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Genetic mapping of the loquat canker resistance gene *pse-c* in loquat (*Eriobotrya japonica*)

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ABSTRACT

Loquat canker disease, caused by *Pseudomonas syringae* pv. *eriobotryae*, is one of the most serious diseases of loquat (*Eriobotrya japonica* (Thunb.) Lindl.). We identified the linkage group and position of the resistance gene *pse-c*, which confers resistance to Group C of the pathogen, by using 141 seedlings derived from self-pollinated 'Nagasakiwase' (*Pse-c/pse-c*). Bulked segregant analysis revealed two RAPD markers, RAPD-OPY-03 and RAPD-OPA-18, with significant linkages to *pse-c*. The nucleotide sequences of these markers showed high similarity to the genome sequence of chromosomes 3 and 11 of 'Golden Delicious' apple. Of 448 SSR markers based on those genome sequences, five had significant linkages to *pse-c*. A linkage group including the *pse-c* gene, the two RAPD markers, and the five SSR markers spanned 37.9 cM with an average interval of 5.4 cM, and corresponded to the top of chromosome 3 of apple. The 170-bp allele of marker Chr3-ssr85 and the 184-bp allele of Chr3-ssr91 showed tight linkages with *pse-c*. The development of molecular markers linked to loquat canker resistance genes would be an effective way to establish marker-assisted selection and to pyramid several resistance genes in loquat breeding programs.

1. Introduction

Loquat canker disease, caused by *Pseudomonas syringae* pv. *eriobotryae*, causes serious damage in loquat cultivation. The pathogen attacks all parts of the tree, resulting in weak growth and loss of commercial value of the fruit. Damage due to the disease has been reported in Argentina, Australia, China, Japan, New Zealand, and the USA (Alippi and Alippi, 1990; Lai et al., 1971; Lin et al., 1999; McRae and Hale, 1986; Mukoo, 1952; Wimalajeewa et al., 1978). Morita (1978) classified 109 isolates of *P. syringae* pv. *eriobotryae* collected from orchards throughout Japan into three groups, A, B, and C, based on canker brown pigmentation and pathogenicity to mesophyll. Group A strains produce no pigment and are not pathogenic to mesophyll. Group B strains produce no pigment and are pathogenic to mesophyll. Group C strains produce brown pigment and are not pathogenic to mesophyll. All three groups are widespread in commercial orchards in Japan.

Resistance to Group A is controlled by a single dominant gene, *Pse-a* (Hiehata et al., 2002). Resistance to Group C is controlled by a single recessive gene, *pse-c*, but there are few genetic resources showing such resistance (Hiehata et al., 2012). Hiehata et al. (2007) evaluated 52 loquat cultivars for resistance to Groups A, B, and C. Twenty-five cultivars were resistant and 22 were susceptible to Group A. Most of these showed the same responses to Group B. This result suggests that the resistance gene *Pse-a* controls resistance to Groups A and B. Only three cultivars showed resistance to Group C. These three cultivars may show durable resistance to all three strains. Since many commercially important cultivars are susceptible to the disease, it will be important to breed new cultivars with durable resistance. However, it is time- and labor-consuming to select seedlings carrying both resistance genes by traditional breeding techniques. Apple scab disease is caused by *Venturia inaequalis*. Apple breeders have tried for many years to improve scab resistance by introducing major resistance genes derived from wild apple, including *Vf*, *Vr*, *Vb*, *Va*, *Vm*, and *Vbj* (MacHardy, 1996). Through the use of molecular markers associated with these genes, multiple genes can be pyramided and introgressed into elite cultivars. Similarly, the use of molecular

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markers linked to *Pse-a* and *pse-c* will greatly accelerate the selection of resistant loquats for breeding new elite cultivars showing durable resistance to loquat canker disease. We previously developed several DNA markers linked to *Pse-a* from random amplified polymorphic DNA (RAPD) markers OPAY02/OPAY16 by bulked segregant analysis (BSA) (Fukuda et al., 2005). However, molecular markers associated with *pse-c* have not been reported.

Loquat belongs to the subtribe Pyrinae of the tribe Pyreae (Potter et al., 2007; Campbell et al., 2007) in the subfamily Spiraeoideae of the family Rosaceae, along with pears (*Pyrus* spp.) and apples (*Malus* spp.). Simple sequence repeat (SSR) markers from apple and pear have been used to evaluate the genetic diversity of loquat (Fukuda et al., 2013) and to construct genetic linkage maps (Gisbert et al., 2009). The results show that the positions and linkage groups (LGs) of SSR loci are conserved between loquat and apple. The use of genome sequences of 'Golden Delicious' apple (Velasco et al., 2010) facilitated the development of new molecular markers for specific genome regions of loquat. These markers allowed us to identify *Pse-a* of Taiwan loquat (*Eriobotrya deflexa*), which we mapped to the top of LG 10 (Fukuda et al., 2014). Comparison of the linkage map of loquat LG 10 with those of the European pear 'Bartlett' (Yamamoto et al., 2007) and the apple rootstock 'Sanashi 63′ (Moriya et al., 2011) revealed high co-linearity among LG 10 of loquat, pear, and apple.

In this study, we identified *pse-c* in loquat and mapped it to the top of LG 3 using newly developed SSR markers based on the apple genome sequence. We discussed the use of molecular markers linked to *pse-c* for marker-assisted selection of resistance to loquat canker disease in loquat breeding.

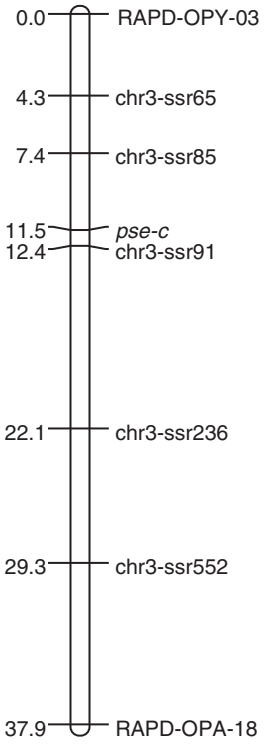

Fig. 1. Linkage group (LG) 3 of loquat 'Nagasakiwase', including the loquat canker resistance locus *pse-c*. Genetic distances (cM) are shown on the left, and locus names on the right.

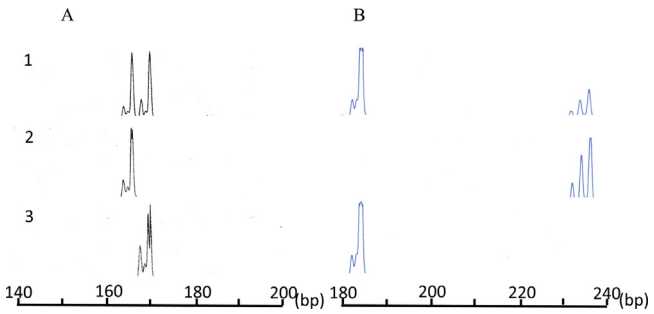

Fig. 2. Amplified fragment patterns of Chr3-ssr85(A) and Chr3-ssr91(B) from 3 loquat cultivars. Lanes 1–3 display amplified products the following cultivars. Lane 1: Mogi; 2: Tanaka; 3: Shiromogi.

2. Materials and methods

2.1. Plant materials and DNA isolation

Selfing of 'Nagasakiwase' loquat (*Pse-c/pse-c*) produced 141 seedlings that we used for genetic analysis for *pse-c*. To determine the allele compositions of molecular markers, we used eight cultivars ('Mogi', 'Nagasakiwase', 'Natsutayori', 'Obusa', 'Shiromogi', 'Suzukaze', 'Tanaka', and 'Yougyoku'; Table 1). To genotype parents, we evaluated 475 F1 seedlings derived from crosses between 'Nagasakiwase' as the female parent and 14 susceptible cultivars ('Arakiwase', 'Kawaharashiro', 'Kawaharataika', 'Moriyasutaika', 'Murotowase', 'Obama-Hayashida', 'Onowase', 'Saitama-Shimomura', 'Satomi', 'Shiroishi', 'Toi', 'Wasedai', 'Yougowase', and 'Yukawa') as the pollen parent. All plants were maintained at the Fruit Tree Research Division of the Agriculture and Forestry Technical Development Center of the Nagasaki Prefectural Government (Nagasaki, Japan). Genomic DNA was isolated from fresh leaves of all plant materials by using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). The DNA was quantified in a DyNA Quant 200 DNA Mini-Fluorometer (Hoefer, USA) and diluted to 10 ng/μL for SSR-PCR (polymerase chain reaction) analysis.

2.2. Evaluation of loquat canker resistance

Plants were inoculated with strain CG001 of *P. syringae* pv. *eriobotryae* (Group C) as previously described (Hiehata et al., 2012). Strain CG001 was isolated from cankers on loquat leaves at the Fruit Tree Research Division. The bacteria were cultured on PSA agar (decoction of 300 g of potato in 1 L of water, 0.5 g $Ca(NO_3)_2$, 2 g $Na_2HPO_4{\cdot}12H_2O$, 15 g sucrose, 5 g polypeptone, 15 g agar; pH 7.0) for 2 days at 25 °C. Bacteria were collected immediately before inoculation and resuspended in sterile distilled water (10^8 cfu/mL) containing 0.02% Tween 20. The suspension was needle-inoculated at six to nine sites per leaf along the midrib on the abaxial surface. The inoculated leaves were covered with polyethylene bags for 24 h to maintain high humidity. The plants were held in a greenhouse to avoid infection by other pathogens. Canker symptoms were evaluated 2 months after inoculation. Plants with black-brown cankers were identified as susceptible; those without were resistant. The inoculation test was conducted in 2006 and again in 2007.

2.3. RAPD analysis

Bulked segregant analysis was used in RAPD analysis (Michelmore et al., 1991). A total of 1080 RAPD primers (Eurofins) were tested. Two bulked DNA sets containing five susceptible or five resistant progeny of 'Nagasakiwase' were used for primer screening. Primers producing polymorphic fragments specific to