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Note

Conservation of a Resistance Gene-like Fragment ADG2 Related to Potato Y Potyvirus Resistance Gene Ry_{adg} in Diploid and Tetrasomic Tetraploid Potato Lines

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Potato Y potyvirus is globally one of the most important viral pathogens of the cultivated potato (*Solanum tuberosum* L.) and can cause yield loss of up to 80% (Hooker 1981). Incorporation of resistance genes into potato cultivars from disease-resistant varieties is the most efficient way of controlling this virus (Gebhardt and Valkonen 2001, Ross 1986, Watanabe *et al.* 1995). Two types of monogenically inherited resistance genes have been found in cultivated and wild potato species (*Solanum* spp.) (Ross 1986, Valkonen 1994). *Ny* genes are PVY strain-specific and express hypersensitive resistance (H) in infected plants, which produce necrotic lesions or systemic necrosis where infected. The other is *Ry* genes conferring extreme resistance (E) to all strains of PVY. In inoculated plants expressing *Ry*, no symptom develops except for limited systemic necrosis occurring after graft-inoculation in some genotypes, and appearance of an ELISA-detectable PVY titer (Ross 1986, Valkonen *et al.* 1996). Two *Ry* genes had been utilized in potato PVY resistance breeding programs, i.e., Ry_{sto} from *Solanum stoloniferum* Schlechtd. et Bché (Ross 1986) and Ry_{adg} from *Solanum tuberosum* subsp. *andigena* Hawkes (Kasai *et al.* 2000, Muñoz *et al.* 1975, Watanabe *et al.* 1996a, 1996b).

Disease resistance is a defense response of the plant to its pathogens controlled by resistance (R) genes (Bergelson *et al.* 2001, Dangl and Jones 2001). The structure similarity and motif conserved observed among various plant *R* genes provide attractive targets for PCR-amplification and isolation of similar sequences in other plant species. Some resistance gene-like fragments (RGL) have been isolated and characterized by this method (Aarts *et al.* 1998, Kanazin *et al.* 1996, Leister *et al.* 1996, Meyers *et al.* 1999, Shen *et al.* 1998, Speulman *et al.* 1998, Yu *et al.* 1996). A 355 bp RGL fragment (ADG2) was amplified from potato with primers having sequence conservation between gene *N* from *Nicotiana glutinosa* (Whitham *et al.* 1994) and gene *RPS2* from *Arabidopsis thaliana* (Bent *et al.* 1994, Mindrinos *et al.* 1994) in a domain spanning the predicated kinase-2 and kinase-3a motifs downstream of the P-loop. It was mapped on chromosome XI and was found to be co-segregated with Ry_{adg} in a F_1 population with 77 individuals (Hämäläinen *et al.* 1998). Sequence comparison revealed a 12 nucleotide-difference between the ADG2 fragments derived from a PVY resistant line 2x(v-2)7 (Ry_{adg} carrying) and a susceptible line 84.194.30. One of the differences located within the kinase-3a motif has been developed as the CAPS (cleaved amplified polymorphic sequences) marker which could distinguish the potato lines carrying Ry_{adg} from those lacking this gene (Sorri *et al.* 1999). Because ADG2 was originally amplified from mRNA pools and showed 77% homology with the corresponding region of the gene *N* from *Nicotiana glutinous* (Whither *et al.* 1994) and 53% homology with gene *RPP5* from *Arabidopsis thaliana*, both are transcriptional R genes (Leister *et al.* 1996), Sorri *et al.* (1999) argued that ADG2 was probably part of the Ry_{adg} conferring extreme resistance to PVY.

Further sequence analyses of ADG2 fragments amplified from diverse potato lines may be of value in understanding the molecular basis of Ry_{adg} function, developing new markers such as SCAR (sequence characterized amplified region) (Kasai *et al.* 2000) or providing pedigree information for parental line selection in breeding programs. Here we report the sequences of ADG2 fragments from 24 potato lines.

Sequencing with various potato genotypes

Totally 24 potato lines with a diverse genetic background were amplified by PCR and the sequences of ADG2 fragments analyzed. As a response to potato Y potyvirus, eleven of them showed extreme resistance, three were hypersensitive and the remaining ten were susceptible. The extreme resistance was controlled by an Ry_{adg} gene in eight lines, while the others were derived from Ry_{sto} or Ry_{chc}. The potato lines and their ploidy, resistance phenotype, resistance donor species and related references are listed in Table 1.

Total DNA was extracted from pathogen-free *in vitro*

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grown plants by the CTAB method (Doyle and Doyle 1987). ADG2 was amplified from total DNA with ADG2-specific primer pair 3.3.3s (5′-ATACACTCATCTAAATTTGATGG-3′) and 3.3.3as (5′-ACTTAACTGCATCATGTTCAAG-3′) (Hämäläinen *et al.* 1998) using a Thermal Cycler (MJ Research, USA). The reaction mixture (50 µl) contained 50 ng template DNA, 1x GeneAmp PCR buffer (Perkin Elmer, USA), 0.1 mM each of dNTPs, 0.25 µM primers and 1 unit *Taq* DNA polymerase (AmpliTaq Gold, Perkin Elmer, USA). The expected fragments were amplified by PCR as follows: preheated at 94°C for 10 min, followed by 35 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 80 sec. a final extension was performed at 72°C for 10 min.

PCR products were cloned into a pGEM-T vector (Promega, USA), and these clones were mapped prior or after the sequencing to confirm the fragments localized at the same chromosomal region in Chromosome XI by using the existing tomato map with RFLP markers (Tanksley *et al.* 1992). The confirmed PCR products were sequenced using an automated DNA sequencer (ABI Prism, Perkin Elmer, USA) and a Cycle Sequencing Kit (ABI Prism, Perkin Elmer, USA). The resulting sequences were aligned, converted to amino acid sequences using the GENETYX-MAC 10.1 software (Software development Co., Ltd., Japan). At least 5 clones containing ADG2 from each potato line were selected for sequencing.

ADG2 fragments have small but specific variation

The sequence comparison of ADG2 fragments from a PVY-resistant line 2x(V-2)7 and a susceptible line 84.194.30 revealed a single nucleotide substitution C to G that could be developed as a CAPS marker to distinguish Ry_{adg}-carrying potato lines from the non-Ry_{adg}-carrying lines in 50 selected potato cultivars and breeding lines. This substitution was located in the predicted kinase-3a motif and resulted in an amino acid substitution, Thr to Ser, in predicated gene products (Sorri *et al.* 1999). Here we tentatively refer the ADG2 fragment having a C nucleotide (Thr residue in amino acid sequence) within the kinase-3a motif to R allele, while the S alleles have a substituted G nucleotide or Ser residue within the corresponding region.

ADG2 fragments were amplified from 24 potato lines: six diploids and eighteen tetraploids, respectively, with a diverse genetic background, as well as different response to

Table 1. Plant materials

Potato line	Ploidy	Resistance phenotype[1)]	Resistance donor species[2)]	Referrences
2x(V-2)7	2x	E	*adg*	Valkonen *et al.* 1994a, Watanabe *et al.* 1994a
(V-2)62[a)]	2x	E	*adg*	Sorri *et al.* 1999
7XY.1	4x	E	*adg*	Iwanaga *et al.* 1991, Watanabe *et al.* 1994b
TA3.5.3.6[b)]	4x	E	*adg*	Watanabe *et al.* 1992
NY103	4x	E	*adg*	Kasai *et al.* 2000
NY121	4x	E	*adg*	Kasai *et al.* 2000
NY123	4x	E	*adg*	Kasai *et al.* 2000
84.194.30	2x	S		Valkonen *et al.* 1994a, Watanabe *et al.* 1994a
AA3	4x	E	*adg*	Iwanaga *et al.* 1991, Watanabe *et al.* 1994b
DG81-68	2x	S		Swiezynski *et al.* 1989
NY99	4x	S		Hamalainen *et al.* 1997
NY109	4x	S		Kasai *et al.* 2000
NY115	4x	S		Kasai *et al.* 2000
acl 7-8	4x	S		S. Slack, personal communication
HHI9.1CD	4x	S		E. Fernandez-Northcote, personal communication
I12.1	4x	S		E. Fernandez-Northcote, personal communication
Papa Amarilla	2x	S		CIP[3)] 1998
Russet Burbank	4x	S		CIP[3)] 1998
Pentland Crown	4x	H		Jones 1990, Valkonen *et al.* 1994b
Pentland Ivory	4x	H	*tbr*	Jones 1990, Stegemann and Schnick 1982
Yukon Gold	4x	H		Valkonen 1997
86.61.26	2x	E	*sto*	Valkonen *et al.* 1994a, Watanabe *et al.* 1994a
Konafubuki	4x	E	*chc*	HKAES[4)] 1998
Sakurafubuki	4x	E	*chc*	HKAES[4)] 1998

[1)] E: extreme resistance, S: susceptible, H: hypersensitive resistance

[2)] *adg*: *Solanum tuberosum* subsp. *andigena*, *chc*: *S. chacoense*, *sto*: *S. stoloniferum*, *tbr*: *S. tuberosum*

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[a)] (V-2)62 is F_1 hybrid derived from the cross of 2x(V-2)7x84.194.30

[b)] TA3.5.3.6 was produced using the potato line AA3 (7XY.1x *S. acaule* 954.3CA) as the female in cross with an interspecific hybrid DG 81.68. E to PVY in TA line is conferred by Ryadg derived from 7XY.1

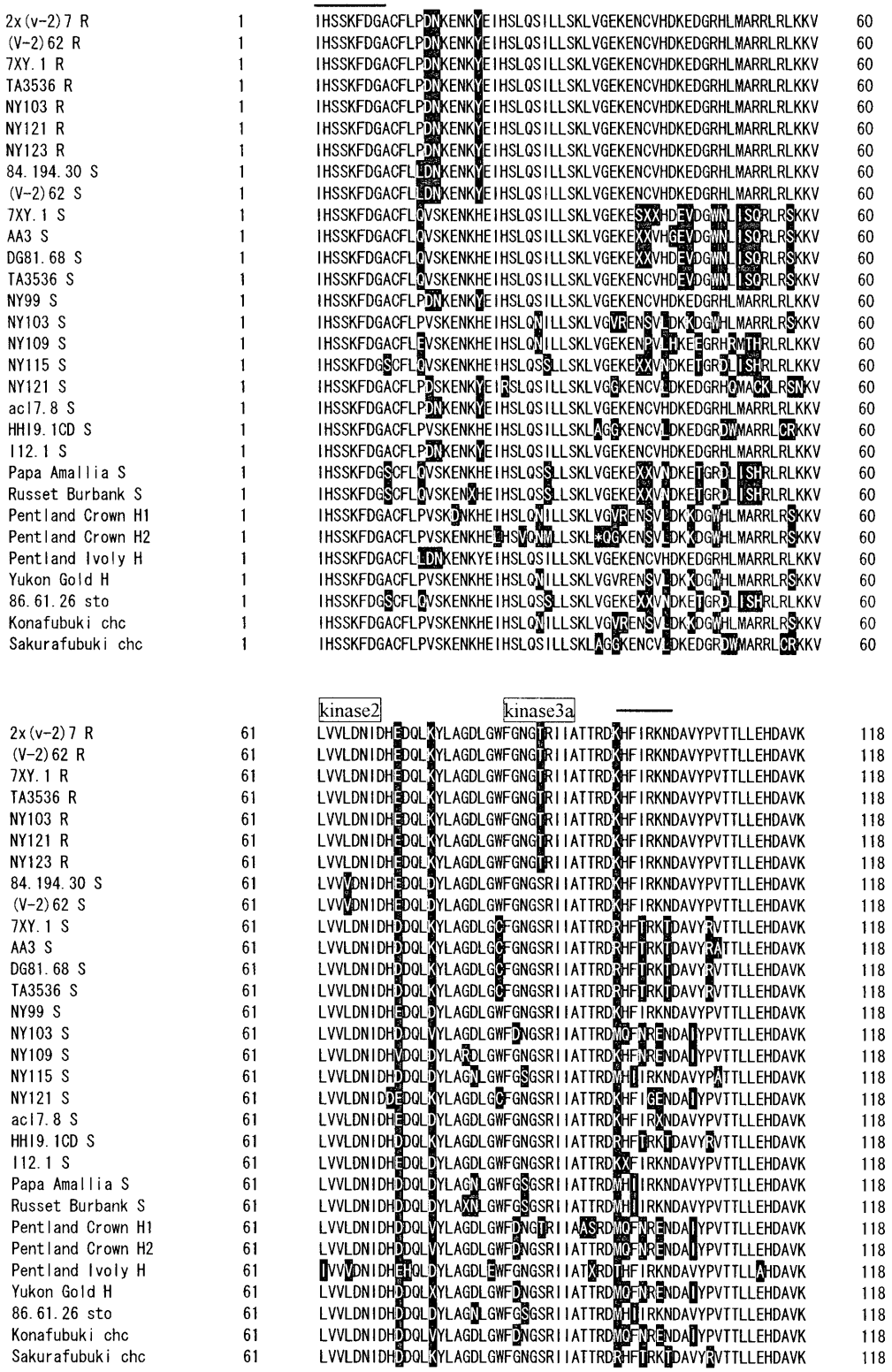

Fig. 1. Alignment of the deduced amino acid sequence of ADG2 fragments amplified from potato. The letter R and S following the potato lines represent the tentative R and S alleles of Ry_{adg}, respectively. H indicates the hypersensitive response to PVY. Two alleles were amplified from Pentland Crown: H1 has Thr in kinase-3a motif while H2 has a Ser. *sto* and *chc* refer to the E resistance conferred by Ry_{sto} and Ry_{chc}, respectively. The highlighted white letters indicate the difference among the potato alleles. The kinase-2 and kinase-3a motifs are indicated, respectively. The over-lined sequences are primer binding regions for SCAR marker (Kasai *et al.* 2000).

PVY, i.e., extreme resistance, hypersensitive or susceptible. Over ten and more than twenty cloned ADG2 fragments for diploid and tetraploids, respectively, from each potato line, were sequenced, and the outcome of the deduced ADG2 amino acid sequence with R or S allele from all the 24 potato lines were aligned and shown in Figure 1. As for Ry_{adg} genotypes, the occurrence of R type allele and S type allele were fitted with 1 : 1 ratio, and in tetraploids with Ry_{adg}, the R to S ratio corresponded to 1 : 3 by the chi-square test for goodness of fit at the 5% level. Thus, these diploids and tetraploids with Ry_{adg} were deduced as heterozygous and simplex for Ry_{adg}, respectively. Indeed, this inference was well correlated with the pedigree and progeny testing conducted previously (Watanabe *et al.* 1992, 1994a, 1994b, R. L. Plaisted, personal communication on NY clones in Table 1).

As summarized in Figure 1, at least one R allele was found to be amplified from each Ry_{adg}-carrying line, whereas all the ADG2 in non-Ry_{adg}-carrying lines were amplified from the S allele with nucleotide C substituted by G. An exception was Pentland Crown, which had an allele with nucleotide G in the kinase-3a motif but many variations occurred in other regions. The sequence comparison also showed that the R alleles were well conserved among Ry_{adg} carrying lines that derived from different genotypes of *S. tuberosum* ssp. *andigena* (Watanabe *et al.* 1992, 1994a, 1994b, R. L. Plaisted, personal communication on NY clones in Table 1).

Application of the sequence information to marker-assisted selection (MAS) of potato

The sequence comparison between the AGD2 fragments from 2x(V-2)7 and 84.194.30 led to the identification of CAPS (Sorri *et al.* 1999) and SCAR (Kasai *et al.* 2000) markers which could distinguish the potato lines carrying Ry_{adg} from those lacking this gene and thus should be applicable in marker-assistant breeding. A wide comparison of ADG2 fragments from diverse potato genotypes with a different background in this study provided more information about this fragment.

ADG2 amplified from all Ry_{adg}-carrying potatoes shared a common allele (R allele to PVY) with unique sequence pattern. As compared to the R allele, differences at various locations within ADG2 were found among the S alleles amplified from non-Ry_{adg}-carrying or Ry_{adg}-heterozygous lines. The S allele from 84.194.30 had the highest similarity to R allele. It differed from R alleles in 12 nucleotides (Sorri *et al.* 1999), the deduced amino acid sequence only showed four residue differences (Fig. 1). One of them occurred in kinase-2 motif and the other in kinase-3a motif. The difference between R and S in kinase-3a was the molecular base of CAPS marker developed by Sorri and her colleagues. Although most of the S alleles confer Ser in the kinase-3a motif, one allele from Pentland Crown conferring hypersensitive to PVY had Thr as the R allele (Fig. 1). This allele (Pentland Crown, H1 in Fig. 1), however, had many other variations as compared to R allele and thus might be the reason for the lack of the extreme resistance to PVY as conferred by Ry_{adg}. This observation suggests that conservation of the entire ADG2 region (not only kinase-3a) is essential for a functional Ry_{adg} gene.

Kasai *et al.* (2000) used the nucleotide difference of ADG2 between 2x(v-2)7 and 84.194.30 in the position of 297-321 bp to design primers for SCARs. The S alleles from other potato lines showed much more variation in this region (Fig. 1). Considering the exception of Pentland Crown in kinase-3a which may complicate the CAPS analysis, the SCARs should be reliable as genetic markers in marker-assistant breeding and indeed, the sequence analysis of representative potato genotypes in this report support the high association of R and S phenotypes with SCAR markers.

ADG2 fragments were amplified using primers homologous to the downstream region of P-loops (phosphate-binding loop or kinase-1a) present in gene *N* from *Nicotiana glutinous* (Whitham *et al.* 1994) and gene *RPS2* from *Arabidopsis thaliana* (Bent *et al.* 1994, Mindrinos *et al.* 1994). This is a nucleotide binding site (NBS) conserved in many resistance genes (Leister *et al.* 1998, Meyers *et al.* 1999, Wang *et al.* 2002). NBS is extensively defined as the domain encompassing the kinase-1a (P-loop) and is followed by kinase-2 and kinase-3a motifs in R proteins which are distinct from those found in protein quinces (Taylor *et al.* 1993, Hammond-Kosack and Jones 1997, Wang *et al.* 2002). The NBS domain may activate the plant's defense by altering the interaction between R gene products and other members of the defense signal transduction cascade (Dangl and Jones 2001). Site-specific mutations that alter key residues within the proposed NBS have been found to eliminate the function of *R* genes (reviewed by Bent 1996). The present sequence data also suggested that residue substitution in kinase-2 and kinase-3, as well as a certain amount of variation occurring in NBS may cause the loss of resistance conferred by Ry_{adg} in potato lines.

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