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Quantitative Inheritance of Resistance to Loquat Canker (*Pseudomonas syringae* pv. *eriobotryae*, Group C) in Loquat Progenies from Crosses between a Resistant Cultivar, 'Champagne', and Susceptible Cultivars

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***Abstract*. Loquat canker, caused by *Pseudomonas syringae* pv. *eriobotryae*, is a serious disease of loquat [*Eriobotrya japonica* (Thunb.) Lindl.] in some countries such as Japan. Therefore, improved canker resistance is an important objective for loquat breeding. The resistance to loquat canker Group C in descendants of 'Shiromogi' was expressed only in homozygotes with a recessive gene at a single locus, which was designated *pse-c/pse-c*. 'Champagne', which is distantly related to 'Shiromogi', is another cultivar with resistance to Group C. The inheritance of this resistance in progenies of crosses between 'Champagne' and susceptible cultivars was examined. The offspring seedlings from 14 crosses between 'Champagne' (*pse-c*/*pse-c*) and 12 susceptible cultivars (*Pse-c*/*Pse-c* or *Pse-c*/*pse-c*) were classified into two types of resistant and susceptible. All of the hybrid progenies between 'Champagne' (*pse-c*/*pse-c*) and *Pse-c*/*Pse-c* parents showed two types of resistant and susceptible. The proportion of resistant offspring showed great differences significantly, depending on the hybrid combinations. It ranged from 0.203 to 0.596 with an average of 0.407. It indicated that the resistance was controlled by one or more additional genes or loci other than the *Pse-c* (*pse-c*) locus. In addition, the proportion of resistant offspring from crosses between 'Champagne' (*pse-c*/*pse-c*) and *Pse-c*/*pse-c* parents ranged from 0.463 to 0.701 (and averaged 0.601), which seriously deviated from the segregation of 1:1, indicating that the segregation was both Mendelian and polygenic in a threshold character. The proportion of resistant seedlings cannot be predicted by the phenotype and the genotype in the *Pse-c* (*pse-c*) locus. Therefore, the general combining ability of 'Champagne' resulting from the additional gene effect was estimated, which was 0.407 and 0.101 for 'Champagne' × *Pse-c*/*Pse-c* and 'Champagne' × *Pse-c*/*pse-c* cultivars, respectively. The gene effect of susceptible cultivars ranged from –0.204 ('Yougyoku') to +0.189 ('Togoshi') for 'Champagne' × *Pse-c*/*Pse-c* cultivars and from –0.138 ('Taisho') to +0.089 ('Nagasakiwase') for 'Champagne' × *Pse-c*/*pse-c* cultivars.**

Loquat canker, caused by *Pseudomonas syringae* pv. *eriobotryae*, is a bacterial disease that has been reported for nearly a century in Japan (Ikata, 1927; Nakata, 1934). The disease has also been reported in China (Lin et al., 1999), the United States (Lai et al., 1971), Australia (Wimalajeewa et al., 1978), New Zealand (McRae and Hale, 1986), and Argentina (Alippi and Alippi, 1990). The disease attacks the buds, shoots, leaves, and fruit of the loquat tree (Morita, 1988; Mukoo, 1952), and it has a detrimental effect on vegetative growth and fruit production (Morita, 1991). It is currently the most serious disease of the loquat in Japan (Nesumi, 2006). The disease attacks not only the above-ground parts of the loquat, but also its below-ground parts, leading to seedling production decline in nurseries (Suga et al., 2007).

Because there were no completely resistant varieties grown commercially in Japan, bactericides were widely used in loquat orchards. However, controlling the disease in this way was difficult because of the high labor requirements and cost. Therefore, improvement of canker resistance of loquat in Japan is one of the most important goals in loquat breeding. In addition, requirements for disease-resistant cultivars continue to increase as a result of increasing public concerns of environmental responsibility. To support resistance breeding, a screening assay based on inoculation and marker-assisted selection for loquat canker resistance has been developed (Fukuda et al., 2005; Morita, 1988). This assay has been used to select resistant seedlings at the nursery stage in the loquat breeding program at the Agricultural and Forestry Technical Development Center, Nagasaki, Japan (Hiehata et al., 2002a).

The pathogen has been classified into three groups (A, B, and C) based on the presence of a brown pigment and pathogenicity in mesophyll tissue (Morita, 1978). Group A strains produce no pigment and are not pathogenic, Group B strains produce no pigment and are pathogenic, and Group C strains produce brown pigment and are not pathogenic. Progress has been made in breeding for resistance to Groups A and B because many resistant materials have been identified (Hiehata et al., 2002b, 2007; Morita, 1988) and the resistance to these two groups is based on a single dominant gene (Hiehata et al., 2002b; Morita et al., 1985). We have successfully developed three cultivars that are resistant to both groups: 'Reigetsu' (Terai et al., 2007), 'Ryoho' (Hiehata et al., 2008), and 'Natsutayori' (Hiehata et al., 2010). In contrast, there are not enough genetic resources that are resistant to Group C (Hiehata et al., 2007; Morita, 1988); 'Shiromogi' and 'Champagne' are resistant to Group C and have moderate or larger fruit size and good edible fruit quality. Therefore, they are important cultivars with high potential as cross-parents in breeding, which combine high fruit quality and large fruit size with resistance to loquat canker Group C.

Hiehata et al. (2012) elucidated the inheritance of the resistance to Group C derived from 'Shiromogi'. 'Shiromogi' originated as a seedling derived from an open-pollinated 'Mogi' seed irradiated with gamma rays (Ichinose et al., 1982). The resistance to Group C in 'Shiromogi' is currently the most valuable source of resistance to Group C among loquat cultivars, which is inherited with complete dominance at a single locus and expressed only in a recessive homozygote (*pse-c/pse-c*; Hiehata et al., 2012). The gene *pse-c* was probably derived from 'Mogi' (Hiehata et al., 2012), which is currently a major Japanese cultivar and it was a chance seedling found and selected in Japan.

Seedlings resistant to Group C with the *pse-c* gene have been produced by the breeding program at the Agricultural and Forestry Technical Development Center, Nagasaki, Japan. However, inbreeding depression might be a concern in breeding for Group C resistance because the number of resistant parent materials that contain *pse-c* (e.g., 'Mogi', 'Shiromogi') is limited, and there are close relationships among these materials (Fukuda et al., 2013). Although their descendants could be crossed or back-crossed with parents to produce a high proportion of homozygous-recessive seedlings (*pse-c*/*pse-c*) that are resistant to Group C, these crosses involve inbreeding and were therefore likely to exhibit inbreeding

depression with lower tree vigor or yield. Resistant materials which are less closely related to 'Mogi' and 'Shiromogi' should therefore be actively used as parents to improve the effectiveness of breeding for resistance to Group C.

'Champagne', which is of unknown parentage, is one of the few genetic resources that are resistant to Group C. It was selected and introduced to California ≈1908 (Morton, 1987) and was introduced to Japan in 1952. The particular usefulness of this loquat is its complete resistance to Groups A, B, and C (Morita, 1988). 'Champagne' is distantly related to 'Mogi', 'Shiromogi', and Japanese cultivars (Fukuda et al., 2013). Thus, it has high potential as a cross-parent for preventing inbreeding depression in breeding for loquat canker resistance, and especially resistance to Group C. The genotype of 'Champagne' has been estimated to be homozygous-recessive (*pse-c*/*pse-c*) for the locus (Hiehata et al., 2012), but the resistance of 'Champagne' may be controlled by additional genes at another locus different from the *pse-c* locus (Hiehata et al., 2003). The objective of the present study was to clarify the inheritance of the resistance to loquat canker Group C derived from 'Champagne' through crosses between 'Champagne' and susceptible genotypes.

Materials and Methods

Plant materials. 'Champagne' was crossed with 12 cultivars susceptible to loquat canker Group C (Table 1). The genotype of susceptible cultivars is *Pse-c/pse-c* or *Pse-c/Pse-c*, and eight of the 12 cultivars have been characterized at this locus (Table 1; Hiehata et al., 2012). The genotype of the other four cultivars was estimated as follows: 'Obusa' and 'Morimoto' were estimated as *Pse-c/Pse-c* because 'Obusa' was an offspring from 'Tanaka' (*Pse-c/Pse-c*) × 'Kusunoki' (*Pse-c/Pse-c*), and 'Morimoto' is a late-maturing bud sport from 'Tanaka' (*Pse-c/Pse-c*). 'Suzukaze' and 'Togoshi' were estimated as *Pse-c/Pse-c* because a cross of 'Suzukaze' with 87-222, which has the genotype *Pse-c/pse-c*, and selfing of 'Togoshi' both produced no resistant seedlings (N. Hiehata, S. Fukuda, and O. Terai, unpublished data).

The crosses were performed in 1996, 1999, and 2002 at the Fruit Tree Research Division, Agricultural and Forestry Technical Development Center, Nagasaki, Japan, by using standard techniques. Nine of the susceptible parents were used as the male parent and five were used as the female parent in crosses with 'Champagne', including two sets of reciprocal crosses (Tables 2 and 3).

Fruit from the crosses were harvested at full maturity. The seeds were extracted and sown in plastic flats filled with a mixture containing an equal volume of peatmoss and *kanuma-tsuchi* (Japanese pumice that is widely used for horticulture) after rinsing but without stratification. Seedlings at the second- or third-leaf stage were potted individually in plastic pots (0.6 L) containing the same medium. The following spring, the plants were transplanted into bigger plastic pots (5.7 L) containing the same medium. Compound fertilizer (18N–4.8P–9.1K) was added to the pots every month during seedling growth in the plastic pots. All seedlings were placed in a greenhouse from the time of sowing to inoculation and were watered as needed. Seven hundred thirty-six seedlings were produced from 14 crosses between 'Champagne' and the 12 susceptible genotypes.

Inoculum preparation and inoculation tests. We inoculated 2-year-old seedlings with *P. syringae* pv. *eriobotryae* (Group C). The inoculum source, methods, and evaluation for pathogenicity of loquat canker Group C were the same as in Hiehata et al. (2012). The number of resistant or susceptible seedlings in each progeny was determined using an inoculation test with strain CG001. The bacteria were cultured at 25 °C on potato sucrose agar medium [decoction of 300 g potato in 1 L of water, 0.5 g $Ca(NO_3)_2$, 2 g $NaHPO_4 \cdot 12\ H_2O$, 15 g sucrose, 5 g polypeptone, 15 g agar, pH 7.0] for 2 d before inoculation. Immediately before inoculation, the bacteria were collected and suspended in sterile distilled water to give a concentration of $\approx 10^8$ colony-forming units/mL, and 0.02% Tween 20 was added as a surfactant. The loquat seedlings were inoculated in the greenhouse to avoid infection by other pathogens. Two actively growing, half-expanded leaves were selected from each seedling. The bacterial suspension was needle-inoculated at six to nine sites per leaf at the midribs on the abaxial surface of the selected leaves. The inoculated leaves were covered with a polyethylene bag for 24 h to maintain high humidity. Canker incidence was evaluated ≈2 months after inoculation. Seedlings could be classified as either resistant or susceptible according to the absence or presence of black–brown cankers because the response to the inoculation of loquat canker is qualitative (Hiehata et al., 2002b, 2012). Small or unclear cankers that were difficult to classify in appearance were sliced off and evaluated based on the presence of lesions in the midrib tissue.

Statistical analysis. We used the χ^2 test to compare the observed and expected segregation ratios for each cross that produced both resistant and susceptible seedlings and to determine the inheritance of the loquat canker resistance. Because the proportion of resistant seedlings can be approximated as a binomial distribution, we calculated confidence limits for the obtained proportion of resistant seedlings (p_i for cross i) at $P = 0.95$ as $p_i \pm 1.96\sqrt{[p_i(1-p_i)/n_i]}$ (Snedecor and Cochran, 1967). Homogeneity of the proportion of resistant seedlings over the crosses was tested using the χ^2 test for each of the 'Champagne' × *Pse-c/Pse-c* and 'Champagne' × *Pse-c/pse-c* groups (Snedecor and Cochran, 1967).

Results and Discussion

Segregation for the resistance to loquat canker Group C in seedlings from 10 crosses between 'Champagne' (pse-c/pse-c) and Pse-c/Pse-c cultivars. All the seedlings from crosses between 'Champagne' (*pse-c/pse-c*) and *Pse-c/Pse-c* cultivars were expected to have no segregation; that is, all would have a susceptible *Pse-c/pse-c* genotype (Hiehata et al., 2012). However, the phenotype of the seedlings segregated into both resistant and susceptible offspring for all crosses between 'Champagne' and the *Pse-c/Pse-c* cultivars (Table 2).

The proportion of resistant seedlings varied widely among the crosses, ranging from 0.203 ('Yougyoku' as the male parent) to 0.596 ('Togoshi' as the male parent) and averaged 0.407 (Table 2). 'Fusahikari' was used in reciprocal crosses with 'Champagne', and the proportions of resistant seedlings were 0.256 and 0.414 when 'Fusahikari' was used as the male parent and the female parent, respectively. These proportions were not significantly different (χ^2 test).

The proportion of resistant seedlings differed significantly between different crosses (χ^2 test, $P < 0.001$; Table 2). We calculated the confidence interval for the proportion of resistant seedlings for each cross at $P = 0.95$ based on a binomial distribution (Table 2). We assumed a significant difference when the confidence interval did not overlap for a pair of crosses. The proportions of resistant seedlings in crosses with 'Yougyoku' and 'Fusahikari' as the male parents and 'Suzukaze' as the female parent were lower than 0.26 and were significantly lower than those of 'Kusunoki' and 'Togoshi' as male parents; both of them had proportions higher than 0.52. Furthermore, the proportion of resistant seedlings with 'Yougyoku' as the male parent was significantly lower than those of 'Fukuharawase' and 'Morimoto' as the male parents and 'Tanaka' as the female parent.

The unexpected production of resistant seedlings in all of these crosses clearly indicated that the inheritance of resistance to loquat canker Group C was controlled by one or more additional genes or loci other than the *Pse-c* (*pse-c*) locus. The large difference in the proportion of resistant seedlings from cross to cross suggested that the inheritance was controlled by unknown factors that were

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not evident phenotypically in the parents, possibly with additive and/or dominant effects. Genetic control by some oligogenes or polygenes indicates that the resistance to loquat canker Group C, which is a qualitative reaction based on the absence or presence of black–brown cankers (Hiehata et al., 2012), may exhibit threshold characteristics (Falconer, 1981). Thus, the proportion of resistant seedlings can only be estimated by observing the offspring of the crosses, and breeders must choose parents and crosses that yield a high proportion of resistant seedlings based on the combining ability of the parents in each cross.

The proportion of the resistant seedlings from 'Champagne' × 'Fusahikari' (0.256) did not differ significantly from that in the reciprocal cross, 'Fusahikari' × 'Champagne' (0.414). It suggested that the inheritance is not controlled cytoplasmically, but rather is controlled by nuclear genes.

*Segregation for the resistance to loquat canker Group C in seedlings from four crosses between 'Champagne' (*pse-c/pse-c*) and Pse-c/pse-c cultivars.* If we assume that the genetic control of Group C resistance is solely associated with the *Pse-c* (*pse-c*) locus, the seedlings from crosses between 'Champagne' (*pse-c/pse-c*) and susceptible heterozygous (*Pse-c/pse-c*) cultivars should segregate into resistant (*pse-c/pse-c*) and susceptible (*Pse-c/pse-c*) offspring with a 1:1 ratio (i.e., a proportion of 0.500 for the resistant seedlings). The actual proportion of resistant seedlings from these four crosses ranged from 0.463 (with 'Taisho' as the male parent) to 0.701 (with 'Mogi' as the pollen parent) and averaged 0.601 (Table 3). The observed segregations in two of the four crosses fit the expected 1:1 ratio of resistant to susceptible seedlings, but in 'Champagne' × 'Mogi' and 'Nagasakiwase' × 'Champagne', the proportions of resistant seedlings were significantly higher than 0.5, and the segregation therefore did not fit the expected ratio. In addition, the proportions of resistant seedlings differed significantly among the four crosses (χ^2 test,

Table 1. Parental cultivars used to produce progenies for determining the inheritance of the resistance to loquat canker Group C of 'Champagne' and their origin, evaluation, and genotype.

Cultivars	Origin	Evaluation	Genotype
Champagne	Selected and introduced to California ≈1908	Resistant	*pse-c/pse-c*[z]
Fukuharawase	'Mizuho' × a seedling of an unknown Chinese loquat	Susceptible	*Pse-c/Pse-c*[z]
Fusahikari	'Mizuho' × 'Tanaka'	Susceptible	*Pse-c/Pse-c*[z]
Kusunoki	Derived from a seedling of an unknown Chinese loquat	Susceptible	*Pse-c/Pse-c*[z]
Mogi	Derived from a seedling of an unknown Chinese loquat	Susceptible	*Pse-c/pse-c*[z]
Morimoto	Bud mutant of 'Tanaka'	Susceptible	*Pse-c/Pse-c*[y]
Nagasakiwase	'Mogi' × 'Hondawase'	Susceptible	*Pse-c/pse-c*[z]
Obusa	'Tanaka' × 'Kusunoki'	Susceptible	*Pse-c/Pse-c*[x]
Suzukaze	'Kusunoki' × 'Mogi'	Susceptible	*Pse-c/Pse-c*[w]
Taisho	Bud mutant of 'Mogi'	Susceptible	*Pse-c/pse-c*[z]
Tanaka	Derived from a seedling of an unknown Chinese loquat	Susceptible	*Pse-c/Pse-c*[z]
Togoshi	'Mogi' × 'Tanaka'	Susceptible	*Pse-c/Pse-c*[w]
Yougyoku	'Mogi' × 'Morimoto'	Susceptible	*Pse-c/Pse-c*[z]

[z]Hiehata et al. (2012).
[y]It is likely that 'Morimoto' is homozygous-dominant because it is a bud mutant of 'Tanaka', which is homozygous-dominant.
[x]The genotype of 'Obusa' seems to be homozygous-dominant because it is a F_1 of 'Tanaka' × 'Kusunoki', both of which are homozygous-dominant.
[w]It has been confirmed that genotypes of 'Suzukaze' and 'Togoshi' are homozygous-dominant by progeny tests (N. Hiehata, S. Fukuda, and O. Terai, unpublished data).

Table 2. Segregation of resistance to loquat canker Group C in the progenies between 'Champagne' (*pse-c*/*pse-c*) and susceptible cultivars with homozygous-dominant genotype (*Pse-c*/*Pse-c*) for the locus controlling the resistance of 'Shiromogi'.

Cross	*i*	No. of seedlings evaluated (n_i)	No. of seedlings		Proportion of resistant seedlings ($p_i = a_i/n_i$)	Confidence limit ($P = 0.95$)[z]	
			Resistant (a_i)	Susceptible		Lower	Upper
Champagne × Yougyoku	1	69	14	55	0.203 a[y]	0.108	0.298
Suzukaze × Champagne	2	29	7	22	0.241 ab	0.086	0.397
Champagne × Fusahikari	3	43	11	32	0.256 ab	0.125	0.386
Champagne × Obusa	4	51	19	32	0.373 abc	0.240	0.505
Fusahikari × Champagne	5	58	24	34	0.414 abc	0.287	0.541
Champagne × Fukuharawase	6	72	31	41	0.431 bc	0.316	0.545
Tanaka × Champagne	7	53	24	29	0.453 bc	0.319	0.587
Champagne × Morimoto	8	61	30	31	0.492 bc	0.366	0.617
Champagne × Kusunoki	9	70	37	33	0.529 c	0.412	0.646
Champagne × Togoshi	10	47	28	19	0.596 c	0.455	0.736
Total		553 (N)	225 (A)	328			
Average					0.407 (p)[x]		
χ^2 (9 df) = $(\Sigma a_i p_i - Ap)/[p(1-p)]$ = 33.21***							

[z]Confidence limit of proportion of resistant seedlings at $P = 0.95$ calculated as $p_i \pm 1.96\sqrt{[p_i(1-p_i)/n_i]}$.
[y]Rows with the same letter are not significantly different based on the confident limits.
[x]$p = A/N$.
***Significant at $P < 0.001$ by χ^2 test.

Table 3. Segregation of resistance to loquat canker Group C in progenies between 'Champagne' *(pse-c/pse-c)* and susceptible cultivars with heterozygous genotype *(Pse-clpse-c*) for the locus controlling the resistance of 'Shiromogi'.

Cross	*i*	No. of seedlings evaluated (n_i)	No. of seedlings		χ^{2z}	*P*	Proportion of resistant seedlings ($p_i = a_i/n_i$)	Confidence limit ($P = 0.95$)[y]	
			Resistant (a_i)	Susceptible				Lower	Upper
Champagne × Taisho	1	41	19	22	0.220	0.639	0.463	0.311	0.616
Mogi × Champagne	2	46	24	22	0.087	0.768	0.522	0.377	0.666
Nagasakiwase × Champagne	3	29	20	9	4.172	0.041	0.690	0.521	0.858
Champagne × Mogi	4	67	47	20	10.881	0.001	0.701	0.592	0.811
Total		183 (N)	110 (A)	73					
Average							0.601 (p)[x]		
χ^2 (3 df) = $(\Sigma a_i p_i - Ap)/[p(1-p)]$ = 8.21*									

[z]Data tested for goodness-of-fit to a 1:1 ratio.
[y]Confidence limit of proportion of resistant seedlings at $P = 0.95$ calculated as $p_i \pm 1.96\sqrt{[p_i(1-p_i)/n_i]}$.
[x]$p = A/N$.
*Significant at $P < 0.05$ by χ^2 test.

$P < 0.05$). These results also indicated that one or more additional genes or loci affect the resistance to Group C, as was the case in the crosses between 'Champagne' and the *Pse-c/Pse-c* cultivars.

We calculated the confidence interval for the proportion of resistant seedlings for each cross at $P = 0.95$ based on a binomial distribution (Table 3). The confidence intervals all overlapped, so we found no pair of crosses that differed significantly from the others in the proportion of resistant seedlings.

The proportion of resistant seedlings from 'Champagne' × 'Mogi' was 0.701, which was higher than that (0.522) in 'Mogi' × 'Champagne' (Table 3). However, the proportions in the reciprocal crosses did not differ significantly, suggesting that the inheritance was not controlled cytoplasmically, but rather was controlled by nuclear genes.

Estimation of the combining abilities in crosses between 'Champagne' and susceptible cultivars. The *Pse-c* (*pse-c*) locus, with a large genetic effect, controls the inheritance of resistance to loquat canker Group C qualitatively (Hiehata et al., 2012), and it appears based on the present results that one or more additional quantitative genes modified the phenotype in the offspring. In crosses between 'Champagne' (*pse-c/pse-c*) and the *Pse-c/Pse-c* cultivars, all of the seedlings have the genotype *Pse-c/pse-c*, meaning that any resistance would not result from inheritance controlled solely by the *Pse-c* (*pse-c*) locus. However, the results in Table 2 showed a high proportion of resistant seedlings (0.407 on average), which represents a large deviation from the value (0.000) that would be expected if inheritance was controlled solely by the *Pse-c* (*pse-c*) locus. The magnitude of the deviation varied significantly from cross to cross, ranging from 0.203 to 0.596.

In breeding, it is important for breeders to be able to predict the proportion of resistant seedlings from a cross so they can choose crosses that are expected to produce a high proportion of resistant seedlings. Based on the present results, this proportion can only be estimated by performing the crosses and observing segregation in the offspring from each cross, because the parental phenotypes did not differ and therefore provided no clues that would let breeders predict the proportion of resistance in the progeny. To provide a more quantitative estimate of the expected segregation, we calculated the general combining ability (*GCA*) in each cross from the actual results of the crosses as follows:

$$PRS_i = PRS_E + GCA_C + (GCA_i + SCA_i) \quad (1)$$

where PRS_i = the actual proportion of resistant seedlings in offspring for each cross between 'Champagne' and susceptible cultivar *i*; PRS_E = the expected proportion of resistant seedlings derived from the *Pse-c* (*pse-c*) locus genotype; GCA_C = the general combining ability of 'Champagne'; GCA_i = the general combining ability for susceptible cultivar *i*; and SCA_i = the specific combining ability for each cross between 'Champagne' and susceptible cultivar *i*.

GCA_i and SCA_i were assumed to result from additive and dominance gene effects, respectively.

Estimation of the combining abilities of crosses between 'Champagne' and the Pse-c/Pse-c cultivars. First, we obtained the observed proportion of resistant seedlings for each cross between 'Champagne' and susceptible cultivar *i* (PRS_i). Second, we assumed that PRS_E was zero for crosses between 'Champagne' and the *Pse-c*/*Pse-c* cultivars. Third, we estimated GCA_C as the value that remains after subtracting the expected proportion of resistant seedlings ($PRS_E = 0.000$) from the average proportion of resistant seedlings for all crosses between 'Champagne' and the *Pse-c*/*Pse-c* cultivars weighted by the number of seedlings in each cross; the weighted average for all cultivars combined was 0.407 (Table 4). We included the results of the reciprocal crosses between 'Champagne' and 'Fusahikari' in this calculation and estimated the weighted average, because they did not differ significantly. Fourth, we estimated the total combining ability ($GCA_i + SCA_i$) by rearranging Eq. [1] so that the value equaled ($PRS_i - PRS_E - GCA_C$). $GCA_i + SCA_i$ cannot be separated into GCA_i and SCA_i based on the available data. Fortunately, $GCA_i + SCA_i$ is sufficient to guide breeders to choose superior crosses.

GCA_C (0.407) differed greatly from PRS_E (0.000). However, ($GCA_i + SCA_i$) was not large, ranging from –0.204 to +0.189 (Table 4), indicating a minor modification of the proportion of resistant seedlings, although there were significant differences among the crosses (Table 2).

$GCA_i + SCA_i$ was high for 'Champagne' × 'Togoshi' and 'Champagne' × 'Kusunoki' at 0.189 and 0.122, respectively. In contrast, 'Champagne' × 'Yougyoku' and 'Suzukaze' × 'Champagne' had low $GCA_i + SCA_i$, at –0.204 and –0.166, respectively. $GCA_i + SCA_i$ did not differ greatly among the other crosses. We should note that the $GCA_i + SCA_i$ estimates contain an error component that results from the number of evaluated seedlings in each cross (sampling error).

Estimation of the combining abilities in crosses between 'Champagne' and the Pse-c/pse-c cultivars. We estimated PRS_E, GCA_C, and $GCA_i + SCA_i$ in the same way as in the crosses between 'Champagne' and the *Pse-c/Pse-c* cultivars. PRS_E was 0.500, and GCA_C was estimated at 0.101, which was much smaller than GCA_C in the crosses between 'Champagne' and the *Pse-c/Pse-c* cultivars (0.407). $GCA_i + SCA_i$ ranged from –0.138 to +0.089; the highest value was in 'Champagne' × 'Nagasakiwase' and the lowest was in 'Champagne' × 'Taisho'. However, the $GCA_i + SCA_i$ estimates were much lower than PRS_E (0.500), even for 'Nagasakiwase', which indicates a small effect of $GCA_i + SCA_i$ on the production of resistant seedlings. The $GCA_i + SCA_i$ estimates, therefore, appeared to provide little guidance for choosing crosses.

Possible involvement of multiple genetic factors that control the resistance to loquat canker Group C. In our previous research on the inheritance of resistance to loquat canker, it was found that the resistance to Group A was controlled by a single dominant gene, *Pse-a* (Hiehata et al., 2002b). Although the genotypes of the parents used in previous tests with Group B have not been identified, the resistance to Group B seemed to be a dominant trait that segregates with values near the expected values in classical Mendelian inheritance (Morita et al., 1985). In contrast, the resistance to Group C in progenies derived from 'Shiromogi' was conferred by a single recessive gene (*pse-c*) in a different manner from Group A resistance (Hiehata et al., 2012). In the present study, resistant seedlings were produced from crosses between 'Champagne' (*pse-c*/*pse-c*) and homozygous-dominant cultivars (*Pse-c*/*Pse-c*), although all of the progeny would be heterozygous and, thus, would be expected to be susceptible if resistance were determined by a single recessive allele. In addition, the proportion of resistant seedlings varied widely among the crosses, and the differences were statistically significant.

The resistance in the progenies of crosses between 'Champagne' and susceptible cultivars clearly indicates the existence of quantitative trait loci, like in the case of resistance

Table 4. $GCA_i + SCA_i$ estimates of susceptible cultivars with *Pse-c*/*Pse-c* and *Pse-c*/*pse-c* for the locus controlling the resistance of 'Shiromogi' to loquat canker Group C in crossing with 'Champagne'.

Cultivar	No. of seedlings		Proportion of resistant seedlings (PRS_i)	PRS_E	GCA_C	$GCA_i + SCA_i$
	Evaluated	Resistant				
Pse-c/*Pse-c* cultivar						
Fukuharawase	72	31	0.431	0.000	0.407	0.024
Fusahikari	101	35	0.347	0.000	0.407	–0.060
Kusunoki	70	37	0.529	0.000	0.407	0.122
Morimoto	61	30	0.492	0.000	0.407	0.085
Obusa	51	19	0.373	0.000	0.407	–0.034
Suzukaze	29	7	0.241	0.000	0.407	–0.166
Tanaka	53	24	0.453	0.000	0.407	0.046
Togoshi	47	28	0.596	0.000	0.407	0.189
Yougyoku	69	14	0.203	0.000	0.407	–0.204
Weighted average			0.407			
Pse-c/pse-c cultivar						
Mogi	113	71	0.628	0.500	0.101	0.027
Nagasakiwase	29	20	0.690	0.500	0.101	0.089
Taisho	41	19	0.463	0.500	0.101	–0.138
Weighted average			0.601			

to downy mildew (*Plasmopara viticola*) in grapevine (*Vitis* spp.; Brown et al., 1999), necrotic scab (*Venturia nashicola*) in japanese pear [*Pyrus pyrifolia* (Burm. f.) Nakai] and chinese pear (*Pyrus bretschneideri* Rehder; Abe and Kotobuki, 1998), and scab (*Venturia inaequalis*) in apple (*Malus pumila* Mill.; Williams and Kuc, 1969). In our previous study, the observed segregation in most crosses between 'Shiromogi' (*pse-c*/*pse-c*) and the *Pse-c*/*pse-c* cultivars fit the expected ratio (one resistant:one susceptible), but we observed significant segregation distortion in two crosses (Hiehata et al., 2012). This bad fit may be explained by the existence of one or more quantitative trait loci in addition to the *Pse-c* (*pse-c*) locus.

Therefore, we conclude the resistance to loquat canker Group C is polygenic or controlled by one or more oligogenes in addition to *pse-c*. The inheritance of one or more recessive genes and of one or more polygenes has been reported in the resistance to fusarium wilt (*Fusarium oxysporum* f. sp. *melonis*) in melon (*Cucumis melo* L.; Nakazumi and Hirai, 2004), which is similar to the results obtained in the present study. There were some reports that resistance to scab in apple and pear is controlled by both a dominant major gene and polygenes (Abe et al., 2000; Lamb et al., 1985).

There were many reports about the inheritance of resistance to pathovars of *Pseudomonas syringae* in several crops besides loquat. Resistance controlled by major genes has been described in tomato (*Solanum lycopersicum* L.; Martin et al., 1993) to *P. syringae* pv. *tomato*, in soybean [*Glycine max* (L.) Merrill; Keen and Buzzell, 1991] to *P. syringae* pv. *glycinea*, in maize (*Zea mays* L.; Xu et al., 2009) to *P. syringae* pv. *syringae* Van Holl, and in cucumber (*Cucumis sativus* L.; Olczak-Woltman et al., 2009) to *P. syringae* pv. *lachrymans*. In contrast, resistance of bean (*Phaseolus vulgaris* L.; Yaish et al., 2006) to *P. syringae* pv. *phaseolicola* and of pea (*Pisum sativum* L.; Fondevilla et al., 2012) to *P. syringae* pv. *syringae* is under polygenic control. On the other hand, Taylor et al. (1996) reported that race-specific resistance to *P. syringae* pv. *phaseolicola* in bean was controlled by major genes, whereas non-race-specific resistance exhibited quantitative inheritance. Considering that 'Champagne' exhibits resistance to all three groups of loquat canker, it might have non-race-specific resistance.

Implications for breeding for resistance to loquat canker. Because the resistance to loquat canker Group C from 'Shiromogi' is a recessive trait, it was more difficult to breed cultivars resistant to Group C than that of Groups A and B, for which the resistance is dominant (Hiehata et al., 2002b; Morita et al., 1985). In the present study, we found that 'Champagne' had a high *GCA* and consequently produced resistant plants in all crosses with homozygous-dominant cultivars, even for cultivars that did not produce resistant seedlings in crosses with 'Shiromogi' (Hiehata et al., 2012). However, the proportion of resistant seedlings varied among the crosses, as was the case for scab in apple (Kellerhals et al., 1993). This information will facilitate the breeding of resistant cultivars because 'Champagne' could produce resistant seedlings through a single cross, even crossed with cultivars that did not produce resistant seedlings in crosses with 'Shiromogi'.

The quality of fruit produced by 'Champagne' is not as good as that of accessions that possess *pse-c* derived from 'Mogi' (e.g., 'Shiromogi', 'Nagasakiwase'). However, the fruit maturity stage of 'Champagne' is earlier than that of these cultivars derived from 'Mogi' (Nagato et al., 1996). Because 'Champagne' is homozygous-dominant for *Pse-a* (Hiehata et al., 2002b), it is a valuable breeding material to produce cultivars with precocious character. Also, 'Champagne' is less closely related genetically to the mentioned materials that possess *pse-c* derived from 'Mogi' (Fukuda et al., 2013), it may help breeders to avoid the inbreeding depression that has been reported in fruit bushes and trees such as rabbiteye blueberry (*Vaccinium ashei* Reede; Lyrene, 1983), persimmon (*Diospyros kaki* Thunb.; Yamada, 1993), and japanese pear (Sato et al., 2008). In addition, introgression of the resistance from 'Champagne' into existing cultivars would decrease the possibility of breakdown of the Group C resistance conferred by a single major gene (*pse-c*), which has been reported for some diseases (Kiyosawa, 1982; Parisi et al., 1993). 'Champagne' is therefore a valuable breeding resource for canker resistance.

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