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ORIGINAL PAPER

Genetic mapping of the pear scab resistance gene *Vnk* of Japanese pear cultivar Kinchaku

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Abstract Pear scab (caused by *Venturia nashicola*) is one of the most harmful diseases of pears, especially Japanese and Chinese pear species. The molecular identification and early selection of resistant plants could greatly improve pear breeding. We have identified the position of the scab resistance gene, designated *Vnk* in an indigenous Japanese pear cultivar Kinchaku, within the pear genome by using simple sequence repeat (SSR) markers derived from pear and apple. The position of *Vnk* was identified in the central region of linkage group 1 of Kinchaku. Several amplified fragment length polymorphism (AFLP) markers linked to *Vnk* were obtained by bulked segregant analysis. Among them, the AFLP marker closest to *Vnk* was converted into a sequence tagged site (STS) marker. Four random amplified polymorphic DNA (RAPD) markers previously found to be loosely associated with *Vnk* (Iketani et al. 2001) were successfully converted into STS markers. Six markers (one SSR Hi02c07 and five STSs converted from AFLP and RAPD) showed tight linkages to *Vnk*, being mapped with distances ranging from 2.4 to 12.4 cM. The SSR CH-Vf2, which was isolated from a BAC clone of the contig containing the apple scab gene *Vf*, was mapped at the bottom of linkage group 1 in Kinchaku, suggesting that the *Vnk* and *Vf* loci are located in different genomic regions of the same homologous linkage group.

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Introduction

Pears (*Pyrus* spp.) have been cultivated for more than 2,000 years and are among the most important fruits in about 50 countries in the world's temperate zones (Bell 1990; Bell et al. 1996). The Japanese pear (*Pyrus pyrifolia* Nakai), the European pear (*P. communis* L.), and the Chinese pears (*P. bretschneideri* Rehd., *P. ussuriensis* Maxim.) are the major edible species commercially grown for fruit production. The Japanese and Chinese pears are cultivated in East Asia, and the European pear is grown in Europe, North America, and temperate regions of the southern hemisphere.

Pear scab, caused by two species of *Venturia*, viz., *Venturia nashicola* and *Venturia pirina*, is one of the most serious diseases of Asian and European pears, especially Japanese pear. The fungi infect leaves, fruit, and twigs. *V. nashicola* infects Asian pears throughout their natural range, and *V. pirina* occurs in most regions where European pears are grown. These two species are differentiated from morphological, cultural, and pathological characters (Ishii and Yanase 2000). The species are classified in the same genus as *Venturia*

inaequalis, which causes apple scab. *V. nashicola* is pathogenic only on Asian pears and is not pathogenic on European pears (Bell et al. 1996; Ishii et al. 2002). In contrast, Japanese and Chinese pears are generally resistant to *V. pirina* (Bell et al. 1996; Ishii et al. 2002).

None of the major commercial Japanese pear cultivars are resistant to scab disease caused by *V. nashicola* (Ishii et al. 1992; Bell et al. 1996), but no scab symptoms were observed on the indigenous Japanese pear cultivar Kinchaku, the Chinese pear (*P. bretschneideri*) cultivars Hongli and Mili, and the European pear cultivars Flemish Beauty and La France in field conditions under severe disease pressure (Ishii et al. 1992). Kinchaku is a native cultivar, which was grown commercially in the beginning of the last century (Kajiura and Sato 1990). Kinchaku, Hongli, and Mili were described as highly resistant (HR) to pear scab, displaying no visible symptoms after inoculation with scab (Abe and Kotobuki 1998a). Inheritance analysis indicated that this resistance is controlled by a single dominant gene (Abe and Kotobuki 1998a). Abe and Kotobuki (1998b) observed that a necrotic-type reaction characterized by symptoms without sporulation on some Japanese and Chinese cultivars. They suggested that the necrotic reaction to *V. nashicola* was controlled by polygenes (quantitative trait loci). Molecular markers closely linked to scab resistance genes will be greatly useful for improving pear breeding by marker-assisted selection. However, information on molecular markers associated with scab resistance in pear is presently very limited.

In our previous study, genetic linkage maps of the Japanese pear cultivars Kinchaku and Kousui were constructed using random amplified polymorphic DNA (RAPD) markers (Iketani et al. 2001). The Kinchaku map consisted of 120 loci in 18 linkage groups covering a length of ca. 770 cM, in which two disease-related genes associated with resistance to pear scab and susceptibility to black spot (caused by *Alternaria kikuchiana*) were mapped. However, DNA markers tightly linked to the scab resistance gene and its exact position within the pear genome were not identified.

Detailed genetic linkage maps of the Japanese pear cultivar Housui (syn. Hosui) and the European pear cultivar Bartlett (syn. Williams) were constructed based on Amplified Fragment Length Polymorphism markers (AFLPs), and Simple Sequence Repeat markers (SSRs) (from pear, apple, and *Prunus*) by using their F_1 progeny (Yamamoto et al. 2002c, 2004). These two pear maps were successfully aligned to the apple map by using SSR markers, and all pear linkage groups could be anchored to homologous apple groups. The results suggested that positions and linkages of SSR loci are well conserved between pear and apple. Therefore, positions of important traits, including disease resistance, can be compared in pears as well as between pear and apple by using SSR anchored maps.

Resistance to apple scab disease (caused by *V. inaequalis*) is one of the most important traits in apple breeding. Several major scab resistance genes have been identified and introduced into breeding lines (Williams and Kuc 1969). The *Vf* gene originating from *Malus floribunda* 821 has been incorporated into commercial cultivars, and is found in the distal part of linkage group (LG) 1 (Maliepaard et al. 1998). In a transgenic study, Belfanti et al. (2004) reported that *HcrVf2* (homologue of the *Cf* resistance genes of tomato in the apple *Vf* region) cloned by positional cloning, under the control of the 35S promoter induces scab resistance. Other major scab resistance loci have been identified in apple genetic linkage maps: *Vg* and *Vb* (originated from *M.* × *domestica* cultivar Golden Delicious and Hansen's baccata 2) on LG 12 (Durel et al. 2000; Calenge et al. 2004); *Vd* (*M.* × *domestica* cultivar Durello di Forli) on LG 10 (Tartarini et al. 2004), *Vh2, Vh4* (*M. pumila* R12740-7A), *Vbj* (*M. baccata jackii*), *Vh8* (*M. sieversii*), and *Vr2* (GMAL 2473) on LG 2 (Hemmat et al. 2002; Gygax et al. 2004; Patocchi et al. 2004; Bus et al. 2005); and *Vm* (*M. micromalus* 245-38) on LG 17 (Patocchi et al. 2005). Furthermore, quantitative field resistance against apple scab has been reported in greenhouse and field assessment (Durel et al. 2003; Liebhard et al. 2003b; Calenge et al. 2004). Since the genome structure is highly conserved between pear and apple (both are classified in the family Rosaceae and the subfamily Pomoideae) and their scab diseases are caused by fungal pathogens belonging to the same genus, comparing scab resistance genes (loci) between pear and apple may speed up the identification of resistance gene sources.

In this study, the map position of the scab resistance gene *Vnk* of the Japanese pear cultivar Kinchaku is identified, and several DNA markers showing significant linkage to the gene are obtained. The relationships between pear and apple scab resistance genes are examined.

Materials and methods

Plant materials and DNA extraction

Two mapping populations obtained from three-way crosses in Japanese pear (*Pyrus pyrifolia* Nakai) were used for genetic mapping of the pear scab resistance gene of Kinchaku (Fig. 1). The scab resistance gene of

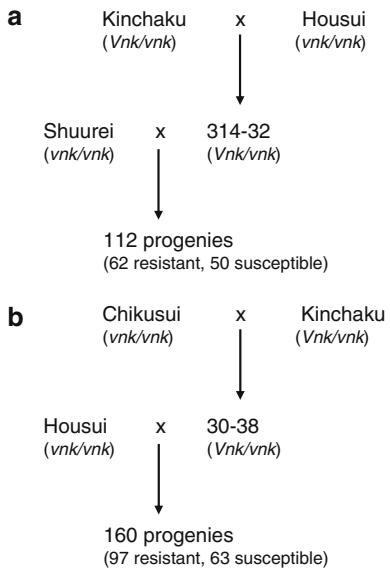

Fig. 1 Three-way crosses performed to generate the two mapping populations (**a**: Shuurei × 314-32; **b**: Housui × 30–38) used to map *Vnk*

Kinchaku was designated as *Vnk* in this study. One hundred and twelve F_1 individuals were obtained from a cross between Shuurei and hybrid No. 314-32 (Kinchaku × Housui). Another mapping population derived from a cross between Housui and hybrid No. 30-38 (Chikusui × Kinchaku) consisted of 160 F_1 progeny. Hybrids 314-32 and 30-38 are heterozygous for the *Vnk* gene inherited from Kinchaku. Shuurei, Housui, and Chikusui are susceptible to pear scab disease (Fig. 1).

Evaluation of scab resistance

The resistance of all F_1 progeny of both populations and their parents and ancestors to pear scab disease was evaluated according to the methods of Abe and Kotobuki (1998a) and Iketani et al. (2001) with slight modifications. Conidia of *V. nashicola* were collected from naturally infected leaves of the Japanese pear cultivar Chojuro. After washing with centrifugation, a conidial suspension (2.5×10^5 per ml, 0.2% sucrose) was prepared. Drops (ca. 10 µl) of the conidial suspension were placed onto five areas on the upper epidermis of each of five young leaves of 3-month-old seedlings. After air drying, the inoculated plantlets were incubated in a moist chamber at 20°C for 2 days, and then transferred to a greenhouse. The appearance of scab symptoms was evaluated at 30–40 days after inoculation. The degree of resistance was classified as either resistant (no visible symptoms) or susceptible (lesions with abundant sporulation).

SSR markers from pear, apple, and *Prunus*

Sixty-five SSR markers originating from pear were screened to identify the linkage group carrying the scab resistance gene *Vnk* (Yamamoto et al. 2002a–c). One hundred and five SSR markers developed from apple, whose positions were identified in apple genetic linkage maps (Guilford et al. 1997; Gianfranceschi et al. 1998; Liebhard et al. 2002), were screened for detection of the scab resistance locus. Seven SSR markers derived from peach or cherry, which were previously identified in pear maps, were also tested (Yamamoto et al. 2002c).

Parents and ancestors (Kinchaku, Housui, 314-32, Shuurei) of the mapping population of Shuurei × 314-32 were tested with SSR markers from pear, apple, and *Prunus* in order to identify SSRs that could be mapped in Kinchaku. These selected SSRs were then scored in all F_1 individuals of Shuurei × 314-32. The SSR markers which showed significant linkage to *Vnk* and previously identified in the same linkage groups were used for the Housui × 30-38 population.

Polymerase chain reaction (PCR) amplification was performed in a 20-µl solution of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM each dNTP, 10 pmol of each forward primer labeled with a fluorescent chemical (Fam/Tet/Hex or Fam/Vic/Ned) and unlabeled reverse primer, 10 ng of genomic DNA, and 0.5 units of *Taq* polymerase (Invitrogen). Amplification was performed in 35 cycles at 94°C for 1 min (denaturing), 50–55°C for 1 min (annealing) and 72°C for 2 min (primer extension). The PCR products were separated and detected using a PRISM 377 or Genetic Analyzer 3100 DNA sequencer (Applied Biosystems). The size of the amplified bands was determined on the basis of an internal standard DNA (GeneScan-350TAMRA or ROX HD-400, Applied Biosystems) with GeneScan software (Applied Biosystems).

SSR markers associated with the apple Vf gene

Two apple SSR markers, CH-Vf1 and CH-Vf2, isolated from BAC clones of the contig spanning the region containing the apple scab resistance gene *Vf* (Vinatzer et al. 2004), were tested for our pear mapping progeny. The apple SSR marker Hi02c07 (Silfverberg-Dilworth et al. 2006), positioned in the middle region of apple LG 1, was also tested. Amplification and detection of the SSR fragments were performed under the same conditions as described for the pear SSRs.

AFLP analysis

Bulked segregant analysis (Michelmore et al. 1991) was conducted to identify AFLP fragments linked to *Vnk*. Four sets of five resistant F_1 plantlets (Shuurei × 314-32), four sets of five susceptible F_1 plantlets, and parents and ancestors (Shuurei, 314-32, Kinchaku, Housui) were tested. Primer combinations producing polymorphic bands between resistant and susceptible bulks were tested in all F_1 progeny, parents and ancestors.

AFLP reaction was performed with an AFLP Analysis System II (Invitrogen) according to the supplier's protocol, except that *Eco*RI primers were labeled with fluorescent chemical. Two hundred and fifty nanograms of genomic DNA was digested with two restriction enzymes (*Eco*RI, *Mse*I), and then the DNA fragments were ligated to adaptors. Pre-amplification reactions were performed with a pre-amp primer mix. Selective amplification was performed with 128 primer combinations of 16 Fam-labeled *Eco*RI primers (E-AA, E-AT, E-AG, E-AC, E-TA, E-TT, E-TG, E-TC, E-GA, E-GT, E-GG, E-GC, E-CA, E-CT, E-CG, E-CC) and eight *Mse*I primers (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, M-CTT). The PCR products were separated and detected using a Genetic Analyzer 3100 DNA sequencer (Applied Biosystems). The size of the amplified bands was determined on the basis of an internal standard DNA (ROX HD-400, Applied Biosystems) with GeneScan software. The designation of the AFLP markers is based on the primer combination and the sizes in bp, shown as *Eco* primer/*Mse* primer-size.

RAPD analysis

Seven RAPD primers (OPAI-20, OPAW-13, OPAQ-11, OPO-09, OPW-02, OPU-17, OPN-17), which were previously found to amplify fragments associated with *Vnk* of Kinchaku (Iketani et al. 2001), were used. These RAPD markers had been screened and identified from 1300 RAPD primers by using 82 F_1 progeny of a cross Kinchaku and Kousui (Iketani et al. 2001). RAPD analysis was conducted on both mapping populations (Shuurei × 314-32 and Housui × 30-38), as described by Iketani et al. (2001).

Conversion of RAPD and AFLP markers to sequence targeted side markers

Four polymorphic RAPD fragments obtained from Kinchaku, the 995-bp band with OPW-02, the 2106-bp band with OPO-09, the 815-bp band with OPAW-13, and the 564-bp band with OPAQ-11, were cloned and sequenced according to Yamamoto and Hayashi (2002). A polymorphic AFLP fragment derived from Kinchaku, the 260-bp fragment obtained from a primer combination of E-CT and M-CTA, was excised from the polyacrylamide gels in order to be re-amplified and sequenced, according to Calenge et al. (2005). The sequence tagged site (STS) primer sets corresponding to cloned sequences were designed using the software Oligo ver. 6 (Molecular Biology Insights, Inc.).

STS-PCR amplification was performed in a 20-μL solution of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM each dNTP, 0.5 μM of each forward and reverse primer, 10 ng genomic DNA, and 0.5 units of *Taq* polymerase (Invitrogen, USA). Amplification was performed in 35 cycles at 94°C for 1 min, 57°C for 1 min, and 72°C for 2 min. The PCR products were separated in 1.5% agarose gels. To reveal the polymorphism between resistant and susceptible genomes, the amplicon produced by the marker STS-OPO9 was digested with *Sal*I, and was then separated in 1.5% agarose gels. The gels were stained with ethidium bromide and then visualized with ultraviolet light.

Linkage analysis

JoinMap ver. 3.0 (Stam and van Ooijen 1995) was used to identify linkage between scab resistance and DNA markers and for constructing a genetic linkage map of Kinchaku. A LOD score of 5.0 was used to define the linkage group. The Kosambi mapping function was used to convert recombination units into genetic distances.

Results

Evaluation of scab resistance

Sixty-two out of 112 F_1 progeny of Shuurei × 314-32 (Kinchaku × Housui) were scored as resistant to pear scab (caused by *V. nashicola*), as no disease symptoms were visible at 30–40 days after inoculation. The other 50 F_1 plantlets showed sporulating lesions, and were judged susceptible. Kinchaku and 314-32 showed no disease symptoms, while Shuurei and Housui showed severe symptoms (Fig. 2). No necrotic reactions or intermediate types were observed in F_1 progeny or their parents. According to the scale used for apple scab evaluation (Chevalier et al. 1991; Bénaouf and Parisi 2000), only class 0 (no symptoms) and class 4 (susceptibility, abundant sporulation) were observed

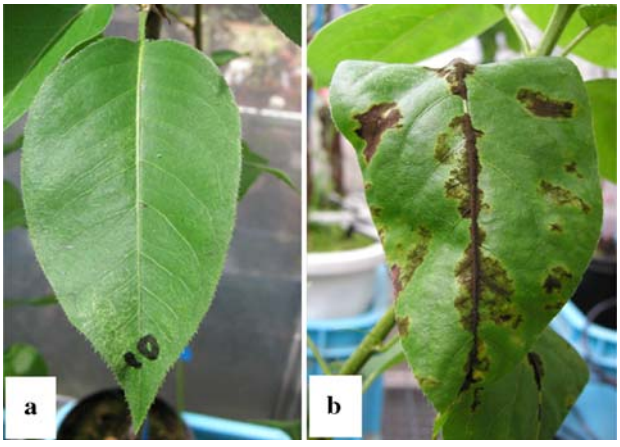

Fig. 2 Pear scab symptoms of Kinchaku (*Vnk/vnk*, **a**) and Housui (*vnk/vnk*, **b**) at 40 days after inoculation with *Venturia nashicola*. The resistant cultivar Kinchaku shows no disease symptoms, while the susceptible cultivar Housui shows lesions with heavy sporulation

among the progeny. Segregation of resistance and susceptibility in this cross fitted the ratio of 1:1 based on the Chi-square test ($\chi^2 = 1.29$), indicating a monogenic inheritance.

No disease symptoms were observed in 97 F_1 plantlets of Housui × 30-38 (Chikusui × Kinchaku), whereas the other 63 progeny showed sporulating lesions judged as susceptible. No intermediate types were observed. Segregation of resistance and susceptibility showed a slight distortion from the 1:1 ratio at the 1% level. Both segregations agree well with that obtained by Abe and Kotobuki (1998a), who reported that the resistance of Kinchaku was controlled by a single dominant gene.

DNA marker analysis

SSRs from pear, apple and Prunus

A total of 180 SSR markers derived from pear, apple, and *Prunus* were tested to evaluate their linkage to the scab resistance gene *Vnk*, using the Shuurei × 314-32 population. All tested SSR markers had already been mapped in pear or apple genetic linkage maps (Yamamoto et al. 2004; Liebhard et al. 2003a). About two-fifths of the SSR markers produced polymorphic fragments derived from Kinchaku. Two of them, PS12A02 and NH013a, showed significant linkage to *Vnk* with recombination values of 0.259 (LOD 5.64) and 0.241 (LOD 6.59), respectively. All other SSR markers showed no significant linkage to *Vnk* (data not shown). NH013a, developed from pear, had already been found on LG 1 in genetic linkage maps of Bartlett (Yamamoto et al. 2004). PS12A02, developed from cherry, is also located on LG 1 of Bartlett (Yamamoto et al. 2004). These results indicate that the pear scab resistance gene *Vnk* is on LG 1 of pear, which is homologous to apple LG 1.

The SSR CH-Vf2, derived from the *Vf* region, amplified a polymorphic allele of 86 bp which displayed a significant linkage to *Vnk*. In the Shuurei × 314-32 population, the recombination value between *Vnk* and CH-Vf2 is 0.288 (LOD 4.51) The other SSR associated with *Vf*, CH-Vf1, did not produce polymorphic bands. These results suggest that scab resistance genes *Vf* in apple and *Vnk* in pear are both located on LG 1.

SSR markers previously identified on LG 1 were evaluated for linkages to *Vnk* by using the second mapping population Housui × 30-38. Three apple SSR markers Hi02c07, CH-Vf2, and AG04, amplified polymorphic alleles and displayed significant linkages to *Vnk*. Among them, Hi02c07 (Silfverberg-Dilworth et al. 2006) showed a tight linkage to *Vnk* with a recombination value of 0.057 (LOD 31.54).

AFLPs

One hundred and twenty-eight primer combinations amplified 269 informative polymorphic AFLP fragments, i.e., band presence for Kinchaku and 314-32, and absence for Shuurei and Housui. Twelve primer combinations showed polymorphism between the resistant and the susceptible bulks, as well as between the parents. The 12 AFLP fragments were scored in all F_1 progeny, and seven AFLP markers showed significant linkages to *Vnk* (Fig. 4). The AFLP marker CT/CTA-260, which is the 260-bp fragment obtained from the primer combination of E-CT and M-CTA, showed a tight linkage to *Vnk* with a recombination value of 0.036. This AFLP marker was successfully converted into an STS marker, designated STS-CT/CTA (Table 1). Two AFLP markers, GG/CAC-123 and TA/CTG-83, had recombination values of 0.098 and 0.107, respectively, to *Vnk*. Significant linkages between the remaining four AFLP markers (CT/CAC-77, TT/CTT-170, GT/CAG-205, AC/CAC-118) and *Vnk* were observed, and their recombination values ranged from 0.14 to 0.25.

RAPD and STS markers

Four out of seven RAPD markers previously found to be associated with *Vnk* (Iketani et al. 2001) were polymorphic in the two mapping populations Shuurei × 314-32 and Housui × 30-38 (Table 1). Kinchaku and 30-38 (Chikusui × Kinchaku) amplified the

Table 1 STS markers developed from polymorphic RAPDs linked to the *Vnk* gene

STS marker name	RAPD (or AFLP) primer	RAPD (or AFLP) fragment (bp)[a]	Primer sequences (5′-3′)	Size of the alleles coupled with *Vnk* (bp)
STS-OPW2	OPW2	995	F: TTGGTAGGGGACCATGACTC R: CCAAAGAAGAGGCAGAGTGG	803
STS-OPO9	OPO9	2,106	F: AAGCACCAAGACAGCACAAC R: CATGTATCAGGCACACGAAC	790 (267, 523)[b]
STS-OPAW13	OPAW13	815	F: TCTCACCACCTGTCATTCGT R: GACGGGCCCAACTTATTAGC	714
STS-OPAQ11	OPAQ11	564	F: CTTGGCCATCATGCATCTGT R: GAATTTTCCTTTTCGCAGGT	112
STS-CT/CTA	E-CT/M-CTA	260	F: GCTGTAAGGTAGGAACTGCAAAC R: GGCTAAAATCCGGCAGTTC	181

[a] The size refers to amplified fragment from Kinchaku

[b] Fragment sizes after digestion with *Sal*I are indicated in parenthesis

995-bp band with OPW-02, the 2106-bp band with OPO-09, the 815-bp band with OPAW-13, and the 564-bp band with OPAQ-11 (Table 1), but these bands were not observed in the susceptible parents/ancestors Housui and Chikusui. OPW-02, OPO-09, OPAW-13, and OPAQ-11 showed significant linkages to *Vnk*, with recombination values of 0.088, 0.050, 0.057, and 0.119, respectively.

The former three RAPDs (OPW-02, OPO-09 and OPAW-13) also produced informative polymorphic bands in the progeny of Shuurei × 314-32. Tight linkages to *Vnk* were found, with recombination values of 0.107, 0.045, and 0.054, respectively.

The four polymorphic RAPD fragments showing tight linkages to *Vnk* were amplified from Kinchaku, cloned and sequenced, and then successfully converted into STS markers (Table 1; Fig. 3). STS-OPW2, STS-OPAW13, and STS-OPAQ11 produced amplified fragments of 803, 714, and 112 bp, respectively, in Kinchaku, 30-38 and 314-32. However, no amplification was obtained from 314-32 with STS-OPAQ11. STS-OPO9 amplified the 790-bp band in all lines used in this study. *Sal*I restriction digestion of the allele of Kinchaku (*Vnk*) showed a polymorphism (production of two fragments of 267 and 523 bp), which was absent in the susceptible cultivars Shuurei, Housui, and Chikusui. The four STS markers exhibited identical patterns to their original RAPDs in all progeny of Shuurei × 314-32 and Housui × 30-38 (data not shown).

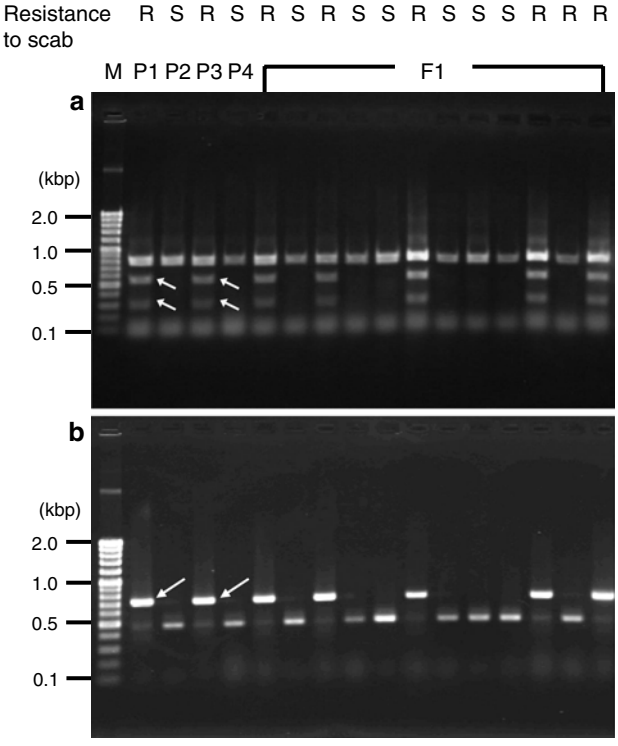

Fig. 3 Banding patterns of parental and ancestral cultivars and F_1 progeny of the Housui × 30-38 population analyzed with STS-OPO9 (**a**) and STS-OPAW13 (**b**). The fragments indicated by *white arrows*, 267 and 523 bp after *Sal*I digestion of STS-OPO9 (**a**) and the 714-bp band of STS-OPAW13 (**b**) are coupled with *Vnk* resistance. Response to pear scab disease is indicated as R for resistance or S for susceptibility. *P1*, *P2*, *P3*, *P4*, and *F1* indicate Kinchaku, Chikusui, 30-38, Housui, and hybrid progeny of Housui × 30-38, respectively. *M* 100-bp ladder marker (100–2,000 bp)

Comparison of LG 1 of apple and pear

LG 1 of Kinchaku was determined in the two mapping populations Shuurei × 314-32 and Housui × 30-38, designated Kin1a and Kin1b, respectively (Fig. 4). In both maps *Vnk* was mapped in the central part of LG 1. We compared the four LGs (Fig. 4), i.e., Kin1a and Kin1b of Kinchaku, LG 1 of the pear cultivar Bartlett (Yamamoto et al. 2004; Yamamoto, unpublished data), and LG 1 of the apple cultivar Discovery

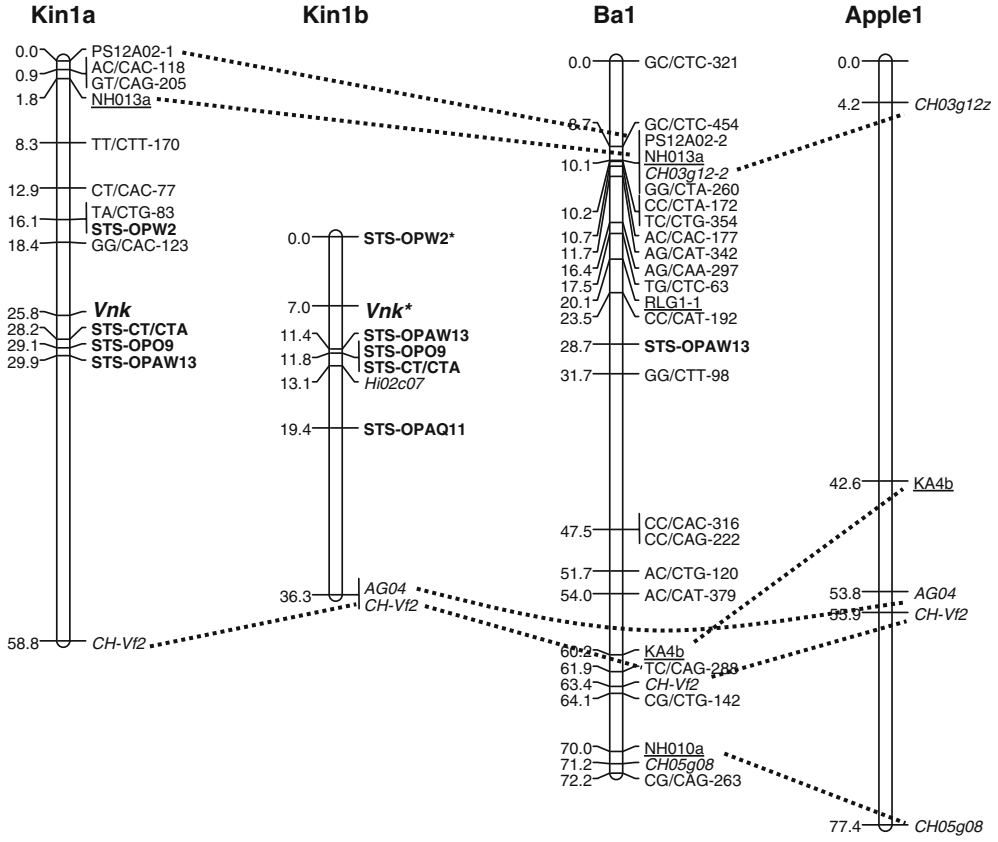

Fig. 4 Linkage groups 1 of the Japanese pear Kinchaku (Kin1a, Kin1b), the European pear Bartlett (Ba1, Yamamoto et al. 2004; Yamamoto, unpublished data), and the apple cultivar Discovery (Apple1, Silfverberg-Dilworth et al. 2006). Kin1a and Kin1b are constructed from the populations of Shuurei × 314-32 and Housui × 30-38, respectively. The pear scab resistance locus is denoted by *Vnk* in groups Kin1a and Kin1b. STS markers are indicated in *bold*. SSR loci from pear and apple are *underlined* and *italicized*, respectively. PS12A02 is an SSR locus derived from cherry. The designation of the AFLP is based on the primer combination and the sizes in bp, shown as *Eco* primer/*Mse* primer-size. * shows distorted segregation at 1% level

(Silfverberg-Dilworth et al. 2006) using six SSRs. SSR CH03g12 was found at the top of LG 1 of Bartlett and apple. PS12A02 and NH013a were anchored at the top of LG 1 of Kinchaku (Kin1a) and Bartlett. CH05g08 and KA4b were aligned at the bottom of LG 1 of Bartlett and apple. CH-Vf2, associated with *Vf*, was mapped at the bottom of LG 1 of Kinchaku (Kin1a, Kin1b), Bartlett, and apple. Since genetic linkage maps of pear are successfully aligned to the apple map, it was possible to compare the position of *Vnk* and *Vf* and establish that the two genes are at two different loci (Fig. 4).

Discussion

Two genetic linkage maps of the *Vnk* region of pear cultivar Kinchaku were constructed (Fig. 4). The maps were successfully aligned to established linkage maps of the European pear Bartlett (Yamamoto et al. 2004) and the apple reference map (Silfverberg-Dilworth et al. 2006) by using several co-dominant SSR markers derived from apple, pear, and cherry. This allowed us to discover that *Vnk* maps on LG 1 of pear. Since LG 1 of Bartlett and the apple reference map span 72–77 cM, and that of Kinchaku encompasses 60 cM, we estimated that LG 1 of Kinchaku covers almost all regions of this linkage group.

One SSR, five STSs (developed from four RAPD and one AFLP markers), and two AFLP markers showed tight linkages to *Vnk*, with recombination values below 0.12. Iketani et al. (2001) mapped scab resistance of Kinchaku on a RAPD-based genetic linkage map. However, the closest marker associated with *Vnk* was mapped at 17.4 cM, and since no SSRs were mapped in that study, it was not possible to identify the LG carrying the scab resistance gene. In the present study, in contrast, we have identified several SSR and STS markers tightly linked to *Vnk*. These molecular markers can now be efficiently used for marker-

assisted selection, and will be useful in the future for pyramiding *Vnk* with other resistance genes.

The degree of scab resistance in pears is classified into three types, i.e., HR (no visible symptoms), necrotic (necrotic and/or chlorotic lesions without sporulation), and susceptible (symptoms with sporulating lesions), and some Japanese and Chinese pears showed necrotic response (Abe and Kotobuki 1998a, 1998b; Abe et al. 2000). The Japanese pear Kinchaku, the Chinese pears Hongli and Mili, and the European pears Flemish Beauty, La France, and Bartlett were evaluated as having high resistance, which was thought to be controlled by a single dominant gene (Abe and Kotobuki 1998a; Abe et al. 2000). Inheritance analyses showed that progeny generated from crosses between Kinchaku and susceptible cultivars segregated into only two phenotypes, i.e., no symptoms (resistant) or abundant sporulation (susceptible) (Abe and Kotobuki 1998a). However, some progeny showing necrotic response in addition to resistant and susceptible types were observed in crosses involving Hongli, Mili, La France, and Bartlett (Abe and Kotobuki 1998a; Abe et al. 2000). These results suggest that scab resistances of different cultivars might be controlled by different genes, or that resistance might be influenced by polygenic factors. Additional studies are required to determine whether scab resistance of different germplasms is controlled by the same gene(s) or not.

Park et al. (2000) examined the infection behavior of *V. nashicola* on resistant cultivar Kinchaku and susceptible cultivar Kousui by light and electron microscopy. Early stages of infection were similar on both cultivars within 3 days after inoculation, and no differences were observed in germination of conidia, formation of appressoria after germ tube elongation, penetration pores observed in the appressoria, and subcuticular hyphae formation. Differences between resistant and susceptible cultivars appeared several days after inoculation. The subcuticular hyphae on the resistant cultivar Kinchaku were modified ultrastructurally and were accompanied by fungal cell death, whereas fungal cells appeared intact in the susceptible cultivar. A similar infection process was documented for the *Vf*-resistance of apple against *V. inaequalis*. The resistance of *Vf* was expressed as an inhibition of stromal growth after 2–3 days (Valsangiacomo and Gessler 1988). On the other hand, symptoms of resistance seem to differ to some extent between *Vnk* and *Vf*. Kinchaku and its resistant progeny showed no visible disease symptoms macroscopically or microscopically (data not shown). Nevertheless, in crosses involving *Vf* apple cultivars, in addition to progeny showing no symptoms, resistant progeny displayed chlorotic or necrotic lesions with or without reduced sporulation (Patocchi et al. 1999). Further study will be necessary to show whether *Vnk* is a *Vf*-like (*HcrVf*-like; Vinatzer et al. 2001) gene or not.

It has been suggested that the genera of the Pomoideae form a distinct and closely related group, based on analyses of chromosome number and the existence of natural generic hybrids (Sax 1931; Kovanda 1965). Some *Malus* SSRs have been successfully used in pear (Yamamoto et al. 2001) and in some other genera of the Pomoideae (Liebhard et al. 2002). Comparative mapping of pear and apple was conducted (Yamamoto et al. 2004). Scab diseases in Asian pears and apple are caused by the fungal pathogens *V. nashicola* and *V. inaequalis*, respectively, which are closely related species. It has been proved that the interaction of *V. inaequalis* with *Malus* genotypes follows a gene-for-gene rule (Bénaouf and Parisi 2000). Recently, isolates (strains) of *V. nashicola* collected from cultivated and wild pears in Japan were categorized by inoculation test into three races (Ishii et al. 2002). Kinchaku showed resistance to all three races, suggesting that *Vnk* may be race-nonspecific (Ishii et al. 2002), or that the specific virulent race has not yet been detected. It was also shown that race-specific resistance genes exist in some pear cultivars (Ishii et al. 2002). It will be necessary to reveal in detail the relationship between resistance genes of different pear germplasms and races of pathogens.

Several scab-resistance genes have been identified and mapped in apple. These genes are distributed in only five linkage groups (LG 1, LG 2, LG 10, LG12, LG17). *Vf*, the most used apple scab resistance gene in apple breeding programs, has been overcome by races six and seven of *V. inaequalis* (Parisi et al. 1993; Bénaouf and Parisi 2000). This finding accentuates the need to use a diverse range of apple scab resistance genes. *Vnk* of Kinchaku, which confers resistance to all three races of *V. nashicola*, has been introgressed into commercial pears. The breakdown of *Vf* resistance in apple suggests that the pyramiding of resistance genes by molecular markers will be necessary for providing durable pear scab resistance in pear. Several tightly linked STS and SSR markers obtained in this study could be efficiently used to pyramid *Vnk* and other resistance genes, as soon these latter will be mapped and molecular markers will be available.

In our preliminary allelism tests, we found that scab resistances of Kinchaku versus Asian pears as well as Asian pears versus European pears may be controlled by different loci (data not shown). This information suggests that, as in apple, scab resistance genes are distributed in different region of the pear genome. The

synteny between pear and apple (Yamamoto et al. 2004) could accelerate the identification of pear scab resistance genes if functional synteny in scab resistance between apple and pear really exists. It is thus very important to identify the position and linkage group of pear scab resistances from different sources in order to verify synteny of resistance genes between apple and pear, and to identify molecular markers suitable to use in pyramiding several different resistance genes to breed pear with durable scab resistance.

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