Theor Appl Genet (1998) 96: 1036–1043

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Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato

Received: 18 December 1997 / Accepted: 30 December 1997

Abstract The gene Ry_{adg} that confers resistance to potato Y potyvirus (PVY) in the cultivated potato [*Solanum tuberosum* subsp. *andigena*, line 2*x*(v-2)7] is located on chromosome XI in a segment that contains three other known resistance genes in other syntenic solanaceous species. One of them is the gene *N* that controls resistance to tobacco mosaic tobamovirus in tobacco and has previously been isolated and sequenced. Three sequence-related, resistance gene-like (RGL) DNA fragments (354–369 bp) highly homologous to the gene *N* were PCR-amplified from the potato line 2*x*(v-2)7. Two RGL fragments (79 and 81% homologous to the *N* gene) co-segregated with Ry_{adg} among the 77 F1 progeny tested. These RGLs may originate from a resistance gene family on chromosome XI. The potato line 2*x*(v-2)7 also expressed resistance to potato A potyvirus (PVA), which was controlled by another locus on chromosome XI mapped ca. 6.8 cM distal to Ry_{adg}.

Key words Potato virus A · Potato virus Y · Resistance gene · RFLP · Diploid potato

Communicated by F. Salamini

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Introduction

During the past 5 years, several disease resistance genes have been cloned from dicotyledenous and monocotyledenous plant species (reviewed in Staskawicz et al. 1995; Jones 1996, 1997). Most of the resistance genes cloned to-date act against fungal and bacterial pathogens (Jones 1996), but the gene *N* cloned from tobacco controls resistance to tobacco mosaic tobamovirus (TMV) (Whitham et al. 1994). It is now well-established that resistance genes occur as gene families or clusters in plants (Martin et al. 1993; Jones et al. 1994; Dixon et al. 1996; Büschges et al. 1997). Many of the resistance gene clusters contain highly homologous genes that, however, contain some differences providing the genes with an ability to recognize specific strains of a pathogen. The strain-specific recognition and activation of resistance responses is hypothesized to depend on a gene-for-gene interaction involving the plant resistance gene product and the pathogen avirulence gene product (Flor 1946). Molecular proof of this theory was recently provided by demonstrating the specific interaction between the product of the gene *Pto* of tomato and the *avrPto* avirulence gene product of *Pseudomonas syringae* pv *tomato* (Tang et al. 1996).

Many of the cloned resistance genes share similar sequence motives that suggest an involvement of these genes in signal-transduction pathways (Jones 1996, 1997; Lamb 1996). Comparison of the amino-acid sequences, as deduced from the DNA sequences, of 12 isolated resistance genes has revealed leucine-rich repeats (LRR; possibly involved in protein-protein interactions) in nine genes and nucleotide-binding sites (NBS) in six genes (Parker et al. 1997). Based on the conserved domains, oligonucleotides have been

designed and used for the PCR-based isolation of fragments of tentative resistance genes from potato (Leister et al. 1996) and soybean (Kanazin et al. 1996; Yu et al. 1996). When these resistance gene-like (RGL) sequences have been localized on the genetic linkage maps of potato or soybean, many of them have been found to be located in the vicinity of the previously mapped resistance gene clusters (Kanazin et al. 1996; Leister et al. 1996; Yu et al. 1996). Therefore, the RGLs may be used as highly linked RFLP markers to select for disease resistance in breeding programs, and can also be starting points for resistance gene isolation. However, the successful use of RGLs for either purpose has not yet been reported.

In vegetatively propagated crops, such as potato (*Solanum tuberosum* L., family Solanaceae), viruses are serious pests as they are carried to the new crops in infected seed tubers. Potato virus Y (PVY) and potato virus A (PVA) are the most prevalent potyviruses infecting potato crops in most areas where potatoes are grown (Hooker 1981). Potyviruses comprise the largest and economically the most important group of plant viruses. Because they are transmitted non-persistently by aphids in the field, their dispersal in the crops can only be prevented by growing resistant cultivars. Dominant, monogenically controlled, extreme resistance (E) to PVY and PVA is utilized in potato breeding programs (Barker 1996; Valkonen et al. 1996). Potato plants expressing E usually remain symptomless, except for the limited necrosis that may develop in the top leaves of some genotypes following graft-inoculation, and the extremely low virus titers that accumulate in infected cells. Also dominant, monogenically inherited, hypersensitive resistance (H) to PVY and PVA is known in potato. It is expressed as the development of necrotic lesions in inoculated leaves and/or necrosis in systemically infected parts of the plant (Valkonen et al. 1996).

Two genes for E to PVY in potato have been genetically localized, namely Ry_{adg} in *S. tuberosum* subsp. *andigena* (Hämäläinen et al. 1997) and Ry_{sto} that is probably derived from *S. stoloniferum* (Brigneti et al. 1997). Both genes are located in the same segment of chromosome XI. Interestingly, the corresponding region of chromosome XI in tobacco (*Nicotiana tabacum*, family Solanaceae) carries the gene *N* conferring resistance to TMV (Leister et al. 1996). Leister et al. (1996) have amplified three RGL fragments from *S. t. tuberosum* that are located in the region of chromosome XI which we have found to contain the gene Ry_{adg} in *S. t. andigena* (Hämäläinen et al. 1997). The aim of the present study was to test whether any resistance gene-like sequences homologous to the above mentioned RGLs (Leister et al. 1996) could be amplified from the locus actually carrying Ry_{adg} in *S. t. andigena*.

In many potato genotypes, E to PVY is associated with the expression of H to PVA (Cockerham 1970; Barker 1996). Indeed, it was recently found that the parental line $2 \times$ (v-2)7, used for the production of our gene mapping population, also expresses H to PVA (J. P. T. Valkonen, unpublished data). Thus, the second aim of our study was to examine the possible linkage between the loci controlling resistance to PVY and PVA (Cockerham 1970; Barker 1996). Our data indicate that chromosome XI contains two different, but linked, loci that control E to PVY and H to PVA, respectively. Our data also show that two of the three RGL fragments produced in this study are either tightly linked to, or originate from, the locus controlling E to PVY.

Materials and methods

Plant and virus material

A cross was made between the diploid ($2n = 2x = 24$) potato breeding line $2x$(v-2)7 and the diploid potato clone 84.194.30 (Valkonen et al. 1994). The parental lines were provided by The International Potato Center (CIP), Lima, Peru (Watanabe et al. 1994).

Two cuttings of each parental line and progeny individual were tested for virus resistance in two experiments. The isolate PVY^O-UK that belongs to the ordinary strain group of PVY^O (Gibson et al. 1990), the isolate PVY^N-RUS that belongs to the tobacco necrosis strain group of PVY (Puurand and Saarma 1992; Valkonen 1997), and the isolate PVA-U that belongs to the PVA strain group 1 (Valkonen et al. 1995), were used for side-graft inoculation as previously described (Valkonen et al. 1994). PVA-U was also used for mechanical inoculation (Valkonen et al. 1995). Experiments were carried out in the greenhouse under natural daylight supplemented with illumination from sodium halide lamps (photoperiod 18 h). Means of the minimum and maximum temperatures were 19°C and 26°C. The previously described procedures and reagents were used (Valkonen 1997) for testing the uppermost fully expanded leaves for PVY and PVA by DAS-ELISA 26–28 days after graft-inoculation.

RFLP analysis

DNA extraction (Bernatzky and Tanksley 1986), bulked segregant analysis (BSA) (Michelmore et al. 1991) and RFLP analysis (Sambrook et al. 1989) were carried out as previously described (Hämäläinen et al. 1997). In BSA, DNA samples of the progeny were combined into three pools, 9–14 progeny in each pool, based on whether they were extremely resistant, hypersensitive or susceptible to PVY^O. The same three DNA pools were also used for mapping the PVA resistance locus, because all the pooled progeny expressing E to PVY^O were also resistant to PVA, and all the pooled progeny expressing H or S to PVY^O were susceptible to PVA. Bulked DNA was digested with *Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Msp*I, *Pst*I, *Taq*I or *Xba*I. Restriction enzyme digestions and RFLP analysis of individual progeny were done as described previously (Hämäläinen et al. 1997).

Tomato (CD17, TG508, TG651, TG523, CT182) and potato (GP125, CP58) markers were used as probes in RFLP analysis (Gebhardt et al. 1989, 1991; Tanksley et al. 1992). Probes were labelled with digoxigenin and the signal on Southern blots was detected by chemiluminescence following the manufacturer's instructions (Boehringer Mannheim, Germany). Linkage analysis was performed using MAPMAKER/EXP 3.0 software (Lander et al. 1987).

Table 1 Primers used for PCR amplification of the RGLs by PCR

Fragment	Primer combinations[a]	Primer sequences[a]	Fragment size[b]
ADG1	Primer 3.3.13s Primer 3.3.13as	5′-CAC ACT CTC GTA TCA GTT TGA-3′ 5′-ATT TAA TAG CGT GAC AGT CAA C-3′	356 bp
ADG2	Primer 3.3.3s Primer 3.3.3as	5′-ATA CAC TCA TCT AAA TTT GAT GG-3′ 5′-ACT TAA CTG CAT CAT GTT CAA G-3′	354 bp
ADG3	Primer 3.3.1s primer 3.3.1.as	5′-GCA TGG GCT GAT GCA CTT G-3′ 5′- TAC AAC AGA CTT GGA GAG TTT-3′	369 bp

[a] Leister 1995
[b]Determined by sequencing

PCR amplification of resistance gene-like (RGL) fragments

Three RGL fragments (ADG1, ADG2 and ADG3) were amplified by PCR from the total DNA of $2x$(v-2)7 using the primers described by Leister (1995) (Table 1). These primers were selected because the studies of Leister et al. (1996) had shown that the DNA fragments amplified by them co-segregated with a few RFLP markers from chromosome XI that we had found to be linked to Ry_{adg} (Hämäläinen et al. 1997). The reaction mixture consisted of 50 µl of 10 mM TrisHCl (pH 8.8), 4.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 0.1 mM of dNTPs, 0.25 µM of each primer and 1 U of Dynazyme DNA polymerase (Finnzymes Oy, Finland) and 50 ng of total DNA. Amplification conditions were: 2 min at 93°C; 35 cycles with 45 s at 93°C, 45 s at 55°C, 80 s at 72°C; 10 min at 72°C. The amplified fragments were purified using a QIAquick PCR Purification Kit (Qiagen Ltd., UK), cloned in a pGEM-T vector (Promega, USA) and sequenced using an automated ALF DNA Sequencer (Pharmacia, Sweden) and a Thermo Sequenase Kit (Amersham, UK). Probes were labeled with digoxigenin and used as RFLP probes as described above (Hämäläinen et al. 1997). Sequence analyses were carried out using the PCGENE 6.85 software (IntelliGenetics Inc., Switzerland).

Results

Resistance to PVY^O and PVY^N

In a previous study (Valkonen et al. 1994), 54 progeny of the cross $2x$(v-2)7 × 84.194.30 had been tested with an isolate of PVY^O (PVY^O-NA) obtained from North America, whereas in the present study the same progeny were tested with the isolate PVY^O-UK. The phenotypic responses of all 54 progeny were identical using the two PVY^O isolates. In our study, 23 new progeny from the same cross were also tested for resistance to PVY^O-UK, thus extending the size of the mapping population to 77 lines. The numbers of lines expressing extreme resistance (E), hypersensitivity (H) or susceptibility (S) to PVY^O (as previously defined and illustrated by Valkonen et al. 1994) were 51, 14 and 12, respectively. The 51 progeny expressing E to PVY^O also expressed E to PVY^N. In contrast, the 14 progeny expressing H to PVY^O were susceptible to PVY^N. The 12 progeny susceptible to PVY^O were also susceptible to PVY^N.

Resistance to PVA

The parental potato clone $2x$(V-2)7 developed necrotic streaks on the stem, and vein necrosis developed in the top leaves 22–24 days after graft-inoculation with PVA-U. Low, but readily detectable, titres of PVA (ELISA absorbance values $A_{405} = 0.7$–0.9) were observed in the leaves showing necrotic streaks, whereas negative absorbance values similar to the non-inoculated plants ($A_{405} = 0.01$) were observed in the symptomless leaves. A few necrotic lesions were observed in the mechanically inoculated leaves of $2x$(v-2)7, but the upper leaves remained symptomless and no PVA was found in them by ELISA. The parental potato line 84.194.30 showed mild mottle symptoms and the upper leaves contained high titres of PVA as shown by the ELISA ($A_{405} = 2.0$–2.5) 22 days after graft- or mechanical inoculation.

Fifty six progeny were resistant to PVA. None of them showed any symptoms in the inoculated and non-inoculated leaves following mechanical inoculation, and no PVA was found by ELISA. Following graft-inoculation, 30 progeny developed necrotic streaks along a few veins in the upper leaves (Fig. 1) and also necrotic streaks in the stem were sometimes observed. However, the necrotic symptoms in these progeny were usually less extensive than in $2x$(v-2)7. Low titres of PVA, similar to those in $2x$(v-2)7, were observed in the leaves showing vein necrosis, whereas no PVA was found in the symptomless leaves tested by ELISA. In contrast, no symptoms (Fig. 1) were observed and no PVA was detected by ELISA in 26 progeny. Twenty one progeny were susceptible to PVA and their phenotypic response (Fig. 1) to PVA infection was similar to that of 84.194.30.

Linkage of the loci controlling resistance to PVY and PVA

Data showed that most of the 56 progeny that expressed resistance to PVA expressed E to PVY, except for progeny nos. v2-14 and v2-47 that expressed H to

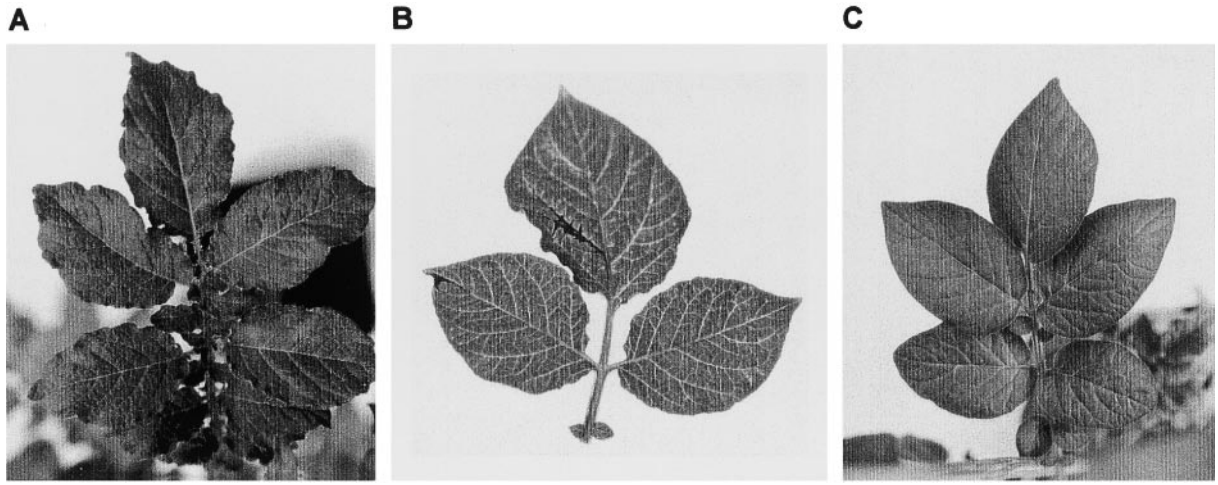

Fig. 1 Phenotypic responses to PVA infection among the progeny lines 22 days after graft-inoculation: a susceptible line showing mild mottle symptoms (**a**); necrotic or hypersensitive response expressed as necrosis along a leaf vein (**b**); and an extremely resistant line expressing no symptoms (**c**)

PVY^O and the progeny nos. v2-11, v2-23 and v2-51 that were susceptible to PVY^O. Of the 21 progeny that were susceptible to PVA, 12 expressed H to PVY^O and nine were susceptible to PVY^O. These data suggested that resistance to PVA was controlled by a locus linked to Ry_{adg} that controls E to PVY ($\chi^2 = 12.9$, $df = 1$, $P < 0.005$). To analyze this further, several RFLP markers from chromosome XI were tested for their linkage to the PVY and PVA resistance loci.

Seven RFLP markers were identified (Fig. 2) that were linked to Ry_{adg}: CP58 (*Eco*RI digest), TG651 (*Hin*dIII), TG523 (*Dra*I), CT182 (*Eco*RI), TG508 (*Eco*RI), CD17 (*Hin*dIII) and GP125 (*Eco*RV), of which the three latter mentioned markers had already previously been reported (Hämäläinen et al. 1997). With each of these RFLP markers, a single fragment of 2–6 kb unique to the extremely resistant phenotypes was observed, whereas the marker band was not observed in the progeny expressing H or S to PVY^O. No recombination was detected between Ry_{adg} and the markers CP58, CT182, CD17 and TG523 (Fig. 2). However, the marker band of TG651 was not detected in progeny no. v2-129 that expressed E to PVY (Table 2). On the other hand, among the 12 progeny susceptible to PVY^O, progeny no. v2-23 had the significant marker band when hybridized with the probes GP125 and TG508. These data indicated that TG651, and GP125 and TG508, were flanking the Ry_{adg} locus (Fig. 2).

Most markers that were linked to Ry_{adg} were also detected in the progeny expressing resistance to PVA, but none of these markers was detected in progeny susceptible to PVA. However, in progeny no. v2-129, the significant marker band was observed with all other probes linked to Ry_{adg} except TG651. In the PVA-resistant progeny no. v2-23 the significant marker band was observed only with the probes TG508 and GP125, and in four PVA-resistant progeny the significant marker band linked to Ry_{adg} was not observed with any probe (Table 2). According to the test for independent assortment, the markers that were linked to Ry_{adg} showed linkage to the PVA resistance locus ($P > 0.005$). The distance between the Ry_{adg} locus and the PVA resistance locus, putatively named Ra_{adg}, was estimated to be 6.8 cM (Fig. 2). The closest linkage with Ra_{adg} was observed with TG508 and GP125 (Fig. 2).

The suggested location of Ry_{adg} and Ra_{adg} on the potato linkage map is based on the linkage maps of

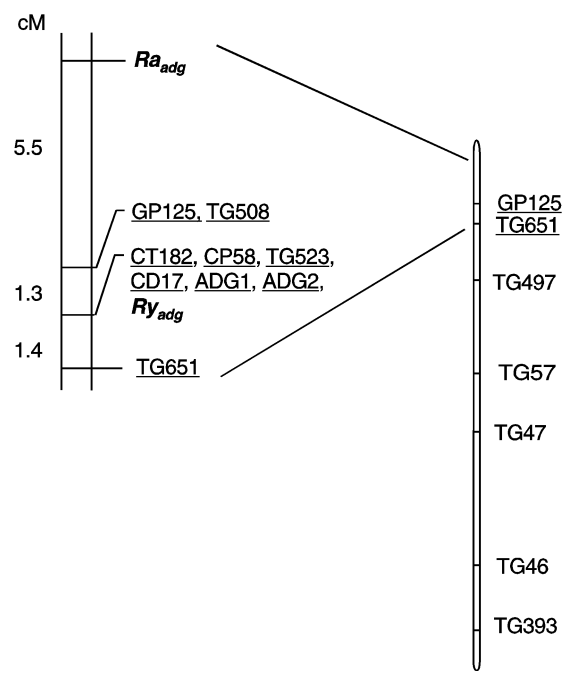

Fig. 2 A genetic map of chromosome XI illustrating the positions of Ry_{adg} and Ra_{adg} (LOD > 3.0). The markers linked to Ry_{adg} are *underlined*

Table 2 Progeny showing probable recombination between the resistance and marker loci

Progeny no.	Virus resistance[a]		Marker								
	PVA	PVY	GP125	TG508	CT182	CP58	TG523	CD17	ADG1	ADG2	TG651
11	E	S	–	–	–	–	–	–	–	–	–
14	E	H	–	–	–	–	–	–	–	–	–
23	E	S	+	+	–	–	–	–	–	–	–
47	E	H	–	–	–	–	–	–	–	–	–
51	E	S	–	–	–	–	–	–	–	–	–
129	E	E	+	+	+	+	+	+	+	+	–

[a] E, extremely resistant; H, necrotic or hypersensitive; S, susceptible

Table 3 Nucleotide-sequence homologies between the RGL fragments amplified in this study (ADG1–3), the corresponding RGL fragments produced by Leister et al. (1996) (3.3.13, 3.3.3, 3.3.1, respectively) and the corresponding region of the *N* gene of tobacco (Whitham et al. 1994)

%	ADG1	ADG2	ADG3	3.3.13[a]	3.3.3[a]	3.3.1[a]
ADG2	79					
ADG3	47	47				
3.3.13	98	79	49			
3.3.3	76	92	45	76		
3.3.1	48	48	91	49	47	
N	81	79	68	80	74	62

[a] EMBL database accession numbers: 3.3.13, U60084; 3.3.3, U60082; 3.3.1, U60079

tomato and potato (Tanksley et al. 1992). The map presented in Fig. 2 includes that the chromosome region bordered by the markers TG497 and CT182 in potato is inverted as compared to tomato (Tanksley et al. 1992).

RGL fragments

The three specific primer combinations (Leister 1995) that we used for PCR-based amplification of the DNA of 2*x*(v-2)7 each produced one fragment, respectively designated as ADG1, ADG2 and ADG3 (Table 1). The nucleotide sequences of all three PCR products were related (47–79% identity) (Table 3). They were initially tested as RFLP probes on the mapping population using BSA. With ADG1 (*Taq*I digest) and ADG2 (*Eco*RI, *Pst*I, *Bst*NI, *Hae*III, *Msp*I or *Taq*I digests), a single band specific to the E pool was observed among the many bands that were detected with each probe. In contrast, no band detected using ADG3 as a probe showed polymorphism between the DNA pools in BSA with the ten restriction enzymes used. Analysis of the parental genotypes and all progeny individuals showed that ADG1 and ADG2 co-segregated with E to PVY (Fig. 3) as well as the markers CT182, CP58, TG523 and CD17 and, thus, were closely linked with Ry_{adg} (Fig. 2).

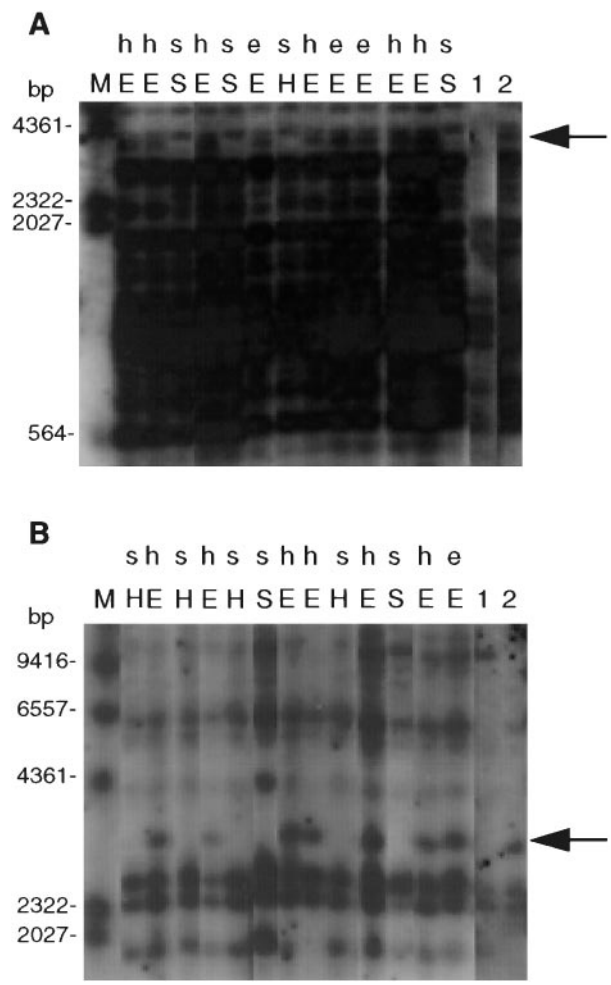

Fig. 3 Hybridization of the RGL fragments ADG1 (**a**) and ADG2 (**b**) to the DNA of individual progeny of the cross 2*x*(v-2)7 × 84.194.30 digested with *Eco*RI and *Taq*I, respectively. 2*x*(v-2)7 (*2*) is extremely resistant *(E)* to PVY and hypersensitive *(H)* to PVA, whereas 84.194.30 *(1)* is susceptible (*S*) to both viruses. The phenotypic response (*E*, *H or S*) of each progeny line to infection with PVY^{O} is indicated above the lane of each line using *capital letters*, whereas the phenotypic response to infection with PVA is indicated using *lower case letters*. The marker band linked to E to PVY and E or H to PVA is indicated by an *arrowhead*

Discussion

This study showed that the diploid potato line $2x$(v-2)7 derived from *S. tuberosum* subsp. *andigena* (Watanabe et al. 1994) is resistant to PVA, and that resistance to PVA is controlled by a chromosomal locus that is different, but not distant, from the locus of Ry_{adg} on chromosome XI (Fig. 2).

The expression of resistance to PVA was intriguing. In $2x$(v-2)7 and 30 progeny, necrotic symptoms developed and the PVA antigen (coat protein) was detected by ELISA in those leaves where necrotic symptoms were observed, although necrosis in the progeny was usually more limited than in $2x$(v-2)7. These data indicated that PVA could replicate in these plants to detectable levels despite the activated resistance response. In contrast, no PVA and symptoms were detected in 26 PVA-inoculated progeny. Both types of resistance responses to PVA infection have been previously reported in cultivated and wild potatoes (Cockerham 1970; Jones 1990; Valkonen et al. 1995; Barker 1996; Valkonen 1997). However, the 'necrotic type' is usually classified as a hypersensitive resistance response (H) controlled by *N* genes, whereas the latter type is consistent with the expression of extreme resistance (E) controlled by *R* genes (Barker 1996; Valkonen 1997). The possibility of two resistance loci (genes) being involved was tested on our data but was not supported by any genetic model. In fact, the marker data suggested that both types of resistance responses to PVA were controlled by the same locus. Also, no genetic model supported possible involvement of an independent locus derived from one of the two parents and which could suppress the expression of the PVA resistance gene in $2x$(v-2)7 and 30 (H) progeny. We feel that the existence of a "suppressor locus" still needs to be considered in further studies, because it has been shown that other genes may be required for (Tang et al. 1996), or do indeed suppress (El-Kharbotly et al. 1996), the resistance response activated by the resistance gene involved in the gene-for-gene interaction with the pathogen. Genetic (Ross 1958) and molecular data (Hämäläinen et al. 1997) have shown that, in a few potato progeny carrying an *Ry* gene, the expression of E to PVY can be converted to a necrotic response resembling H to PVY. This phenomenon has been suggested to be attributable to the expression of genes inherited from the virus-susceptible crossing parent (Ross 1958). Similarly, it is getting evident that the phenotypic expression of resistance to PVA is dependent not only on the resistance gene but also on the genetic background into which the gene is introduced. Therefore, it is often difficult to classify the PVA resistance phenotypes to one of the two types (H or E) (Valkonen et al. 1995; Barker 1996; Rajamäki et al. 1998; J. P. T. Valkonen, unpublished data). Consistent with the above discussed examples on the suppression of resistance gene expression, we have putatively designated the PVA resistance gene in $2x$(v-2)7 as Ra_{adg} as an indication of its potential to produce the E resistance phenotype in a suitable genetic background and, thus, its probable nature as an *R* gene.

Previous studies have shown that resistance to PVY^O in $2x$(v-2)7 is controlled by two dominant genes (Valkonen et al. 1994; Hämäläinen et al. 1997). The gene Ry_{adg} controls E to PVY^O and PVY^N and is located on chromosome XI, as indicated by the eight RFLP markers from chromosome XI that co-segregate with the E phenotype (this study; Hämäläinen et al. 1997). The expression of Ry_{adg} is epistatic to the expression of H to PVY^O controlled specifically by the gene Ny_{adg} also found in $2x$(v-2)7 (Valkonen et al. 1994) (Ny does not confer resistance to PVY^N; Jones 1990). Therefore, E, but no H, to PVY^O is expressed in $2x$(V-2)7 and those progeny that carry both resistance genes, whereas H to PVY^O is expressed in the progeny that contain only Ny_{adg}. The location of Ny_{adg} is not known, but is different from that of the Ry_{adg} locus because the RFLP markers linked to Ry_{adg} are not linked to Ny_{adg} (Hämäläinen et al. 1997). The present study showed that H to PVY^O expressed in 14 progeny of our mapping population was controlled by Ny_{adg} and was not the result of an altered expression of Ry_{adg} (see above) because all the progeny expressing H to PVY^O were susceptible to PVY^N and, as mentioned above, no RFLP or RGL marker linked to Ry_{adg} was detected in the progeny expressing H to PVY^O.

The numbers of virus-susceptible progeny were much less than expected from the model of one locus being responsible for each of the three resistances detected in $2x$(v-2)7, namely resistance to PVA, E to PVY, and H to PVY^O. According to our experience, "distorted proportions" of resistant and susceptible progeny are not uncommon in the crosses made for breeding and genetic mapping on potato. For example, distorted segregation ratios were found for the potato X potexvirus resistance locus *Rx2* on chromosome V (Ritter et al. 1991) and for chromosome XI in one parental line used for crosses by Leister et al. (1996). Reasons for this remain to be resolved in the future.

In addition to Ry_{adg}, a few other resistance genes have also been mapped to the same region of chromosome XI in potato. They include another PVY resistance gene, Ry_{sto}, that was mapped using a tetraploid potato population having the wild potato species *S. stoloniferum* in its genetic background (Brigneti et al. 1997) and the root-knot nematode resistance gene R_{Mc1} in the wild potato species *S. bulbocastanum* (Brown et al. 1996). The previous study of Leister et al. (1996) and the present study have revealed a total of five sequence-related RGL fragments that were also mapped to the above mentioned region of chromosome XI in potato. Furthermore, as only one of the many bands detected on Southern blots using the RGLs as probes could be assigned to chromosome XI, the other

detected bands could be homozygous copies from the same region of chromosome XI, or from other unlinked loci. These data suggest that this region of the potato chromosome contains a cluster of resistance genes, of which homologues are also present in other species of the family Solanaceae. For example, the gene *N* of tobacco is located in a region homologous to this region of potato chromosome XI (Leister et al. 1996).

The RGL fragments amplified by PCR from 2*x*(v-2)7 were quite similar (91–98% identity) to those amplified by Leister et al. (1996) using another potato genotype, and were also quite homologous to the corresponding region of the *N* gene of tobacco (Table 2) that contains two nucleotide-binding sites (NBS) (Whitham et al. 1994). The RFLP analysis showed that the RGL fragments ADG1 and ADG2 produced in this study were linked to Ry_{adg}, without recombination, in our mapping population of 77 progeny (Fig. 2). This infers a physically short distance between the RGL fragments and Ry_{adg}, and the possibility remains that they may actually be parts of Ry_{adg} or other genes in the same gene cluster. However, it needs to be noted that suppressed recombination may occur in some chromosomal regions in potato (Bonierbale et al. 1988), which would mean that the physical distance is greater than expected. On average, one cM equals to approximately 1 Mb in the potato genome (Ballvora et al. 1995). Nevertheless, the RGLs ADG1 and ADG2 serve as highly linked markers that can be used for selection of resistance to PVY (and PVA) in potato breeding programs.

Acknowledgements We are grateful to Dr. S. D. Tanksley for generously providing RFLP markers, and to Anu Miettinen and Ulla Malkamäki for technical assistance. Financial support from the University of Helsinki (grant #932/62/94) and the Academy of Finland (grant #36256) to J.P.T.V. and financial support to K.N.W. (grant #JSPS-RFTF96L00603) are gratefully acknowledged.

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