Original Paper Plant Biotechnology, **16** (5), 361-369 (1999)

Applicability of the Resistance Gene-Like Fragment ADG2 as an RFLP Probe in Selection of Extreme Resistance to Potato Y Potyvirus (PVY)

Akio SHIRANITA*, Kazue KASAI*, Jaana H. HÄMÄLÄINEN**, Jari P. T. VALKONEN**, and Kazuo N. WATANABE*[1)]

**Department of Biotechnological Science, Kinki University, Uchita, Wakayama, 649-6493, Japan*

***Genetic Centre, Swedish University of Agricultural Sciences (SLU), P.O. Box 7025, S-75007 Uppsala, Sweden*

[1)]*Corresponding author* E-mail: watanabe@bio.waka.kindai.ac.jp

Received 9 February 1999; accepted 24 August 1999

Abstract

Resistance gene-like fragment ADG2 has been shown to co-segregate with Ry_{adg}, which controls extreme resistance to potato Y potyvirus (PVY) in *Solanum tuberosum* subsp. *andigena* (Hämäläinen *et al.* 1997, 1998). Applicability tests of ADG2 as a selection tool for resistance to PVY were carried out by genomic Southern analysis on a broad range of cultivars and breeding lines of potato and some other solanaceous species. A new marker band (10.5 kb) associating with *Ry* was found in addition to previously reported 3.5 kb marker band. While the 3.5 kb marker was specifically linked to Ry_{adg} with extremely high association (96 %), the newly identified 10.5 kb marker band was detected in genotypes containing Ry_{adg} and other *Ry* genes. Some signals with ADG2 were also observed in other species within family *Solanaceae* inferring with the diversity of the corresponding chromosome region(s) among solanaceous species.

1. Introduction

PVY is a member of the genus *Potyvirus* which contains ca. 180 of the total of 200 virus species belonging to the family *Potyviridae* that, in turn, contains ca. 25 % of all known plant viruses (Shukla *et al.* 1994). PVY is transmitted by aphids in the field in a non-persistent manner and naturally infects potato, pepper, tomato, and other solanaceous plant species (de Bokx and Huttinga 1981). PVY is characterized by occurrence under an extremely wide range of environmental conditions.

Globally, potato is the fourth most important food crop after wheat, corn and rice (Hawkes 1990). In Northern Europe, Russia, North and South America and Japan, PVY is a particularly important viral pathogen of cultivated potatoes and causes serious problems for potato production as it can reduce yields up to 80 % (Hooker *et al.* 1981).

Potato viruses such as PVY are transmitted to succeeding potato generations through tubers (Valkonen *et al.* 1996). Therefore, the important methods for control of PVY in potato crops include the use of virus-free seed potatoes and pesticides that kill virus vectors (de Bokx and van der Want 1987). However, there are some problems as pesticides have harmful effects on environment. Despite these control efforts, susceptible cultivars become infected with PVY in the field, and the cost of renewing virus-free seed potatoes can not be avoided.

Potato cultivars with the resistance to viruses are the best option for virus control. However, development of resistant cultivars has been difficult because the tetraploid potatoes (2n = 4x = 48) show a complicated inheritance of traits and breeding has consequently required considerable labor and time. Marker-assisted selection (MAS) for resistance can alleviate these problems and improve the cost-effectiveness by speeding up the introgression of resistant genes (Watanabe *et al.* 1994a, 1995a).

Two major types of monogenically inherited resistance to potato Y potyvirus (PVY) are known in cultivated and wild potato species (*Solanum* spp.), namely extreme resistance (E) and hypersensitive resistance (H). E is controlled by *Ry* genes and are effective against all strains of PVY, whereas H controlled by *Ny* genes is often PVY strain group-specific. Following infection with PVY, potato

plants expressing *Ny* develop necrotic lesions in infected leaves and/or necrosis in systemically infected parts, whereas the plants expressing *Ry* remain symptomless, except that limited necrosis may develop in the systemically infected leaves in a few genotypes following graft-inoculation, and no PVY titers detectable with ELISA develop in inoculated plants expressing *Ry* (Cockerham 1970, Ross 1986, Jones 1990, Valkonen *et al.* 1996). The expression of *Ry* is epistatic to the expression of *Ny* (Valkonen *et al.* 1994a).

The gene Ry_{adg} controls E to PVY in the cultivated potato *S. tuberosum* subsp. *andigena* and is located on chromosome XI (Hämäläinen *et al.* 1997, 1998). Another *Ry* gene is also located at the close position on chromosome XI (Brigneti *et al.* 1997) as are many additional disease resistance genes in other potato species and in *Nicotiana* species (reviewed by Hämäläinen *et al.* 1998).

Up to now some RFLP markers that co-segregate with Ry_{adg} have been identified (Hämäläinen *et al.* 1997, 1998). Also PCR-based markers such as CAPS (Cleaved Amplified Polymorphic Sequences) and SCAR (Sequence Characterized Amplified Regions) are available (Sorri *et al.* 1999, Kasai *et al.* 1999). However, these markers are considered Ry_{adg}-specific.

A DNA fragment designated as ADG2 has been previously amplified from *S. tuberosum* subsp. *andigena* with primers that are designed according to highly similar regions at isolated disease resistance genes (Leister *et al.* 1996, Hämäläinen *et al.* 1998, Sorri *et al.* 1999, Kasai *et al.* 1999). This fragment co-segregates with Ry_{adg} among the 77 progeny without recombination and, thus, identifies the locus on chromosome XI that is known to contain resistance clusters in many species of *Solanaceae* (Leister *et al.* 1996, Hämäläinen *et al.* 1998).

Although DNA fragment ADG2 can be amplified from PVY susceptible potato genotypes, twelve nucleotide change was found between ADG2 fragments of PVY resistant $2x(V-2)_7$ and PVY susceptible 84.194.30. ADG2 fragment from $2x(V-2)_7$, which contain Ry_{adg}, shows 77 % nucleotide sequence homology with the corresponding region of the gene *N* for resistance to tobacco mosaic virus in *Nicotiana glutinosa*, and 53 % homology with *RPP5* for resistance to *Peronospora parasitica* in *Arabidopsis thaliana* (Hämäläinen *et al.* 1998, Sorri *et al.* 1999). Moreover, two out of twelve nucleotide differences were located in predicted kinase-2 and kinase-3a motif causing deduced amino acid changes (Sorri *et al.* 1999). Taking these findings into consideration, it has been suggested that ADG2 fragment may be parts of Ry_{adg}. Therefore, it is meaningful to search for homologues of the ADG2 fragment in other solanuceous species to associate with the potential existence of a resistance gene (Watterson *et al.* 1993).

In this study, applicability of MAS for resistance to PVY using ADG2 as an RFLP probe was tested on a genetically diverse range of the potato cultivars and breeding lines, and also other solanaceous crop species that are affected by PVY.

2. Materials and Methods

2.1 Plant materials

A total of 117 diploid and tetraploid potato breeding lines; and diploid, tetraploid, and pentaploid potato cultivars with various genetic backgrounds (**Table 1**), were tested with ADG2 as an RFLP probe using restriction with *Eco*RI. The ADG2 was further tested on a total of 12 cultivars/genotypes of tomato (*Lycopersicon*), pepper (*Capsicum*), and eggplant (*Solanum*) (**Table 4**).

Potato breeding lines and cultivars were maintained as *in vitro* cultures on Murashige and Skoog medium (Murashige and Skoog 1962) at pH 5.8. Seeds of the cultivars/genotypes of other solanaceous crops were planted to and grown in soil (Perlite : Vermiculite : Peatmoss = 1 : 1 : 2).

2.2 DNA preparation

Total DNA for RFLP analysis was extracted with hexadecyl trimethylammonium bromide (CTAB) method with some modification as described by Landry (1993). Total DNAs were digested with restriction enzyme *Eco*RI (Boehringer Mannheim, Germany) according to the manufacturer's instruction, and 2 μg of the digested DNA was loaded and separated on 0.8 % agarose gel using electrophoresis. Southern blotting was carried out as described by Sambrook *et al.* (1989).

2.3 PCR amplification of ADG2 fragment

The ADG2 was amplified by PCR from the total DNA of diploid potato breeding line $2x(V-2)_7$ carrying Ry_{adg} as previously described (Hämäläinen *et al.* 1998). The amplified fragment (355 bp) was cloned in pGEM-T® Easy Vector (Promega co., USA). The clone was digested with *Eco*RI and electrophoresed in 2 % agarose gel to separate the inserted DNA and the vector. The inserted DNA was extracted from agarose gel using a QIAquick™ Gel Extraction Kit (QIAGEN GmbH, Germany) to use for the RFLP probe.

Table 1. Examination of potato cultivars and breeding lines and other solanaceous species tested with ADG2 as an RFLP marker

Potato Clone	Ploidy	Resistance donor species[1]	Resistance phenotype[2]	Presence of marker band[3]		References
				10.5kb	3.5kb	
$2x(V-2)_7$	2x	*adg*	E	+	+	Valkonen *et al.* 1994a, Watanabe *et al.* 1994a
$\{2x(V-2)_7 \times 84.194.30\}_{\epsilon 2}$	2x	*adg*	E	+	+	Sorri *et al.* 1999
84.194.30	2x		S	−	−	Valkonen *et al.* 1994a, Watanabe *et al.* 1994a
7XY.1	4x	*adg*	E	+	+	Iwanaga *et al.* 1991, Watanabe *et al.* 1994b
84.35.7	2x		S	−	−	Watanabe *et al.* 1994a
84.36.29	2x		S	−	−	Watanabe *et al.* 1994a
85.37.38	2x		S	−	−	Watanabe *et al.* 1994a
86.54.18	4x	*sto*	E*	+	−	CIP[4] 1998
86.61.26	2x	*sto*	E*	+	−	Valkonen *et al.* 1994a, Watanabe *et al.* 1994a
87HW13.7	2x		S	−	−	Valkonen *et al.* 1995
90.30.47	2x		S	−	−	E. Fernandez-Northcote, personal communication
90.31.42	2x		S	−	−	E. Fernandez-Northcote, personal communication
954.3CA	4x		S	−	−	Watanabe *et al.* 1994b
AA-3	4x	*adg*	E	+	+	Iwanaga *et al.* 1991, Watanabe *et al.* 1994b
acl7-8	4x		S	−	−	S. Slack, personal communication
BW5.116	4x		S	−	−	E. Fernandez-Northcote, personal communication
CPC2451	2x	*brd*	E*	−	−	Valkonen *et al.* 1995, Watanabe *et al.* 1995b
DG81-68	2x		S	−	−	Swiezynski *et al.* 1989
E74-7	4x	*adg*	E	+	+	Hämäläinen *et al.* 1997
F_1-1	2x	*chc*	E*	+	−	Hosaka and Hanneman 1994
HHI-9.3CD	4x		S	−	−	E. Fernandez-Northcote, personal communication
I12.1	4x		S	+	−	E. Fernandez-Northcote, personal communication
IvP35	2x	*phu*	E*	+	−	Valkonen *et al.* 1995
N140-201	4x	*adg*	E	+	+	Hämäläinen *et al.* 1997
Q237-8	4x	*adg*	E	+	+	Hämäläinen *et al.* 1997
S48-6	4x		H	−	−	R. L. Plaisted, unpublished
TA3.5.3.6	4x	*adg*	E	+	+	Watanabe *et al.* 1992
TA3.5.3.7	4x	*adg*	E	+	+	Watanabe *et al.* 1992
TA3.8.3.3	4x	*adg*	E	+	+	Watanabe *et al.* 1992
TET38.2	2x		H	−	−	Valkonen *et al.* 1995, Watanabe *et al.* 1995b
TET38.9	2x		E*	−	+	Valkonen *et al.* 1995, Watanabe *et al.* 1995b
TET38.12	2x		E*	−	−	Valkonen *et al.* 1995, Watanabe *et al.* 1995b
TET38.13	2x		H	−	−	Valkonen *et al.* 1995, Watanabe *et al.* 1995b
A6	5x		S	+	−	Russo and Slack 1998
All Blue	4x		S	+	−	CIP 1998
Alpha	4x		S	−	−	CIP 1998
Andover	4x		S	−	−	Plaisted *et al.* 1998
Arran Banner	4x		S	+	−	A. Arihara, personal communication
Astarte	4x		S	−	−	HKAES[5] 1998
Atzimba	4x		S or H	−	−	CIP 1998
Atlantic	4x		S	−	−	Valkonen *et al.* 1994b, Russo and Slack 1998
Benimaru	4x		S	−	−	HKAES 1998
Bintje	4x		S	−	−	Valkonen and Palohuhta 1996, Stegemann and Schnick 1982
Charles Downing	4x		S	−	−	A. Arihara, personal communication
Chidiwa	4x		S	−	−	HKAES 1998
Chieftain	4x		S	−	−	CIP 1998
Cutetia	4x		S	−	−	A. Arihara, personal communication
Dannsyaku	4x		S	+	+	HKAES 1998
Dejima	4x		S	−	−	HKAES 1998
Deodara	4x		S	−	−	HKAES 1998
Desiree	4x	*tbr*	H	−	−	Jones 1990, Stegemann and Schnick 1982
Early Gem	4x		S	−	−	A. Arihara, personal communication
Eniwa	4x		S	−	−	HKAES 1998
Erd manner	4x		S	−	−	A. Arihara, personal communication
Ezoakari	4x		S	−	−	HKAES 1998

Table 1. continued

Potato Clone	Ploidy	Resistance donor species[1]	Resistance phenotype[2]	Presence of marker band[3]		References
				10.5kb	3.5kb	
Fabricia	4x		S	–	–	HKAES 1998
Fina	4x		S	–	–	HKAES 1998
Firmula	4x		S	–	–	A. Arihara, personal communication
Furore	4x		S	–	–	A. Arihara, personal communication
Gabriela	4x		S	–	–	A. Arihara, personal communication
Gineke	4x		S	–	–	CIP 1998
Greta	4x		S	–	–	CIP 1998
Hatsufubuki	4x		S	–	–	HKAES 1998
Hokkaishiro	4x		S	–	–	HKAES 1998
Inka-no-Hoshi	4x		S	–	–	HKAES 1998
Jakko	4x		S	+	–	A. Arihara, personal communication
King Edward	4x		S	–	–	Jones 1990, Stegemann and Schnick 1982
Kitaakari	4x		S	–	–	HKAES 1998
Konafubuki	4x	*chc*	E*	+	–	HKAES 1998
La Soda	4x		S	–	–	A. Arihara, personal communication
M. Hindenburg	4x		S	+	–	CIP 1998
Matilda	4x		S	–	–	Valkonen and Palohuhta 1996
May Queen	4x		S	–	–	HKAES 1998
Mentor	4x		S	+	–	CIP 1998
Musamaru	4x		S	–	–	HKAES 1998
Myojo	4x		S	+	–	HKAES 1998
Norchip	4x		S	–	–	CIP 1998
NY99	4x		S	+	+	Hämäläinen *et al.* 1997
NY103	4x	*adg*	E	–	+	CIP 1998
NY109	4x		S	+	–	CIP 1998
NY115	4x		S	+	–	CIP 1998
NY121	4x	*adg*	E	+	+	CIP 1998
NY123	4x	*adg*	E	–	+	CIP 1998
Ohjiro	4x		S	–	–	HKAES 1998
P. Wohitman	4x		S	–	–	A. Arihara, personal communication
Papa Amarilla	2x		S	–	–	CIP 1998
Panther	4x		S	–	–	A. Arihara, personal communication
Parnassia	4x		S	–	–	A. Arihara, personal communication
Pentland Ace	4x		S	–	–	CIP 1998
Pentland Crown	4x		H	–	–	Jones 1990, Valkonen *et al.* 1994b
Pentland Dell	4x		H	+	–	Stegemann and Schnick 1982, Valkonen *et al.* 1994b, 1998
Pentland Ivory	4x	*tbr*	H	–	–	Jones 1990, Stegemann and Schnick 1982
Pito	4x	*tbr*	H	–	–	Valkonen 1997, Stegemann and Schnick 1982
Pukara	4x		S	+	–	A. Arihara, personal communication
Pungo	4x		S	–	–	A. Arihara, personal communication
Purple Peruvian	4x		S	+	–	CIP 1998
Puskin	4x		S	–	–	A. Arihara, personal communication
Rishiri	4x		S	–	+	HKAES 1998
Red Andes	4x		S	–	–	A. Arihara, personal communication
Russet Burbank	4x		S	–	–	CIP 1998
Russet Rural	4x		S	–	–	CIP 1998
Sakurafubuki	4x	*chc*	E*	+	+	HKAES 1998
Sequoia	4x		S	–	–	A. Arihara, personal communication
Serrana	4x		H	–	–	CIP 1998
Shimabara	4x		S	–	–	HKAES 1998
Shiretoko	4x		S	–	–	HKAES 1998
Snowden	4x		S	–	–	CIP 1998
Stirling	4x		S	–	–	CIP 1998
Touya	4x		S	–	–	HKAES 1998
Toyoakari	4x		S	–	–	HKAES 1998

Table 1. continued

Potato Clone	Ploidy	Resistance donor species[1]	Resistance phenotype[2]	Presence of marler band[3] 10.5kb	3.5kb	References
Toyoshiro	4x		S	−	−	HKAES 1998
Tsunika	4x		S	−	−	HKAES 1998
Waseshiro	4x		S	−	−	HKAES 1998
Yagana	4x		S	−	−	CIP 1998
Yellow Fin	4x		S	−	−	S. Slack, personal communication
Yukijiro	4x		S	−	−	HKAES 1998
Yukon Gold	4x		H	−	−	Valkonen 1997

[1] *adg: Solanum tuberosum* subsp. *andigena, brd: S. brevidens, chc: S. chacoense, sto: S. stoloniferum, phu: S. phureja, tbr : S. tuberosum*

[2] E, extreme resistant by Ry_{adg} : E*, extreme resistant by different genes from Ry_{adg} ; H, hypersensitive; S, susceptible

[3] Marker band detected (+) or not detected(−)

[4] International Potato Center

[5] Hokkaido Konsen Agricultural Experimental Station, Japan

2.4 RFLP analysis

Hybridization was done overnight at 65 ℃. The membranes were washed with the stringency wash buffer (2 M Urea, 0.1 % SDS, 50 mM Na phosphate, 150 mM NaCl, 10 mM $MgCl_2$). The first wash was carried out at 55 ℃ for 5 min, and the second wash was carried out at 58 ℃ for 5 min. Chemiluminescent detection of the probe using AlkPhos Direct™ non-radioactive system and Hyperfilm™ (Amersham Pharmacia, UK) was carried out according the manufacturer's instructions.

3. Results and Discussion

A new signals specific to the E phenotype was observed at 10.5 kb among the many bands detected in the tested potato genotypes **(Fig. 1, Table 1)**. It has already been reported that the 3.5 kb band co-segregated with Ry_{adg} in a mapping population consisted of 77 progeny (Hämäläinen *et al.* 1998). The 10.5 kb signal was newly recognized to be associated with Ry_{adg} in this study.

Applicability test for the 3.5 kb marker using various potato genotypes revealed that the 3.5 kb marker was specific to genotypes carrying Ry_{adg} **(Table 1)**. The 3.5 kb signal was detected in 13 of 13 genotypes carrying Ry_{adg}, but not detected at 99 of 104 genotypes lacking this gene. The association between the 3.5 kb signal and the Ry_{adg} was 96 % **(Table 2)**. Thus, it was confirmed that the 3.5 kb marker can be used specifically for the selection on the Ry_{adg} gene, regardless of the diverse genetic background in materials, as previously suggested (Hämäläinen *et al.* 1998).

On the other hand, the 10.5 kb signal was also

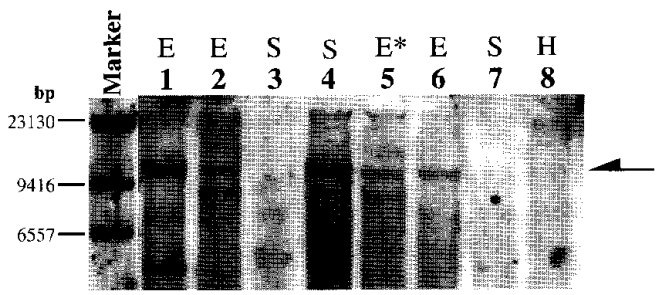

Fig. 1 Southern hybridization with the ADG2 to *Eco*RI-digested genomic DNA of diploid and tetraploid potato breeding lines and tetraploid potato cultivars which are extremely resistant (E), susceptible (S) to PVY. Lane 1: $2x(V-2)_7$ (E), 2: $\{2x(V-2)_7 \times 84.194.30\}_{62}$ (E), 3: 84.194.30 (S), 4: NY115(S), 5: IvP35 (E*), 6: NY121(E), 7: Stirling (S), 8: Pito (H). Marker: lambda DNA *Hind* III digested. The E phenotype in IvP35 is supposed to be controlled by a resistance gene derived from *S. phureja*.

detected in the E phenotypes supposed to be controlled by resistance genes derived from *S. phureja* (IvP35), *S. chacoense* (F_1-1, Konafubuki, and Sakurafubuki), and *S. stoloniferum* (86.54.18 and 86.61.26) (Watanabe *et al.* 1994a) in addition to genotypes carrying Ry_{adg}, **(Table 1)**. This signal was detected in 17 of 22 genotype carrying Ry_{adg} and other *Ry* genes, and the association between the 10.5 kb signal and the phenotypes was 83 % **(Table 3)**. Therefore, the 10.5 kb marker could select E phenotypes in potato cultivars and lines with various genetic backgrounds. This is the complementary function of 10.5 kb signals to 3.5 kb signals which specifically linked to Ry_{adg}.

As for some potato clones with E phenotype such

Table 2. Correspondence between ADG2/ *Eco* RI signals and Ry_{adg}

	10.5kb signal[1]	3.5kb signal
Ry_{adg}	11/13	13/13
other *Ry*	6/9	2/9
S and H[2]	15/95	3/95
Association[3]	94/117=80%	112/117=96%

[1] a denominator: total number of tested genotpe, a numerator. total of genetype detected signal

[2] S: susceptible, H; hypersesitive

[3] Correlations between Ry_{adg} and marker-signal were calculated as follow; A numenrator is sum of the number of the line exhibited signal in genotypes carrying Ry_{adg} and the number of line lacking marker-signal in S, H and other *Ry*. A denominator is sum of number of tested lines.

Table 3. Correspondence between ADG2/*Eco* RI signals and PVY resistance phenotype.

PVY resistant phenotype[1]	10.5kb signal[2]	3.5kb signal[2]
E	17/22	15/22
S and H	15/95	3/95
Association[3]	97/117=83%	107/117=91%

[1] E: Extreme resistance, S: susceptible, H: hypersensitive

[2] a denominator; total of tested phenotype, a numerator: total of phenotype detected signal

[3] Correlations between PVY resistant phenotype and marker-signal were calculated as follow: A numenrator is sum of the number of the line exhibited signal in E phenotype and the number of line lacking marker-signal in S and H. A denominator is sum of number of tested lines.

as CPC2451 and TET38.12, which did not show the positive signals, suggestions shall be referred to Watanabe *et al.* (1995b) and (Valkonen *et al.* 1995). Distantly related species (CPC2451, *S. brevidens*) and its interspecific hybrid (TET38.12) with a diploid potato breeding line Watanabe *et al.* (1995b), could have alternative genetics and resistance gene(s) conferring E phenotype (Valkonen *et al.* 1995). It should also be noted that there were some cultivars with S phenotype such as Dansyaku and NY99, which showed the positive signals. While they are exception in the percentage (**Tables 2 and 3**), extra caution shall be taken when these genotypes and progenies are to be used for MAS with the markers discussed in this paper. Although ADG2-*Eco*RI RFLP fragments have high association for the distinction of the E phenotypes with PVY resistance, the ADG2 fragment may not be the part of an *Ry* gene. It may be adjacent to an *Ry* locus but with a distance which allows recombination as derivatives such as Dansyaku and NY99 exist, or it is independent with a similar motif like an *Ry* gene (Watterson *et al.* 1993).

An application of markers that is reliable and easy to use is preferable in practical use which includes a large number of samples and time-consuming steps. Obtaining two indexes from one procedure is a very attractive feature of the RFLP marker ADG2, even though some optimizations of the hybridization condition will be required. Moreover, the intriguing findings in this report should be addressed here; MAS using the marker ADG2/*Eco*RI can be carried out on a relatively wide range of resistance sources based on the 10.5 kb marker, whereas specific selection for the Ry_{adg} can be done with the 3.5 kb marker. Thereafter, our survey results clearly indicate that the RFLP marker ADG2 provides a powerful tool in breeding of PVY resistant potato cultivars.

As the ADG2 as an RFLP probe was applied to *Eco*RI-digested genomic DNA of tomato (*Lycopersicon*), pepper (*Capsicum*), and eggplant (*Solanum*), which are representative species within the family *Solanaceae* and susceptible to PVY, signals were detected in at least one line of each of the tested species (**Table 4**). Twelve signals with different sizes were detected in these lines. In 'Fox Face' (eggplant), only one signal was detected, whereas in

Table 4. Distribution of signals detected by ADG2

Material name	No. of signal	Detected signals[1]										
		2.5	3.0	3.5[2]	4.0	4.5	5.0	5.5	7.0	7.5	9.0	10.5[2] (kb)
Lycopersicon												
Rejina	3	−	−	+	−	−	+	−	−	−	−	+
Ogata-tomato	4	+	−	+	−	−	+	−	−	−	−	+
Sugar Lamp	7	+	−	+	−	−	+	+	−	+	+	+
Home-Momotaro	3	+	+	−	+	−	−	−	−	−	−	−
Pepe	2	+	+	−	−	−	−	−	−	−	−	−
Yellow Cherry	2	−	+	−	−	−	+	−	−	−	−	−
Capsicum												
Ornamental Capsicum	0	−	−	−	−	−	−	−	−	−	−	−
Fushimi-Amanaga	0	−	−	−	−	−	−	−	−	−	−	−
Shishito	3	+	−	−	−	+	−	+	−	−	−	−
Kyomidori	2	−	+	−	−	−	+	−	−	−	−	−
Solanum												
Fox Face	1	−	−	−	−	−	−	−	+	−	−	−
Bay-nasu	0	−	−	−	−	−	−	−	−	−	−	−

[1] +: detected, − : not detected

[2] 3.5kb and 10.5kb signals were showed to be linked to PVY resistance in potatoes.

'Sugar Lamp' (tomato) seven signals were detected. Other genotypes had from two to six signals. Presence of these signals suggested ADG2 homologous regions are also present in solanaceous species. Interestingly, the 3.5 kb signal and the 10.5 kb signal, which were linked to PVY resistance in potato, were also detected in some lines of tomato.

RFLP linkage maps are now available for many plant species, including three important members of the *Solanaceae*, potato, tomato, and pepper, and indicate similarities of the genomes between potatoes and tomatoes (Gebhardt *et al.* 1991, Prince *et al.* 1993, Tanksley *et al.* 1992). Indeed, some RFLP markers derived from tomato genomic DNA or cDNA are linked to PVY resistance genes in potatoes (Hämäläinen *et al.* 1997). Thus, the ADG2 may be useful for searching PVY resistance in the non-tuber bearing species within the family *Solanaceae*. This needs to be tested in a future study.

Acknowledgments

DNA samples of potato cultivars were provided from CIP, Cornell University and Hokuren Federation of Agricultural Cooperatives. We thank Dr. Kazuyoshi Hosaka, Exp. Stn., Fac. Agr., Kobe Univ. for his helpful advice and for providing some plant materials for conducting this experiment. We are grateful to Yasuhiko Takeda, Youko Morikawa and Junko Kyotani for technical assistance. This research was supported by the grant JSPS-RFTF96L00603 to KNW and KK, and was partially supported by a grant-in-aid No. 09760007 from the Ministry of Education, Science, Sports and Culture, Japan, and by the Academy of Finland (grant #36256).

References

Brigneti, G., Garcia-Mas, J., Baulcombe, D. C., 1997. Molecular mapping of the potato virus Y resistance gene Ry_{sto} in potato. Theor. Appl. Genet., **94**: 198-203.

CIP, 1998. List of Pathogen Tested Potato Genotypes. International Potato Center, Lima, Peru.

Cockerham, G., 1970. Genetical studies on resistance to potato viruses X and Y. Heredity, **25**: 309-348.

de Bokx, J. A., Huttinga, H., 1981. Potato virus Y. CMI/AAB Descriptions of Plant Viruses no. 242.

de Bokx, J. A., van der Want, J. P. H., 1987. Viruses of potatoes and seed-potato production. PUDOC, Wageningen, The Netherlands.

Gebhardt, C., Ritter, E., Barone, A., Debener, T., Walkemeier, B., Schachtschabel, U., Kaufmann, H., Thompson, R. D., Bonierbale, M. W., Ganal, M. W., Tanksley, S. D., Salamini, F., 1991. RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor. Appl. Genet., **83**: 49-57.

Hawkes, J. G., 1990. The Potato. Evolution, Biodiversity and Genetic Resources. Smithsonian Institution, Washington, D. C.

Hämäläinen, J. H., Watanabe, K. N., Valkonen, J. P. T., Arihara, A., Plaisted, R. L., Pehu, E., Miller, L., Slack, S. A., 1997. Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. Theor. Appl. Genet., **94**: 192-197.

Hämäläinen, J. H., Sorri, V. A., Watanabe, K. N., Gebhardt,

C., Valkonen, J. P. T., 1998. Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato. Theor. Appl. Genet., **96**: 1036-1043.

HKAES, 1998. Report on Potato Breeding 1996. Hokkaido Konsen Agricultural Experimental Station, Japan. (In Japanese)

Hooker, W. J., 1981. Compendium of Potato Diseases. APS Press, St. Paul, Minnesota.

Hosaka, K., Hanneman, R. E., Jr., 1994. Random amplified polymorphic DNA markers detected in a segregating hybrid population of *Solanum chacoense* X *S. phureja*. Jpn. J. Genet., **69**: 53-66.

Iwanaga, M., Freyre, R., Watanabe, K., 1991. Breaking the crossabillity barriers between disomic tetraploid *Solanum acaule* and tetrasomic tetraploid *S. tuberosum*. Euphytica, **52**: 183-191.

Jones, R. A. C., 1990. Strain grope specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. Ann. Appl. Biol., **117**: 93-105.

Kasai, K., Morikawa, Y., Sorri, V. A., Valkonen, J. P. T., Gebhardt, C., Watanabe, K., 1999. Development of SCAR markers to the PVY resistance gene Ry_{adg} based on a common feature of plant disease resistance genes. Genome Accepted.

Landry, B. S., 1993. DNA Mapping in Plants, In: Glick, B.R. Thompson, J.E. (Eds): Methods in Plant Molecular Biology and Biotechnology, 267 - 285. CRC Press, Florida.

Leister, D., Ballvora, A., Salamini, F., Gebhardt, C., 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genetics, **14**: 421-429.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant., **15**: 473-497.

Plaisted, R. L., Halseth, D. E., Brodie, B. B., Slack, S. A., Sieczka, J. B., Christ, B. J., Paddock, K. M., Peck, M. W., 1998. Andover: An early to midseason golden nematode resistant variety for use as chipstock or tablestock. Amer. J. Potato Res., **75**: 113-116.

Prince, J. P., Pochard, E., Tanksley, S. D., 1993. Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. Genome, **36**: 404-417.

Ross, H., 1986. Potato breeding - Problems and Perspectives. J. Plant Breed. Suppl, **13**: 132.

Russo, P., Slack, S. A., 1998. Tissue culture methods for the screening and analysis if putative virus-resistant transgenic potato plants. Virology, **88**: 437-441.

Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, New York.

Shukla, D. D., Ward, C. W., Brunt, A. A., 1994. The Potyviridae. CAB International, Wallingford.

Sorri, V. A., Watanabe, K. N., Valkonen, J. P. T., 1999. Predicted kinase 3a motif of a resistance gene analogue as a unique marker for virus resistance. Theor. Appl. Genet., **99**: 164-170.

Stegmann, H., Schick, D., 1982. Index 1982 of European potato varieties. Biologische Bundesanstait fur Land- und Forstwirtschaht, Institut fur Biochemie, Braunschweig, Berlin.

Swiezynski, K. M., Dziewonska, M. A., Ostrowska, K., 1989. Resistance to the potato leafroll virus (PLRV) in diploid potatoes. Plant Breed., **103**: 221-227.

Tanksley, S. D., Ganal, M. W., Prince, J. P., Vicente, M. C. d., Bonierbale, M. W., Broun, P., Fulton, T. M., Giovannoni, J. J., Pineda, O., R_der, M. S., Wing, R. A., Wu, W., Young, N. D., 1992. High density molecular linkage maps of the tomato and potato genomes. Genetics, **132**: 1141-1160.

Valkonen, J. P. T., Slack, S. A., Plaisted, R. L., Watanabe, K. N., 1994a. Extreme resistance is epistatic to hypersensitive resistance to potato virus Y^o in a *Solanum tuberosum* subsp. *andigena* - derived potato genotype. Plant Disease, **78**: 1177-1180.

Valkonen, J. P. T., Slack, S. A., Plaisted, R. L., 1994b. Use of the virus strain group concept to characterize the resistance to PVX and PVYo in the potato cv "Allegany". Am. Potato J., **71**: 507-516.

Valkonen, J. P. T., Orrillo, M., Slack, S. A., Plaisted, R. L., Watanabe, K. N., 1995. Resistance to viruses in F_1 hybrids produced by direct crossing between diploid *Solanum* series *Tuberosa* and diploid *S. brevidens* (series *Etuberosa*) using *S. phureja* for rescue pollination. Plant Breed., **114**: 421-426.

Valkonen, J. P. T., Jones, R. A. C., Slack, S. A., Watanabe, K. N., 1996. Resistance specificities to virus in potato: Standardization of nomenclature. Plant Breed., **115**: 433-438.

Valkonen, J. P. T., Palohuhta, J. P., 1996. Resistance to potato virus A and Potato virus Y in potato cultivars grown in Finland. Agricultural and Food Science in Finland., **5**: 57-62.

Valkonen, J. P. T., 1997. Novel resistance to four potyviruses in tuber-bearing potato species, and temperature-sensitive expression of hypersensitive resistance to potato virus Y. Ann. Appl. Biol., **130**: 91-104.

Valkonen, J. P. T., Rokka, V.-M., 1998. Combination and expression of two virus resistance mechanisms in interspecific somatic hybrids of potato. Plant Science, **131**: 85-94.

Watanabe, K., Vega, S., Orrillo, M., 1992. Characterization on *S. acaule* introgression lines. Am. Potato J., **69**: 613-614.

Watanabe, K., Orrillo, M., Iwanaga, M., Ortiz, R., Freyre, R., Perez, S., 1994a. Diploid potato germplasm derived from wild and land race genetic resources. Am. Potato J., **71**: 599-604.

Watanabe, K., Orrillo, M., Vega, S., Masuelli, R., Ishiki, K., 1994b. Potato germplasm enhancement with disomic tetraploid *Solanum acaule*. II. Assessment of breeding value of tetraploid F_1 hybrids between tetrasomic tetraploid *S. tuberosum* and *S. acaule*. Theor. Appl. Genet., **88**: 135-140.

Watanabe, K. N., Orrillo, M., Golmirzaie, A. M., 1995a.

Potato germplasm enhancement for resistance to biotic stresses at CIP. Conventional and biotechnology-assisted approaches using a wide range of *Solanum* species. Euphytica, **85**: 457-464.

Watanabe, K. N., Orrillo, M., Vega, S., Valkonen, J. P. T., Pehu, E., Hurtado, A., and Tanksley, S. D., 1995b. Overcoming crossing barriers between nontuber-bearing and tuber-bearing *Solanum* species:towards potato germplasm enhancement with a broad spectrum of solanaceaous genetic resources. Genome, **38**: 27-35.

Watterson, J. C., 1993. Development and breeding for resistance to pepper and tomato viruses. In: Kyle, M. M. (Ed.): Resistance to Viral Diseases of Vegetables, 80-101. Timber Press, Portland.