	Shepody	S	-		Canada
29	Yagana	S	-		Chile
30	Serrana	S	-		Argentina
31	Remehue 7	S	-		Chile
32	R91130-05	S	-		Chile
33	RX90020-49	S	-		Chile
34	R90160-5	R	-	nd	Chile
35	R91193-1	S	-		Chile
36	R90213-6	S	-		Chile
37	R91011-1	S	-		Chile
38	R90213-55	S	-		Chile
39	R87009-28	R	+	Ry_{adg}	Chile
40	RX90020-1	S	-		Chile
41	R89067-28	S	-		Chile
42	R89063-84	S	-		Chile
43	Q115-6	S	-		Chile
44	Spunta	S	-		Holland
45	Granola	S	-		Germany

Continued Table 1.

Nº	Clone or cultivar	Phenotype	Presence of RYSC3 marker	PVY resistance gene	Origin
46	Kennebec	S	-		USA
47	Romano	S	-		Holland
48	Heidrun	R	-	Ry_{fsto}	Germany
49	7XY.1	R	+	Ry_{adg}	CIP, Lima, Peru
50	VTN 62.33.3	R	-	nd	Holland
51	Andover	S	-		USA
52	Cordia	R	-	nd	Germany
53	Esta	R	-	Ry_{fsto}	Germany
54	Franzi	R	-	nd	Germany
55	Hudson	S	-		USA
56	MPI 44.1016/10	S	-		Germany
57	MPI 65.346/19	R	-	nd	Germany
58	Pirola	R	-	Ry_{fsto}	Germany
59	Roeslau	R	-	nd	Germany
60	Wega	R	-	Ry_{fsto}	Germany
61	XY. 13	R	+	Ry_{adg}	CIP, Lima, Peru
62	XY. 19	R	+	Ry_{adg}	CIP, Lima, Peru
63	XY. 20	R	+	Ry_{adg}	CIP, Lima, Peru
64	XY. 4	R	+	Ry_{adg}	CIP, Lima, Peru
65	XY. 6	R	+	Ry_{adg}	CIP, Lima, Peru
66	XY. 7	R	+	Ry_{adg}	CIP, Lima, Peru
67	YY. 10	R	+	Ry_{adg}	CIP, Lima, Peru
68	YY. 13	R	+	Ry_{adg}	CIP, Lima, Peru
69	YY. 5	R	+	Ry_{adg}	CIP, Lima, Peru
70	YY. 6	R	+	Ry_{adg}	CIP, Lima, Peru
71	2X(7XY.1)7	R	+	Ry_{adg}	CIP, Lima, Peru

Ry_{adg}: *Solanum tuberosum* spp. andigena; Ry_{fsto}: S. *stoloniferum*; nd = not determined.
CIP: Centro Internacional de la Papa.

a practical way. Progenies of the Eva, Pike, NY123, T88-9, and Chipeta progenitors were used for this (Table 2). The last variety listed is not part of the INIA breeding program; however, it is described as susceptible to PVY (Colorado-Certified, 2007). Plants were obtained from the Eva x Pike (EP), T88-19 x NY123 (TN), and Eva x Chipeta (EC) families, which were inoculated mechanically with PVY, and susceptibility (+) or resistance (-) to the virus were determined through serological detection by DAS-ELISA. Presence of the RYSC3 marker was determined with PCR according to what was previously described. A total of 176, 150, and 134 genotypes of the EP, TN, and EC families, respectively were evaluated. Table 3 shows progeny characterization with regards to the presence or absence of the marker and the PVY resistance or susceptibility phenotype, respectively. At first glance, it can be observed that of a total of 299 genotypes evaluated from the different progenies, only one plant that carried the marker showed a positive serological test of susceptibility to PVY. Furthermore, if it is considered that this could correspond to a false positive of the ELISA test, it could be said that in these progenies, the marker was highly effective, close to 100%, in the identification of genotypes resistant to PVY carriers of the Ry_{adg} gene. However, a total of 36 genotypes, 10 in the TN progeny and 26 in EP, were resistant to PVY and did not show the RYSC3 marker. The aforementioned could be explained as false positives of the DAS-ELISA test, false negatives of the PCR test, segregation of another *Ry* gene resistant to PVY, or recombination between the RYSC3 marker and the Ry_{adg} gene.

A more detailed analysis of the segregation of the Ry_{adg} gene marker and the resistance phenotype to PVY can give more information about the genetic configuration of the progenitors with regard to the number of copies of the resistant gene and/or the marker. In the *S. tuberosum* ($2n = 4x = 48$) species that is autotetraploid, a dominant gene or marker can have one (simplex), two (duplex),

Table 2. Phenotype and probable genotype of progenitors of segregant families resistant to *Potato Virus Y* (PVY).

Progenitor	Number of copies Ry_{adg} gene	Phenotype resistant (R) or susceptible (S) to PVY	Presence of RYSC3 marker
Eva	Simplex	R	+
Pike	-	S	-
NY123	Simplex or duplex	R	+
T88-9	Simplex or duplex	R	+
Chipeta	-	S	ND

ND: not determined.

Table 3. Frequency of the RYSC3 marker and *Potato virus Y* (PVY) resistance in EP, TN, and EC progenies.

		Frequency of individuals			
		RYSC3(+)		RYSC3(-)	
Cross	Type of cross	R	S	R	S
Eva x Chipeta	R x S	90	1	0	43
T88-19 x NY123	R x R	128	0	10	12
Eva x Pike	R x S	81	0	26	69

R: resistant; S: susceptible.

three (triplex), and four (quadruplex) copies. In the absence of double reduction, it is only possible to observe segregation for the simplex and duplex doses where the ratio of gametes would be 1:1 and 5:1, respectively (Hackett *et al.*, 1998). This study did not consider double reduction situations during meiosis which would generate segregation in triplex and slightly modify simplex and duplex segregation; the effect of this phenomenon in tetrasomic segregation is discussed in detail by Luo *et al.* (2000).

In the EP progeny, which is a cross between the resistant genotype (Eva), carrier of the Ry_{adg} gene and the RYSC3 marker, and a susceptible one (Pike), the ratio between carrier individuals of the marker associated to the Ry_{adg} gene vs. non-carriers of the marker is adjusted in the expected ratio of 1:1 (Table 4), indicating that the var. Eva carries only one copy of the Ry_{adg} gene. However, if segregation of the resistance (phenotype) is evaluated, it is confirmed a significant number of resistant genotypes are not carriers of the marker (Tables 3 and 5). This situation could be due to the segregation of the other *Ry* gene present in Eva or to the leaks of the mechanical inoculation technique (false negatives). In the case of the presence of a second major resistance gene (*Ry*) to PVY in Eva, the expected segregation among resistant (R) and susceptible (S) plants is shown in Table 6. It is only possible to know a fraction of the segregation of the second gene *Ry*, shown by the ratio of resistant genotypes without the RYSC3 marker. In the case of independent genes, the expected ratios between R:S is 3:1 and the expected fraction of *Ry* plants without the RYSC3 marker is 1:4. The χ^2 values indicate that both ratios are quite distant from the observed values, indicating that these genes Ry_{adg} and *Ry* could be linked (Table 7). In accordance with the ratio of genotypes *S* and *Ry* without the RYSC3 marker, where the number of individuals S is much greater than *Ry* individuals, it would be a question of linking in the coupling phase. Given that the missing fraction is not known, it is not possible to know the distance between the Ry_{adg} gene and *Ry*. Assuming a balanced recombination, an approximation of *r* would be 2(26/n) (Hackett *et al.*, 1998) which is equal to 29.5 cM (centimorgan). However, it cannot be rejected that these R genotypes without the RYSC3 marker correspond to susceptible leaks that were not infected by the mechanical inoculation method.

In the TN progeny, product of a cross of two parents resistant to PVY carriers of the Ry_{adg} gene, the configuration of the parents could be simplex/simplex (*S*/*S*), duplex/simplex (*D*/*S*) or duplex/duplex (*D*/*D*) with the expected ratios of 3:1, 11:12, and 35:36, respectively. In accordance with the χ^2 values, the ratio of carrier genotypes versus non-carriers of the RYSC3 marker of the Ry_{adg} gene is closer to *D*/*S* than *S*/*S*, though in both cases there is a significant deviation. It is not possible to infer from this experiment which of the two progenitors is duplex or simplex. Furthermore, as for the EP family, a high number of genotypes was observed of non-carriers of the RYSC3 marker that resulted negative for the ELISA test. In both cases, the situation could be explained by reasons such as a PCR defect, recombination between marker and gene, false negatives of the ELISA test, low efficiency in the inoculation of the virus, and/or segregation of another

Table 4. Segregation analysis of RYSC3 marker associated to gene Ry_{adg} to resistance *Potato virus Y* (PVY) in EP, EC, and TN progenies.

Progenies	Total	RYSC3(+)	RYSC3(-)	1:1	5:1	3:1	11:12	35:36
Eva x Pike	176	81	95	1.114ns	176.405**	78.818**	480.008**	1708.366**
Eva x Chipeta	134	91	43	17.194**	22.949**	3.592ns	98.999**	426.310**
T88-19 x NY123	150	128	22	74.907**	0.432ns	8.542**	7.876**	78.507**

* p < 0.05; ** p < 0.01; ns: non significant.

Table 5. Segregation analysis of resistant and susceptible genotypes to *Potato virus Y* (PVY) in EP, EC, and TN progenies.

Progenies	Total	Resistant	Susceptible	1:1	5:1	3:1	11:12	35:36
Eva x Pike	176	107	69	8.205**	64.368**	18.939**	219.579**	864.751**
Eva x Chipeta	134	90	44	15.791**	25.224**	4.388*	105.316**	448.294**
T88-19 x NY123	150	138	12	105.840**	8.112**	23.120**	0.022ns	15.147**

* p < 0.05; ** p < 0.01; ns: non significant.

Ry resistance gene. The PCR defect is not likely given that these same DNA amplified other markers (results not shown). In the remaining situations, recombination between marker and gene is also not likely given that a comparable number of susceptible carrier genotypes of the marker were not observed. This is reinforced by the observations of Vidal *et al*. (2002) who propose that the RYSC3 marker would be contained within the same Ry_{adg} gene. Therefore, the high number of resistant genotypes, non-carriers of the RYSC3 marker, could be due to the segregation of another resistance gene like *Ry*, false negatives in the ELISA test, and/or the low efficiency of PVY virus inoculation. It is important to remember that in this experiment, PVY inoculation was mechanically carried out which is a low efficiency infection system for this virus (Salazar, 1979).

The situation for the EC progeny, Eva x Chipeta, is a more complex case. In the USA, the var. Chipeta according to the variety description profile in Colorado, USA (Colorado-Certified, 2007), behaves as susceptible to PVY. Therefore, it is expected that the segregation of the Ry_{adg} gene and its marker would be similar to the EP progeny. However, in this progeny a high number of carrier genotypes of the marker were observed with a ratio that adjusts to a 3:1 segregation which indicates that it is refers to an RxR cross.

Integrating the Ry_{adg} gene marker in INIA's breeding program

The RYSC3 marker of the extreme resistance Ry_{adg} gene to PVY showed a high effectiveness in identifying potato carrier genotypes of this gene, in both germplasm and segregant progenies. This represents an excellent support tool to increase the frequency of this gene in breeding populations which would allow developing future potato varieties resistant to PVY. It is important to note that in this experiment, the effectiveness of the marker to detect the resistance gene was evaluated in three distinct genetic background environments, and under experimental laboratory, greenhouse, and field conditions of the INIA breeding program, all of which can be distinct in other programs. If we compare the effectiveness of the selection of resistant carrier genotypes of the Ry_{adg} gene using the RYSC3 marker with the massive inoculation biological assays with an abrasive like carborundum (Jayasinghe and Salazar, 1993), the molecular method proved to be a lot more effective, since only one genotype of the 266 evaluated in the progenies turned out to be susceptible, showing a 99.67% effectiveness. However, the biological test identified 36 resistant individuals, non-carriers of the marker, for which effectiveness in identifying carrier genotypes of the Ry_{adg} gene decreased to 87.96%. This loss of effectiveness can be seen as increased in the presence of other PVY resistance genes which are relatively abundant in *S. tuberosum*. Also on this subject, molecular markers have been described for the Ry_{chs}, Ry_{sto}, and $Ry_{f\text{-}sto}$ genes with extreme resistance to PVY from *S. chacoense* and *S. stoloniferum*, respectively (Brigneti *et al*., 1997; Gebhardt *et al*., 2006; Witek *et al*., 2006). The M45 marker of the extreme resistance Ry_{sto} gene described by Brigneti *et al*. (1997) has also been used to evaluate INIA germplasm, found present in 12 of the 71 genotypes evaluated in this study; and only a few of them resulted in RYSC3 (-) (results not shown).

Another important aspect to consider is the efficiency of the selection methods. A molecular test based on PCR only requires a small piece of plant tissue, whether tuber

Table 6. Expected and observed phenotypic ratios for a two-gene resistance model (Ry_{adg} and R*y*) from Eva that segregate in EP progeny.

	Expected frequencies						
		Link					
Possible genotypes	Indep.	Repul.	Coupling	Phenotypes	RSYC3	Classes	Frequency
$Ry_{adg}Ry(r2)_2$	1/4	1/6	3/6	R	+	A	81
$Ry_{adg}r1(r2)_2$	1/4	2/6	0				
$r1Ry(r2)_2$	1/4	2/6	0	R	-	B	26
$(r1r2)_2$	1/4	1/6	3/6	S	-	C	69

Indep.: totally independent genes; Repul.: genes in repulsion phase; Coupling: genes in coupling phase.

Table 7. Segregation analysis for a model of two resistance segregant genes (Ry_{adg} and R*y*) in EP progeny.

Classes				Segregation			
A	B	C	Total	χ^2 Ryadg	χ^2 **Ry**	χ^2 **total**	χ^2 **Link**
81	26	69	176	1.114	19.463**	22.125	1.548

* $p < 0.05$; ** $p < 0.01$.

or foliage, on the other hand, the biological test requires at least one whole plant. As regards the time needed to carry out the diagnosis, and considering the extraction processes for DNA, PCR, and electrophoresis, the molecular test does not need more than 1 day. This time could further be reduced if its amplification were optimized in a real-time PCR system using fluorescent molecules like SYBR-Green (Ponchel *et al*., 2003), which would allow to dispense with the electrophoresis stage. On the other hand, the biological test takes place over a period of months, going from when the plant grows, is inoculated with the virus, the virus is propagated, plant develops new leaves, and ELISA tests are carried out. Even if the cost of equipment and PCR reactives could be a bit higher, these are lower each time and the saving in time and selection effectiveness fully justify selection based on molecular markers. Furthermore, this SCAR RYSC3 marker of the Ry_{adg} gene can be amplified simultaneously with a marker with resistance to golden nematode H1 (Mathias *et al*., 2004).

Extreme resistance is desirable to control recombinant PVY

An alarming situation occurred in Canada and the USA when recombinations of the common and necrotic strains of PVY were detected. These new recombined genotypes could not be detected with the PVY^C, PVY^O, and PVYN specific monoclonal antibodies that use the seed programs of these countries, since they had mutated by recombination for the antibody recognition region (Lorenzen *et al*., 2006). The situation became even more complicated with the rapid dissemination through cultivars like Shepody that is tolerant and practically asymptomatic to PVY. If we consider that this cultivar is used quite a lot by the local frozen French fries industry in the South of Chile, and neighboring countries such as Argentina and Brazil that normally obtain potato seed from Canada and the USA, it becomes evident that Chile is in a risk situation with respect to the entry of these new recombined PVY breeds. In the long-term, the best strategy to control the virus is to use varieties that are carriers of extreme resistance genes that protect against all the strains of the virus. It is important to note that some INIA materials like var. Purén (not included in Table 1) that is resistant to PVY and carrier of the RYSC3 marker associated with the Ry_{adg} gene, have awakened great interest as varieties and progenitors in various USA breeding programs (Secor, G., North Dakota State University, North Dakota, USA, personal communication).

CONCLUSIONS

Of a group of 71 genotypes of potato germplasm of the INIA breeding program inoculated with PVY, 30 showed some type of resistance to the virus, of which 17 (56%) are carriers of the RYSC3 marker specific to the extreme resistance Ry_{adg} gene.

Comparison of the effectiveness of the identification and selection of PVY-resistant individuals, carriers of the Ry_{adg} gene, between the RYSC3 marker assay and mechanical inoculation biological assay followed by detection of the PVY virus by DAS-ELISA, show that

this marker has almost a 100% effectiveness in the three segregant progenies evaluated while biological assays did not reach 90%, which could decrease still more in the presence of other resistance genes.

At present, factors are known that have complicated PVY control in North America, like the virus mutations that modified the recognition region of the antiserums making detection difficult through biological tests. Situations such as this justify the efforts to obtain varieties with extreme resistance to the virus, and which can be done more efficiently by using a neutral marker like SCAR RYSC3.

RESUMEN

Evaluación de un marcador SCAR RYSC3 del gen *Ry$_{adg}$* para seleccionar genotipos resistentes al *Virus Y de la papa* (PVY) en el programa de mejoramiento genético de papa del INIA. El *Virus Y de la papa* (PVY), distribuido mundialmente, es uno de los más dañinos en términos de reducción del rendimiento del cultivo de la papa (*Solanum tuberosum* L.); llegando a producir pérdidas de hasta 80%. Para reducir su impacto, existe gran interés por obtener variedades portadoras del gen *Ry$_{adg}$* que confiere resistencia extrema a este virus. Esto se podría facilitar con la utilización del SCAR RYSC3, mediante selección asistida por marcadores moleculares (MAS). En este estudio se comparó la eficacia del marcador RYSC3 en la detección del gen *Ry$_{adg}$* *vs.* pruebas biológicas, sobre poblaciones del Programa de Mejoramiento Genético de Papa del Instituto de Investigaciones Agropecuarias (INIA), Chile. De un grupo de 71 progenitores analizados, 30 plantas presentaron algún tipo de resistencia al virus, de las cuales 17 son portadores del marcador RYSC3. Estos genotipos provienen de la Universidad de Cornell (EE.UU.), del Centro Internacional de la Papa (CIP, Perú) y del INIA. Del análisis de 460 progenies, provenientes de tres cruzamientos distintos, 299 individuos amplificaron el marcador RYSC3 y presentaron el fenotipo resistente, con la excepción de una planta. En el grupo de plantas no portadoras de RYSC3, un porcentaje significativo (22,5%) presentó un fenotipo resistente, lo que sugiere que en estas progenies segregan otros genes R (ej. hipersensibilidad) que reducen la eficacia de las pruebas biológicas. Estos resultados de alta eficacia (99,7%) en la detección del gen *Ry$_{adg}$*, tanto en progenitores como progenies segregantes, muestran la conveniencia de utilizar RYSC3 en la selección de genotipos portadores del gen *Ry$_{adg}$* en los programas de mejoramiento genético de papa.

Palabras clave: *Solanum tuberosum*, SAMM, resistencia extrema.

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