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J. H. Hämäläinen · K. N. Watanabe
J. P. T. Valkonen · A. Arihara · R. L. Plaisted
E. Pehu · L. Miller · S. A. Slack

Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y

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Abstract The chromosomal location of the major gene Ry_{adg} controlling extreme resistance to potato virus Y (PVY) in *Solanum tuberosum* subsp. *andigena* was identified by RFLP analysis of a diploid potato population. A total of 64 tomato and potato RFLP markers were screened with the bulked segregant analysis (BSA) on segregants extremely resistant, hypersensitive or susceptible to PVY. Four markers TG508, GP125, CD17 and CT168 at the proximal end of chromosome XI showed close linkage with extremely resistant phenotypes. TG508 was identified as the closest marker linked with the Ry_{adg} locus with the maximum map distance estimated as 2.0 cM. The 4 markers linked with the Ry_{adg} locus were tested on independent tetraploid and diploid potato clones and were subsequently found useful for marker-assisted selection for plants containing Ry_{adg}.

Key words Potato virus Y · Resistance gene · *Solanum tuberosum* subsp. *andigena* · Potato · RFLP

[*]The first three authors have contributed equally to this study
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J. H. Hämäläinen[1]
Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA, and Department of Plant Production, P.O. Box 27, FIN-00014 University of Helsinki, Finland

K. N. Watanabe[2]
Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853, USA and International Potato Center (CIP), Lima, Peru

J. P. T. Valkonen (✉)
Institute of Biotechnology, P.O. Box 56, FIN-00014 University of Helsinki, Finland

A. Arihara[3]
Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853, USA

R. L. Plaisted
Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853, USA

E. Pehu
Department of Plant Production, P.O. Box 27, FIN-00014 University of Helsinki, Finland

L. Miller · S. A. Slack
Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA

Present addresses:
[1] Institute of Biotechnology, P.O. Box 56, FIN-00014 University of Helsinki, Finland
[2] Department of Biotechnological Science, Kinki University, Uchita, Wakayama, 649-64, Japan
[3] Hokuren Federation of Agricultural Cooperatives, Naganuma, Hokkaido, 069-13, Japan

Introduction

Potato Y potyvirus PVY is globally one of the most important viral pathogens of the cultivated potato (*Solanum tuberosum* L.) and can reduce yields up to 80% (Hooker 1981). PVY can be controlled using virus-tested seed potatoes. However, PVY-infected volunteer potato plants or weeds usually exist in the proximity of the potato crop and dispersal of PVY by aphids frequently occurs to potatoes in the field. Therefore, resistance to PVY in potato cultivars provides the most effective control against this virus (de Bokx and van der Want 1987).

Two major types of monogenically inherited resistance to PVY are known in cultivated and wild potato species (*Solanum* spp.), namely hypersensitive resistance (H) and extreme resistance (E) (Ross 1986). H controlled by the *Ny* genes is often PVY strain group-specific, whereas E controlled by the *Ry* genes is effective against all strains of PVY (Cockerham 1970; Jones 1990). Following infection with PVY, potato plants expressing *Ny* develop necrotic lesions in infected leaves and/or necrosis in systemically infected parts. Plants expressing *Ry* remain symptomless, except limited necrosis may develop in the systemically infected leaves in a few genotypes following graft-inoculation. No PVY titers detectable with ELISA develop in inoculated plants expressing *Ry* (Ross 1986).

The *Ry* genes known in the cultivated and wild potato species and utilized in potato breeding programs include Ry_{sto} in *S. stoloniferum* Schlechtd. et Bche (Ross 1958; 1986)

and Ry_{adg} in *S. tuberosum* subsp. *andigena* Hawkes (Muñoz et al. 1975). So far, no gene for resistance to PVY has been localized genetically. Resistance for PVY in *S.t. andigena* was previously expected to function at a single locus. However, a few progeny hypersensitive to PVY were observed among extremely resistant and susceptible progeny in the crossing populations of *S.t. andigena*. Thus, Ry_{adg} was thought to follow a 'distorted segregation' (Muñoz et al. 1975; Gálvez et al. 1992). Recently, Valkonen et al. (1994b) found evidence for two resistance genes (Ry_{adg} and Ny_{adg}) in *S.t. andigena.* The expression of Ry_{adg} was found to be epistatic to the expression of Ny_{adg} and consequently, the mapping of the PVY resistance loci in *S.t. andigena* became attractive as the segregation of resistance genes corresponding to discrete phenotypes was expected.

In this study, the linkage maps of potato and tomato (*Lycopersicon esculentum* Mill.) based on restriction fragment length polymorphism (RFLP) (Tanksley et al. 1992) were utilized to identify RFLP markers linked to Ry_{adg} in a diploid potato progeny. The significant markers were applied for marker-assisted selection of extreme resistance to PVY controlled by Ry_{adg} in diploid and tetraploid potatoes.

Materials and methods

Plant material

A (di)haploid (2n=2x=24) potato population (F_1) was produced via a sexual cross between the haploid (2n=2x=24) potato breeding line 2x(V-2)7 containing Ry_{adg} (extremely resistant to PVY) and the diploid (2n=2x=24) potato clone 84.194.30 (susceptible to PVY) (Valkonen et al. 1994b). A total of 54 progeny of this F_1 population, which had previously been tested for phenotypic resistance responses to a PVY isolate belonging to the ordinary strain group of PVY (PVY^O) (Valkonen et al. 1994b), were included in this study for the identification of markers linked to Ry_{adg}. The markers were further tested for selection of PVY resistance using tetraploid potato cultivars and tetraploid and diploid potato breeding lines with different genetic backgrounds (Table 1) obtained from the International Potato Cen-

Table 1 Examination of tetraploid potato cultivars and tetraploid and diploid potato breeding lines using the RFLP markers TG508, GP125, CD17 and CT168 which detected a single band unique for the genotypes carrying the gene Ry_{adg} in the diploid mapping population of this study

Clone	Resistance donor species[a]	Resistance		Presence of the marker band[c]			
		Resistance expression	Reference	TG508	GP125	CD17	CT168
Tetraploid (2n = 2x = 48)							
Allegany	*adg*	H	Valkonen et al. 1994a	–	–	–	–
Andover		S	Anonymous 1994	–	–	–	–
Atlantic		S	Valkonen et al. 1994a	–	–	–	–
Belrus		S	S. A. Slack, unpublished	–	–	nd	nd
Desiree	*tbr*	H	Jones 1990	–	–	nd	nd
Katahdin	*tbr*	H	Jones 1990	–	–	nd	nd
Monona		S	Anonymous 1994	–	–	nd	nd
Norchip		S	Anonymous 1994	–	–	nd	nd
Pentland Ivory	*tbr*	H	Jones 1990	–	–	–	–
Pito	*tbr*	H	Valkonen and Mäkäräinen 1993	–	–	–	–
Sebago	*tbr*	H	This study	–	–	nd	nd
Superior		S	Anonymous 1994	–	–	–	–
NY99		S	R. L. Plaisted, unpublished	–	–	–	–
954.3CA		S	Watanabe et al. 1994a	–	–	–	–
TA.1.27.1.1		S	Watanabe et al. 1992	–	–	nd	nd
E74-7	*adg*	E	This study	+	+	+	+
N140-201	*adg*	E	This study	+	+	+	+
Q237-8	*adg*	E	R. L. Plaisted, unpublished	+	+	+	+
TA3.5.3.6	*adg*	E	Watanabe et al. 1992	+	+	+	+
TA3.5.3.7	*adg*	E	Watanabe et al. 1992	+	+	+	+
TA3.8.3.2	*adg*	E	Watanabe et al. 1992	+	+	nd	nd
TA3.8.3.3	*adg*	E	Watanabe et al. 1992	+	+	nd	nd
TA3.8.3.4	*adg*	E	Watanabe et al. 1992	+	+	nd	nd
Diploid (2n = 2x = 24)							
2x(v-2)7	*adg*	E	Valkonen et al. 1994b	+	+	+	+
2x(v-3)30	*sto*	E	Watanabe et al. 1994b	+	+	–	–
86.61.26	*sto*	E	Valkonen et al. 1994b	+	–	–	–
IvP 35	*phu*	E	Valkonen et al. 1995	–	–	–	–
CPC 2451	*brd*	E	Valkonen et al. 1995	–	–	–	–
84.36.29		S	Watanabe et al. 1994b	–	–	nd	nd
84.194.30		S	Valkonen et al. 1994b	–	–	–	–
J-40		S	Watanabe et al. 1994b	–	–	nd	nd

[a] *adg*, *Solanum tuberosum* subsp. *andigena*; *sto*, *S. stoloniferum*; *phu*, *Solanum phureja*; *brd*, *Solanum brevidens*
[b] E, Extremely resistant; H, hypersensitive; S, susceptible
[c] Marker band detected (+) or not detected (–); nd, not determined

ter (CIP), Lima, Peru; Department of Plant Breeding and Biometry, Cornell University, Ithaca, N.Y., USA; and Cornell Foundation Uihlein Seed Potato Farm, Lake Placid, N.Y., USA. In some of these potato genotypes, resistance to PVY was tested in this study by graft-inoculation with a previously described isolate of PVY^O (Valkonen and Mäkäräinen 1993) under natural daylight in the greenhouse (means of the minimum and maximum temperatures – 19°C and 24°C).

RFLP analysis

DNA was extracted from plants grown in the greenhouse as described by Bernatzky and Tanksley (1986) except that sodium bisulfite (5 g/l) was used instead of mercaptoethanol. RFLP between the progeny was studied using the bulked segregant analysis (BSA) (Michelmore et al. 1991). DNA samples of the progeny were bulked to three pools based on the resistance phenotype (33 progeny with E, 7 progeny with H and 14 progeny susceptible (s) to PVY). Bulked DNA of the progeny and the DNA samples of the parental clones were digested with *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII or *Xba*I (Boehringer Mannheim), and 10 μg of the digested DNA was loaded and separated on a 0.8% agarose gel using electrophoresis. Southern blotting was carried out as described by Sambrook et al. (1989).

A total of 64 tomato and potato genomic and cDNA clones were used as probes (Gebhardt et al. 1989; Tanksley et al. 1992). These selected markers span the entire potato genome and were located at 20-cM intervals. Probes were labelled with digoxigenin or [^{32}P] and hybridized to potato DNA on Southern blots; the signal was detected by chemiluminescence following the manufacturer's instructions (Boehringer Mannheim) or by autoradiography. The putatively polymorphic markers identified based on BSA were hybridized to filters containing DNA of the progeny individuals and the parental lines, each loaded separately.

Linkage analysis was performed using the MAPMAKER/EXP 3.0 software (Whitehead Institute, Cambridge, Mass., USA). Extreme resistance was treated as an extra RFLP fragment being present in the extremely resistant genotypes and absent in the susceptible and hypersensitive genotypes.

Results

Polymorphism between the DNA pools of the extremely resistant, hypersensitive and susceptible progeny was initially detected with TG508 following digestion with *Eco*RI (Fig. 1). Flanking markers close to TG508 in chromosome XI were then used for hybridization, and polymorphism between the three pools was detected with the markers CD17 (*Hin*dIII digest), CT168 (*Dra*I digest) and GP125 (*Eco*RV digest). With all the markers, a single band of 6–8 kb was identified which was unique only to the E phenotype pool and not observed in the pools of the H and S phenotypes. Hybridization of these 4 putatively significant markers against filters containing DNA of the progeny individuals (Fig. 2) showed that all were linked to Ry_{adg} according to the test for independent assortment (TG508: χ^2=51.00; CT168: χ^2=34.38; GP125: χ^2=46.17; CD17: χ^2=39.27; P<0.005). The closest linkage to Ry_{adg} was identified for TG508 (maximum map distance estimated as 2.0 cM). Thus, the data indicated that Ry_{adg} was located at the proximal region of chromosome XI (Fig. 3).

The applicability of CD17, GP125 (Fig. 4), CT168 and TG508 as markers for E to PVY was tested in selected tetraploid potato cultivars and diploid and tetraploid potato breeding lines (Table 1). All markers gave a signal of the correct size in the potato genotypes carrying Ry_{adg}. Additionally, TG508 gave a positive signal in diploid breeding lines 86.61.26 and 2x(V-3)30, which carry the gene Ry_{sto}. GP125 also showed a positive signal in 2x(V-3)30. In contrast, no signal band of the correct size was detected by any marker in *S. phureja* IvP35 and *S. brevidens* CPC 2451, both of which also express E to PVY. Similarly, most of the potato genotypes expressing necrosis (H) following PVY infection were not detected with the markers, except for potato breeding lines N140-201 and E74-7, which showed a positive signal with all four markers (Fig. 4, Table 1). In the test for PVY resistance by graft-inoculation, N140-201 and E74-7 showed necrotic streaks in minor veins, and E74-7 also showed small chlorotic spots with a necrotic centre, but no PVY was detected by ELISA. Thus,

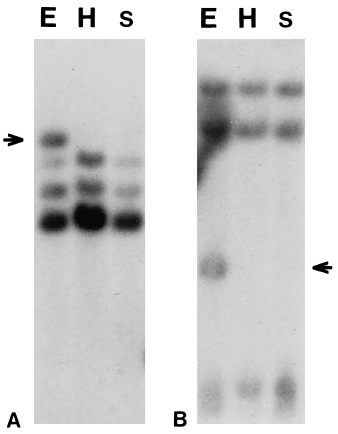

Fig. 1A, B Bulked segregant analysis of diploid potato progeny pooled on the basis of resistance to PVY^O (*E* extreme resistance, *H* hypersensitive resistance, *s* susceptible). DNA pools were digested with *Xba*I (**A**) and *Eco*RI (**B**) and probed with TG508. *Arrowhead* indicates the marker band specific to the extremely resistant progeny carrying Ry_{adg}

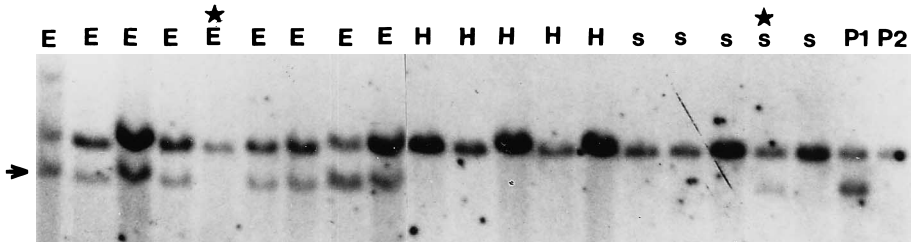

Fig. 2 Hybridization with the marker CD17 to *Hin*dIII-digested individual progeny obtained from the cross 2x(v-2)7 (P1) 84.194.30 (P2). *P1* carries the gene Ry_{adg} for extreme resistance (*E*) and the gene Ny_{adg} for hypersensitive resistance (*H*) to PVY, whereas *P2* is susceptible (*S*) to PVY. The marker band specific to the E genotypes is indicated by an *arrowhead*. Note the two recombinants (marked with *) detected with CD17

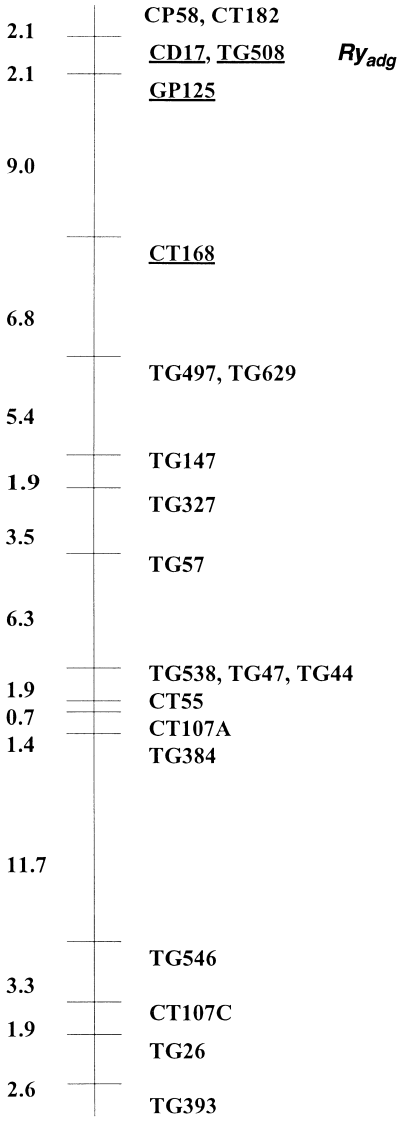

Fig. 3 Composite genetic map of potato chromosome XI showing the predicted position of Ry_{adg}. Map distances are based on segregation in an interspecific cross of diploid potatoes (Tanksley et al. 1992) except for the *underlined* markers, which correspond to linkage analysis of the diploid progeny of this study (LOD>3.0)

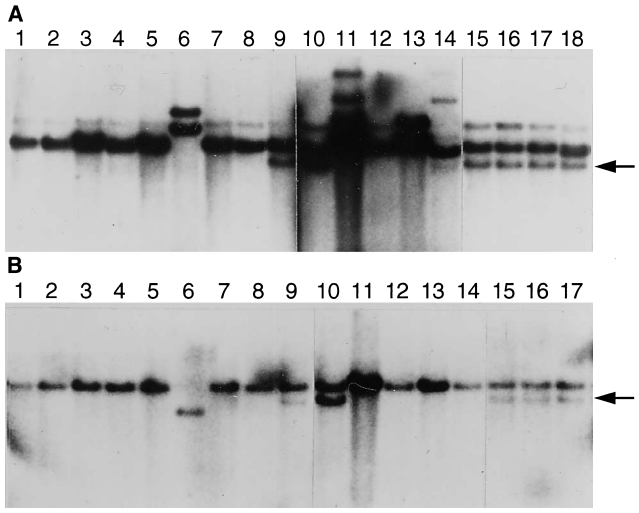

Fig. 4A–B Hybridization with the marker GP125 (**A**) to *Eco*RI-digested and with the marker CD17 (**B**) to the *Hin*dIII-digested DNA of diploid and tetraploid potato breeding lines which are extremely resistant (*E*), hypersensitive (*H*) or susceptible (*s*) to PVY (for detailed description, see Table 1. *Lane 1*, Atlantic (s), *2* Andover (s), *3* NY99 (s), *4* Pentland Ivory (H), *5* Bintje (s), *6 S. brevidens* CPC2451 (E), *7* Allegany (H), *8* Pito (H), *9* E74-7 (H), *10* 2x(v-2)7 (E), *11* 81.61.26 (E), *12* 84.194.30 (s), *13 S. phureja* IvP35 (E), *14* 2x(v-3)30 (E), *15* TA3.5.3.6 (E), *16* TA3.8.3.3 (E), *17* TA3.5.3.7 (E), *18* N140-201 (E)

sensu Ross (1986), both clones were extremely resistant to PVY despite the necrotic symptoms which developed (in contrast, e.g., 2x(V-2)7 carries Ry_{adg} and develops no symptoms following graft-inoculation with PVY; Valkonen et al. 1994b).The marker bands were not observed in any genotype susceptible to PVY.

Discussion

Earlier genetic studies on E and H to PVY in potato species have indicated that these resistances are controlled by single dominant genes (Cockerham 1970; Ross 1986). *S.t. andigena* has been utilized as a source of E to PVY in breeding programs mainly in North and South America (Muñoz et al. 1975; Gálvez et al. 1992). Crosses with *S.t. andigena* have resulted in a few progeny hypersensitive to PVY among the extremely resistant and susceptible progeny. This has been explained as a 'distorted segregation' as the resistance was expected to function at a single locus (Galvez et al. 1992). A recent study by Valkonen et al. (1994b) showed that *S.t. andigena* has two resistance genes, namely Ry_{adg} controlling E to PVY^O and Ny_{adg} controlling H to PVY^O, and that Ry_{adg} is epistatic to Ny_{adg}. Due to epistasis, the genotypes carrying both Ry_{adg} and Ny_{adg} express E to PVY, which is similar to the resistance of the genotypes carrying Ry_{adg} only. In contrast, the genotypes carrying only Ny_{adg} develop necrotic symptoms (Valkonen et al. 1994b). The 4 RFLP markers linked to Ry_{adg} were not associated with the hypersensitive progeny of our mapping population. These data provided further evidence that Ry_{adg} is functional at a locus different from that of Ny_{adg}.

During recent years, diploid potato populations have been utilized for the identification of chromosomal loci controlling disease or nematode resistance in potato (e.g., Barone et al. 1990; Leonards-Schippers et al. 1992; Gebhardt et al. 1993; Pineda et al. 1993; El-Kharbotly et al. 1994, 1996; Gebhardt 1996; Jacobs et al. 1996). Two loci controlling virus resistance in potato have been identified to date, namely *Rx1* and *Rx2* controlling E to potato X potexvirus on chromosomes V and XII, respectively (Ritter et al. 1991). Genetic mapping of the genes for resistance

to PVY and other potato viruses using a wider range of potato species and genotypes will permit the identity of virus resistance genes in potato germplasm to be compared. For example, the positive signals obtained with TG508 and GP125 in potato clones carrying Ry_{sto} in this study illustrate the need for further studies to examine whether Ry_{sto} is also located on chromosome XI, whereas the lack of positive signals in *S. phureja* and *S. brevidens* suggests that the PVY resistance gene(s) of these species may be different or at different chromosomal loci. The region of chromosome XI containing the Ry_{adg} locus seems to carry several resistance genes in the solanaceous species. According to Gebhardt (1996, and personal communication), the marker locus *CP58* (see Fig. 3) is linked to the *N* gene for H to tobacco mosaic virus in tobacco (Whitham et al. 1994). A few DNA fragments amplified by the polymerase chain reaction (PCR) from potato genomic DNA using primers designed according to the *N* gene sequence (Whitham et al. 1994) and the *RPS2* gene sequence of *Arabidopsis* (Bent et al. 1994) map to this region of chromosome XI in potato (Leister et al. 1996). This suggests that resistance genes with different specificities may be arranged in tandem arrays on chromosomes as proposed by Staskawicz et al. (1995) and Gebhardt (1996).

This study reports the localization of Ry_{adg} to chromosome XI, which can serve as a starting point for the positional cloning of Ry_{adg}. For this purpose and for fine mapping, saturation of the genetic map by increasing the amount of markers at the chromosomal region of interest is in progress. Two yeast artificial chromosomes of tomato genomic DNA which contain the GP125 fragment and, thus, are likely to correspond to the region of our interest on chromosome XI are being utilized.

All 4 markers showed a positive signal in breeding lines N140-201 and E74-7 (Fig. 4), which contain Neo-Tuberosum (i.e, *S.t. andigena*). However, these breeding lines expressed necrotic symptoms following graft-inoculation with PVY. It is hypothesized that these 2 breeding lines contain Ry_{adg} but that the genes of the parental, PVY-susceptible potato genotypes interfere with the expression of Ry_{adg}, which thereby changes the expression of the symptomless E phenotype towards a necrotic phenotype. This is consistent with the observations of Ross (1958) who concluded that the polygenes of susceptible potatoes converted E towards H in several progeny containing Ry_{sto}. However, he found no evidence that E or H to PVY could be converted to susceptibility to PVY.

The examples mentioned above show that distinct resistance genes (*Ry* and *Ny*) can have a similar phenotypic expression in some cases. Thus, selection for progeny with the gene of interest is not always possible based on symptom expression following virus inoculation, and molecular markers linked to the gene of interest are required. Compared to the classical crossing schemes (Ross 1986), marker-assisted selection (MAS) for resistance can improve the cost-effectiveness and significantly speed up the introgression of resistance genes to potato cultivars as large-scale screening for resistance phenotypes can be avoided (Tanksley et al. 1989; Watanabe 1994). Localization of Ry_{adg} to the proximal end of chromosome XI in this study is the first step in developing a molecular probe to be used for MAS for PVY resistance in potato breeding lines. Four markers were identified which were linked to Ry_{adg} and which could be used to distinguish diploid and tetraploid potato genotypes carrying Ry_{adg}. MAS using the significant markers could be further enhanced by development of a PCR-based immunoassay (Skerritt and Appels 1995).

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