

## Research Paper

# Inheritance of susceptibility to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach offspring populations derived from Brazilian and Japanese cultivars/selections

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Bacterial spot (caused by *Xanthomonas arboricola* pv. *pruni*) is a serious disease and difficult to control in peach cultivation, and inheritance manner of susceptibility is unclear. Five hundred and fourteen offspring and their parents from 27 peach full-sib families were evaluated for susceptibility to bacterial spot by evaluating lesion length value (LLV) after artificial inoculation to shoots from trees growing in the field. Brazilian cultivars including ‘Chimarrita’ and selections derived from them had notably lower LLVs ranging from 0.302 to 0.490 than those from Japanese cultivars/selections ranging from 0.514 to 1.295. Family means in offspring crossed between Brazilian cultivars/selections (low LLVs) and other cultivars/selections (high LLVs) showed rather low LLVs, whose values were close to Brazilian cultivar/selection parents. These results suggested that the susceptibility was controlled by single major gene and that the Brazilian and Japanese cultivar/selection parents showed dominant and recessive homozygotes, respectively. In contrast, the LLVs of family means were very high ranging from 0.719 to 1.194 in offspring population derived from crosses among Japanese cultivars. Repeated backcrosses of Brazilian cultivars/selections with Japanese cultivars/selections having high fruit quality are proposed as an effective method for developing new cultivars combining bacterial spot resistance and fruit quality in Japan.

**Key Words:** disease resistance, environmental variance, genetic resources, heritability, *Prunus persica*.

## Introduction

Bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* is one of the most important and serious diseases for commercial cultivation in peach (*Prunus persica* (L.) Batsch) in Japan, especially in windy areas with heavy rainfall. The microbe also attacks other stone-fruit crops: Japanese plum (*Prunus salicina* Lindl.), apricot (*Prunus armeniaca* L.) and other *Prunus* spp. (Du Plessis 1988, Kuwatsuka 1921, Werner *et al.* 1986). The disease causes spots on the leaves, twigs, and fruit, resulting in severe defoliation. The presence of spots on fruits seriously reduces their marketability.

Since it is difficult to control this bacterium completely by chemical applications, the use of resistant cultivars would be the most effective way to control this disease; however, immune cultivars are not known.

Varietal differences in susceptibility to bacterial spot for peach, Japanese plum, apricot and other *Prunus* spp. were reported in countries other than Japan by Du Plessis (1988), Keil and Fogle (1974), Martins and Raseira (1996), Medeiros *et al.* (2011), Randhawa and Civerolo (1985), Sherman and Lyrene (1981), and Werner *et al.* (1986). Several different evaluation methods were used in these studies: orchard susceptibility observations (Keil and Fogle 1974, Medeiros *et al.* 2011, Sherman and Lyrene 1981, Werner *et al.* 1986), detached-leaf bioassays (Medeiros *et al.* 2011, Randhawa and Civerolo 1985) and greenhouse inoculation (Du Plessis 1988, Martins and Raseira 1996, Medeiros *et al.* 2011).

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In North Carolina in the USA, breeding for resistance to peach bacterial spot had been carried out with the cooperation of phytopathologists and breeders, and some commercially resistant cultivars have been identified: ‘Whynot’, ‘Candor’, ‘Rubired’, ‘Pekin’, ‘Troy’, ‘Norman’, ‘Winblo’, ‘Biscoe’ and ‘Emery’ (Clayton 1976). The most resistant cultivars from the breeding program were ‘Candor’ and ‘Clayton’ (Okie *et al.* 2008); however, these cultivars have not been introduced to Japan.

The cultivars and genetic resources used in these earlier reports of varietal differences in susceptibility did not include Japanese peach cultivars. The susceptibility in cultivars including economically important peach cultivars in Japan has been observed in several orchards (Kuraoka and Kato 1955, Shiina *et al.* 1966, Takanashi 1978, Yamamoto *et al.* 1953). They suggested varietal differences in susceptibility to bacterial spot, however, those reports had no statistical analyses with experimental designs. The occurrence of bacterial spot fluctuates highly in different environmental conditions including rainfall, wind, temperature and bacterial density of the year prior to the experimental year.

Suesada *et al.* (2013) developed a new artificial shoot inoculation method and elucidated the genetic differences for peach genetic resources in Japan in susceptibility to bacterial spot with appropriate statistical analyses. They selected relatively tolerant cultivars to bacterial spot: ‘Chimarrita’ (a Brazilian cultivar), ‘Nishiki’ (Kajiura *et al.* 1966) and ‘Mochizuki’ (Yamaguchi *et al.* 2001) for canning use, and ‘Tsukikagami’ (Yaegaki *et al.* 2016), a table peach cultivar in Japan. They elucidated the magnitude of environmental variability in the observed values using artificial inoculation and averaged value with repetitions of three shoots repeated over two years had a considerably reduced environmental variance and resulted in broad-sense heritability of 0.71 for the 69 cultivars/selections.

To develop new, resistant cultivars with high fruit quality and productivity, the inheritance of resistance must be elucidated. Sherman and Lyrene (1981) evaluated the susceptibility to bacterial spot in their low-chilling breeding germplasm in Florida and hypothesized that resistance was controlled by a few genes. In addition, Yang *et al.* (2013) investigated the inheritance of resistance using ‘Clayton’, suggesting that resistance to bacterial spot was controlled by quantitative trait loci (QTLs).

Peach breeding with the goal of combining excellent fruit quality for Japanese market with resistance to bacterial spot started recently at the Institute of Fruit Tree and Tea Science, NARO (Tsukuba, Ibaraki, Japan), using peach genetic resources conserved in Japan. The objective of this study was to identify the inheritance of bacterial spot resistance in a seedling population from NARO peach breeding programs so far using the artificial inoculation method, and to propose an effective way to efficiently accelerate the peach resistant breeding to bacterial spot.

## Materials and Methods

### Plant materials

Three to five year-old peach seedling trees of 514 offspring of 27 full-sib families and their cross-parent (4 to 14 year-old) trees of 28 cultivars/selections (**Table 1**) with no tree replications were used in this study. Cultural practices were carried out in the same way for parental cultivars/selections as for the seedling population.

Here, the experiment had no tree replications within each genotype. Generally, tree effects may often be caused by differences in tree vigor; however, here, several 30–40 cm long current-year shoots with basal diameters of about 5 mm were chosen and inoculated artificially (Suesada *et al.* 2013). Thus, sampling was not based on individual trees but multiple shoots with uniform vigor. In addition, the trees used in the experiment were pruned, the flower buds and fruit were thinned, fertilizer was applied, and the trees were irrigated under conditions that kept the trees uniform. Therefore, we assumed a minimum of tree effects within genotype, which were included in the genetic effect, and regarded the tree effects as negligible.

They were grown at the Institute of Fruit Tree and Tea Science, NARO. Their susceptibility to peach bacterial spot was evaluated by the artificial shoot inoculation method (Suesada *et al.* 2013) during 2006–2008. Offspring and cross-parents were evaluated in a single year or repeatedly for two years during 2006–2008, respectively. Suesada *et al.* (2013) reported negligible and non-significant year effects during 2006–2008; therefore, we combined the data from all years.

Depending on the breeding objectives, crosses were divided into “Brazilian crosses” and “Japanese crosses”. Brazilian crosses included the combinations of a Brazilian cultivar ‘Chimarrita’ crossed as a parent, and the combinations that selections (296-16, 332-16, and 333-13) were crossed as parents, derived from Brazilian cultivars ‘Chimarrita’ and ‘Coral’ (**Fig. 1**). Japanese crosses included crosses among Japanese cultivars and selections, some of which were partly derived from American cultivars. The former crosses were made to develop cultivars with a low-chilling requirement and excellent quality for table use in Japan. The latter crosses were aimed at developing new commercial table peach cultivars with a high sugar content, low acidity, large fruit size and attractive appearance at various maturing times. Both Brazilian and Japanese crosses had no specific mating design. Some cultivars were repeatedly used as cross-parents. The number of offspring from a cross varied from 5 to 65 per family. In the present study, we use the term “family” as the full-sib offspring population resulting from a cross.

### Evaluation of peach bacterial spot

Evaluation of expansion resistance to bacterial spot was carried out in the same manner as Suesada *et al.* (2013). *Xanthomonas arboricola* pv. *pruni* (MAFF301420), supplied by the Genetic Resources Center, NARO (Tsukuba,



cultivar/selection ( $i = 1$  to 28,  $j = 1$  to 2). The ANOVA provided estimates of variance components for genetic variance ( $\sigma_{g_l}^2$ ) and environmental variance ( $\sigma_{e_l}^2$ ).

### (2) Evaluation of offspring for estimating between-family and within-family variance in Japanese crosses

Five offspring per full-sib family were randomly chosen from offspring in 21 crosses among Japanese cultivars/selections, and the LLV data of those offspring were subjected to ANOVA in a one-way classification with family as the factor. The model was:

$$P_{ij} = \mu + B_i + W_{ij}$$

$P_{ij}$ : LLV of the  $j$ th offspring in the  $i$ th family (cross),  $\mu$ : overall mean,  $B_i$ : the effect of the  $i$ th family,  $W_{ij}$ : the variance of the  $j$ th offspring of the  $i$ th family ( $i = 1$  to 21,  $j = 1$  to 5).

The homogeneity of within-family variances in LLVs was tested by Bartlett's test (Snedecor and Cochran 1972), and the normal distribution of residual estimates was tested using Kolmogorov-Smirnov one-sample test (Campbell 1974). The homogeneity of the variances was not rejected at  $P = 0.05$ , and the residual distribution approached a normal distribution at  $P = 0.05$ , indicating that ANOVA was applicable to the data.

The ANOVA provided estimates of variance components as follows: between-family variance ( $\sigma_b^2$ ) and within-family variance ( $\sigma_w^2$ ). The  $\sigma_{e_l}^2$  obtained for parental cultivars/selections (1) was used as the within-family environmental variance ( $\sigma_{we}^2$ ), and the within-family genetic variance ( $\sigma_{wg}^2$ ) was calculated by  $\sigma_w^2 - \sigma_{we}^2$ .

### (3) Regression of family mean on mid-parental values and ANOVA for offspring in Japanese crosses

According to methods described by Yamada (2011), Yamada *et al.* (1995, 1997), and Sato *et al.* (2006), ANOVA and estimation of variance components was performed, and regression analysis was performed for family mean (the mean LLV for five offspring in a family) on the mid-parental value, which was the mean LLV for seed and pollen parents for offspring from the 21 crosses among Japanese cultivars/selections. The genetic model was as follows:

$$Y_{ij} = \mu + \beta (X_i - \bar{X}) + d_i + W_{ij}$$

where  $Y_{ij}$ : phenotypic value of the  $j$ th offspring in the  $i$ th family

$\mu$ : overall mean (constant)

$\beta$ : the regression coefficient of family mean on mid-parental value

$X_i$ : mid-parental value in the  $i$ th family

$\bar{X}$ : the mean of all the mid-parental values

$d_i$ : the deviation of the  $i$ th family mean from the regression line

$W_{ij}$ : the within-family effect of offspring in the  $j$ th offspring of the  $i$ th family

The  $W_{ij}$  was divided into  $wg_{ij}$  and  $we_{ij}$ , the genetic and environmental effect of the  $j$ th offspring of the  $i$ th family, respectively.

## Results

### Estimation of environmental variance using parental cultivars/selections

The susceptibility to bacterial spot was evaluated for 28 cross-parents, including four cultivars/selections derived from Brazilian cultivars/selections and 24 cultivars/selections derived from Japanese cultivars/selections (partly from American cultivars). Although bacterial spot lesions showed black necrotic regions for all tested cross-parents (**Supplemental Fig. 1**), necrotic lesion lengths showed differences among cross-parents and were larger than control treatments. The average lesion length for each shoot ( $X$ ; unit is millimeter) was reduced by 5.5 mm and log-transformed to improve normality.

LLVs for 28 cross-parents evaluated repeatedly for two years were subjected to ANOVA in a one-way classification with genotype (cultivar/selection) as the factor. The genetic effect was highly significant (**Table 2**). The genetic ( $\sigma_{g_l}^2$ ) and environmental variances ( $\sigma_{e_l}^2$ ) were estimated at 0.04175 and 0.04395, respectively.

### Susceptibility to bacterial spot for cross-parents

We summarized the LLVs for seed and pollen parents and the family-means, representing the mean value of offspring from a cross, in **Table 3**. The average LLVs for a total of 28 cultivars/selections used as cross-parents for two years in 27 crosses varied from 0.302 (333-13) to 1.295 (346-23) (**Table 3**). For Brazilian crosses, three cultivars/selections of a Brazilian cultivar 'Chimarrita', and selections 333-13 and 296-16 derived from Brazilian cultivars ('Chimarrita' and 'Coral'), had the LLV less than 0.5. Here, we referred to cultivars of Brazilian origin and selections partly derived from them as "Brazilian cultivars/selections". A Brazilian selection, 332-16, with a high LLV (0.981) was crossed with Brazilian selections having low LLVs (296-16 and 333-13).

In contrast, all cross-parents in the Japanese crosses had LLVs of 0.5 or more. 'Mochizuki' and 'Tsukikagami' had relatively low LLVs among Japanese cross-parents, and their LLVs were 0.514 and 0.667, respectively.

### Susceptibility to bacterial spot for Brazilian crosses

The family means of LLVs in the six Brazilian crosses (cross nos. 381, 384, 402, 403, 404 and 405) were generally

**Table 2.** Analysis of variance in a one-way classification with genotype (cultivar/selection) as the factor for LLV data of 28 parental cultivars/selections with two-year repetitions

Source of variation	Sum of squares	Degree of freedom	Mean squares	F-value	Expected mean squares
Genotype	3.44112	27	0.12745	2.900**	$\sigma_{e_l}^2 + 2\sigma_{g_l}^2$
Residual	1.23056	28	0.04395		$\sigma_{e_l}^2$
Total	4.67167	55			

\*\* indicates significant at  $P < 0.01$ .

**Table 3.** Lesion length values (LLV) of cross-parents, mid-parent, and family mean in progenies

Cross number	Seed parent	Pollen parent	Number of evaluated offspring	Lesion length value (LLV) <sup>a</sup>				Mean separation between seed parent and family mean <sup>d</sup>	Mean separation between pollen parent and family mean <sup>d</sup>
				Seed parent <sup>b</sup>	Pollen parent <sup>b</sup>	Mid-parent	Family mean $\pm$ SE <sup>c</sup>		
Crosses having one or both parents derived from Brazilian cultivars									
381	296-16	Chimarrita	6	0.490	0.476	0.483	0.561 $\pm$ 0.062	NS	NS
384	333-13	332-16	9	0.302	0.981	0.642	0.434 $\pm$ 0.100	NS	**
402	296-16	332-16	13	0.490	0.981	0.736	0.576 $\pm$ 0.079	NS	*
403	Kawanakajima Hakutou	Chimarrita	30	1.177	0.476	0.827	0.402 $\pm$ 0.069	**	NS
404	346-23	Chimarrita	9	1.295	0.476	0.886	0.474 $\pm$ 0.044	**	NS
405	296-16	Tsukiakari	11	0.490	1.090	0.790	0.446 $\pm$ 0.075	NS	**
Crosses having both parents derived from Japanese cultivars									
354	Shimizu Hakutou	Momo Tsukuba 119	23	1.052	1.172	1.112	0.817 $\pm$ 0.067		
371	Benikunimi	Himekonatsu	9	1.048	0.955	1.002	0.922 $\pm$ 0.109		
374	Natsuotome	Momo Tsukuba 122	18	1.171	1.119	1.145	0.984 $\pm$ 0.069		
375	Yuzora	Momo Tsukuba 122	5	1.027	1.119	1.073	1.144 $\pm$ 0.169		
386	Hakuhou	Momo Tsukuba 124	7	0.810	0.786	0.798	1.045 $\pm$ 0.090		
387	Akatsuki	Momo Tsukuba 124	7	0.884	0.786	0.835	0.786 $\pm$ 0.066		
390	Akizora	Momo Tsukuba 124	17	1.142	0.786	0.964	0.824 $\pm$ 0.049		
396	Mochizuki	Hakushu	19	0.514	1.172	0.843	0.746 $\pm$ 0.060		
397	Mochizuki	Tsukiakari	29	0.514	1.090	0.802	0.779 $\pm$ 0.054		
398	Masahime	348-35	7	0.827	0.882	0.855	1.050 $\pm$ 0.104		
406	Kawanakajima Hakutou	Yuzora	27	1.177	1.027	1.102	0.742 $\pm$ 0.055		
407	Yuzora	Tsukikagami	65	1.027	0.667	0.847	0.719 $\pm$ 0.033		
410	Natsuotome	Yuzora	23	1.171	1.027	1.099	1.048 $\pm$ 0.034		
411	317-25	Yuzora	15	0.579	1.027	0.803	0.903 $\pm$ 0.038		
413	Yuzora	319-25	22	1.027	1.143	1.085	1.011 $\pm$ 0.052		
414	346-23	Tsukiakari	37	1.295	1.090	1.193	1.194 $\pm$ 0.030		
415	348-35	Tsukiakari	32	0.882	1.090	0.986	0.867 $\pm$ 0.047		
416	Yoshihime	Momo Tsukuba 124	43	1.236	0.786	1.011	0.888 $\pm$ 0.046		
418	Masahime	338-15	17	0.827	0.896	0.862	0.811 $\pm$ 0.049		
419	Natsuotome	338-15	8	1.171	0.896	1.034	0.794 $\pm$ 0.086		
N-128	316-2	Tsukikagami	6	0.973	0.667	0.820	0.830 $\pm$ 0.068		

<sup>a</sup> Lesion length value is the average value of log-transformed data from three shoots.

<sup>b</sup> LLV in seed and pollen parent had the environmental variance ( $\sigma_{eI}^2$ ).

<sup>c</sup> SE was calculated as the square root of each within-family variance divided by number of evaluated offspring in the family.

<sup>d</sup> Behrens-Fisher test with approximate significance level by Cochran (1964) according to Snedecor and Cochran (1972). NS, \*, \*\* indicate non-significant, significant at  $P < 0.05$ , or 0.01, respectively.

low, ranging from 0.402 to 0.576, as compared with the family means of LLVs in Japanese crosses that ranged from 0.719 to 1.194. The family means in six Brazilian crosses were nearly the same as or close to both or one of the Brazilian cultivar/selection parents having low LLVs irrespective of which Brazilian cultivar/selection was the seed or pollen parent.

In the “381” family, which resulted from the cross between both parents having low LLVs (296-16  $\times$  ‘Chimarrita’), the difference between the family mean (0.561) and the seed or pollen parental values (0.490 or 0.476) seemed to be within the expected range for environmental variation.

The other five crosses were between Brazilian cultivars/selections having low LLVs (less than 0.5) and cultivars/selections with high LLVs (0.981 to 1.295). Family means in four crosses of 384, 403, 404 and 405 were less than 0.5. The family mean in the cross 296-16  $\times$  332-16 was 0.576, whose value is much closer to the low LLV of the Brazilian parent 296-16.

The homoscedasticity between the environmental variance ( $\sigma_{eI}^2$ ) in seed or pollen parental LLVs and the within-

family variance was tested by an F-test. Those variances were not significantly different in four crosses including 296-16  $\times$  ‘Chimarrita’, but were significantly different at  $P = 0.05$  for one cross (346-23  $\times$  ‘Chimarrita’) and at  $P = 0.01$  for one cross (‘Kawanakajima Hakutou’  $\times$  ‘Chimarrita’), respectively. Therefore, the difference between the seed or pollen parental values and the family mean was tested using the Behrens-Fisher test with an approximate significance level by Cochran (1964) according to Snedecor and Cochran (1972). As a result, family means were separated from all seed or pollen parental values of the higher LLV cultivars/selections (Table 3).

### Susceptibility to bacterial spot for Japanese crosses

The family means of LLVs in the 21 Japanese crosses ranged from 0.719 to 1.194, whose values were much higher than those in the Brazilian crosses (0.402 to 0.576) (Table 3). ANOVA for five offspring per family in 21 families from Japanese crosses detected a significant effect due to family ( $P < 0.05$ ) (Table 4). The between-family ( $\sigma_b^2$ ) and within-family ( $\sigma_w^2$ ) variances were estimated at 10.992

**Table 4.** Analysis of variance for LLV in a one-way classification with family as the factor of the family and regression of family-mean on mid-parent for 21 families each with five offspring from crosses among Japanese cultivars/selections

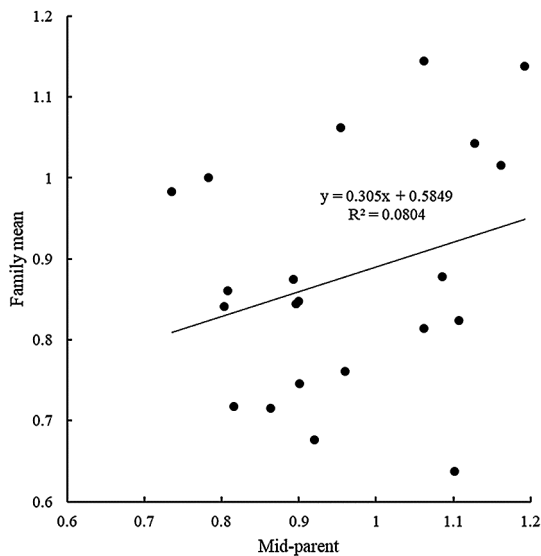
Source of variation	Sum of squares	Degree of freedom	Mean squares	Expected mean squares
Between-family	2.185	20	0.1092 *	$\sigma_w^2 + 5\sigma_b^2$
Regression <sup>a</sup>	0.176	1	0.1757 NS	$\sigma_w^2 + 5\sigma_d^2 + (5 \times 20)\sigma_r^2$
Residual	2.009	19	0.1057 *	$\sigma_w^2 + 5\sigma_d^2$
Within-family	4.560	84	0.0543	$\sigma_w^2$
Total		104	0.0586	

<sup>a</sup> Regression of family mean on mid-parent value.

NS,\* indicate non-significant or significant at  $P < 0.05$ , respectively.

**Table 5.** Estimates of variance components for LLV in 21 families each with five offspring from crosses among Japanese cultivars/selections

Variance components		Estimated value ( $10^{-3}$ )	Percentage of variance components (%)
Between-family	$\sigma_b^2$	10.992	16.8
Regression	$\sigma_r^2$	0.699	1.1
Residual	$\sigma_d^2$	10.293	15.8
Within-family	$\sigma_w^2$	54.280	83.2
Genetic	$\sigma_{wg}^2$	10.332	15.8
Environmental	$\sigma_{we}^2$	43.948	67.3
Total		65.273	100.0



**Fig. 2.** Regression of the family mean of LLV on mid-parental values for offspring in Japanese crosses.

$\times 10^{-3}$  and  $54.280 \times 10^{-3}$ , respectively, of which 16.8% and 83.2% of the total variance was in the entire ANOVA offspring population (Table 5). As within-family environmental variance was assumed to be  $\sigma_{el}^2$  among parental cultivars/selections, the within-family variance was divided into within-family genetic variance ( $\sigma_{wg}^2$ :  $10.332 \times 10^{-3}$ ) and within-family environmental variance ( $\sigma_{we}^2$ :  $43.948 \times 10^{-3}$ ).

Regression of family mean to mid-parental values was

not significant at  $P = 0.05$  (Fig. 2, Table 4). The between-family variance was divided into the variance explained by the regression ( $\sigma_r^2$ :  $0.699 \times 10^{-3}$ ; 6% of the between-family variance) and the residual variance from the regression ( $\sigma_d^2$ :  $10.293 \times 10^{-3}$ ; 94% of the between-family variance). The variance in the mid-parental value was estimated at  $18.889 \times 10^{-3}$ , and the environmental variance of the mid-parental value was estimated at  $\sigma_{el}^2/2$ :  $21.974 \times 10^{-3}$ . Therefore, the genetic variance of the mid-parental value was negligible in the population. This result could be a probable reason for the negligible variance explained by the regression. The regression is associated with additive gene effects (Yamada 2011), which cannot be estimated from the regression in the present study.

The total genetic variance was estimated as  $\sigma_b^2 + \sigma_{wg}^2$  ( $21.324 \times 10^{-3}$ ), which represents only approximately one-half of  $\sigma_{el}^2$  (Table 5). The  $\sigma_b^2$  and  $\sigma_{wg}^2$  accounted for 52% and 48% of the total genetic variance, respectively, indicating that around one-half of the total genetic variation in the offspring population was due to between-family and within-family genetic variation, respectively. The large environmental variation indicated by  $\sigma_{we}^2$  masked the genetic variation. The broad-sense heritability in a family for LLVs for an offspring defined as  $\sigma_{wg}^2 / (\sigma_{wg}^2 + \sigma_{we}^2)$  was estimated at only 0.19.

## Discussion

Since bacterial spot disease is difficult to control under windy and humid climate conditions, resistant cultivars are desired in peach. Although varietal differences in susceptibility were partly reported, the mode of inheritance remains unclear and the breeding program for resistance to bacterial spot has been rarely carried out. In North Carolina, resistant breeding to bacterial spot has been preliminarily carried out over decades, resulting that several resistant cultivars were released (Clayton 1976, Okie *et al.* 2008). However, the well-organized breeding process was not established. In Brazil, peach cultivar/selections Conserva 930, Cascata 1020, A334 and ‘Cristal Taquari’ showed some degree of resistance and were used for breeding programs (Raseira and Bonifacio 2006). In this study, we clarified two patterns of inheritance for resistance to bacterial spot. Resistance derived from Brazilian cultivars including ‘Chimarrita’ is controlled by a QTL with large effect, and another resistance is controlled by QTLs with small effects. The resistance of Japanese peaches ‘Mochizuki’ and ‘Tsukikagami’ may be the latter type. Elucidation of the mode of resistance inheritance will be useful to accelerate the resistant breeding to bacterial spot in peach.

LLVs of family means of Brazilian crosses between Brazilian cultivars/selections having low LLVs and cultivars/selections having high LLVs were low and close to the LLVs of Brazilian cultivar/selection parents. Those family means were separated from all seed or pollen parental values of the higher LLV cultivars/selections. In addition, the

within-family variances were not significantly different from the environmental variance estimate in four Brazilian crosses. These results suggested that bacterial spot resistance is controlled by a QTL with a large effect, and that the low LLV Brazilian cultivar/selection parents are dominant homozygotes (genotype: AA) and the Japanese cultivar/selection and 332-16 parents are recessive homozygotes (aa). In above case, all offspring would be heterozygotes (Aa) in the five crosses of “Brazilian crosses” (family nos. 384, 402, 403, 404 and 405), resulting in the similar phenotypic values for the offspring as the Brazilian cultivar/selection parent having low LLVs. Differences in LLVs between family means and the Brazilian parents having low LLVs may be due to environmental variation and additional minor gene effects.

In the “381” family, family mean did not significantly separate from both seed and pollen parental values. In addition, there were no significant differences between within-family variance and environmental variance ( $\sigma_{el}^2$ ) for the four Brazilian crosses. These results suggested little effect of the additional minor genes for those crosses.

Based on the above results, the 296-16 genotype in the locus was supposed to be a dominant homozygote (AA); however, 296-16 is an offspring from a cross between ‘Yoshihime’ (seed parent; a Japanese cultivar, with an LLV of 1.236 in the present study) and ‘Coral’ (pollen parent; a Brazilian cultivar; its LLV has not been evaluated). Normally, the cross yielded all offspring with Aa or aa genotypes even if the genotype of ‘Coral’ was AA or Aa. Some doubling of a section of chromosome during recombination may happen rarely but is possible. Also, some interactive effect may be possible among genes. In addition, crossing may be very rarely, but possibly, mistaken. There is no information on the response of ‘Coral’ to bacterial spot, and it is unknown whether the resistance of ‘Chimarrita’ and ‘Coral’ originates from their common ancestor. Therefore, the gene effects and genotype in 296-16 are still unknown and should be elucidated in future studies.

For the Japanese crosses, we frequently used the following cultivars as cross-parents that had desirable characteristics related to our breeding objectives: ‘Yuzora’, a late maturing cultivar with a high sugar content; ‘Tsukiakari’, a middle maturing cultivar with a high sugar content, and Momo Tsukuba 124, an early maturing selection with large fruit. ‘Yuzora’, ‘Tsukiakari’ and Momo Tsukuba 124 were used as cross-parents for six, four and four times, respectively, in the present study. These crosses were made without information about bacterial spot resistance in these cultivars/selections. Almost all widely grown cultivars in Japan are descendants of ‘Hakutou’ (Yamamoto *et al.* 2003). Peach does not have self-incompatibility, and inbreeding depression, and selfing and backcrossing has been repeatedly used in the breeding, resulting in very narrow genetic variability (Scorza *et al.* 1985, Yamamoto *et al.* 2003). Most Japanese cultivars/selections used as cross-parents are closely related, which may have resulted in the narrow genetic

variation in mid-parent for LLV and small value of broad-sense heritability (0.19).

‘Mochizuki’ was noticed as having a relatively low LLV ( $0.514 \pm 0.157$ ) in Suesada *et al.* (2013). This cultivar was crossed with cultivars having large LLVs (‘Hakushu’, LLV:1.172; ‘Tsukiakari’, LLV:1.090), and the family-means in families resulting from those crosses were 0.746 (No. 396) and 0.779 (No. 397), values not very close to the LLV of ‘Mochizuki’. In addition, the within-family variance was estimated as very small. Those result indicated that the relatively low LLVs of ‘Mochizuki’ was not inherited to offspring like that of Brazilian parents having low LLVs.

The present study revealed the presence of resistance to bacterial spot controlled by a QTL with a large effect derived from Brazilian cultivars, and offspring with low LLV could be obtained easily from Brazilian crosses with parents having low LLVs. In contrast, it was difficult to obtain offspring with low LLVs from Japanese crosses. We thus state our negative view to idea of obtaining offspring having LLV as low as Brazilian cultivars have by crosses among Japanese cultivars/selections although number of offspring within a family was not many and equal resulted in difficulty in precise discussion based on offspring frequency within a family due to difficulty in estimating error in the present study.

Japanese cultivars/selections have high eating quality and large fruit size, which assumed to be inherited quantitatively. Therefore, Japanese cultivars/selections used as cross-parents are indispensable to developing new cultivars with excellent marketability in Japan. In conclusion, it is an effective way to backcross repeatedly Brazilian cultivar/selection having low LLV with Japanese cultivars/selections having high fruit quality in order to develop new cultivars combined bacterial spot resistance and high fruit quality in Japan.

The Brazilian cultivars, ‘Chimarrita’ and ‘Coral’, were introduced to Japan in 1989 and 1971, respectively. These two cultivars have been used as genetic resources for introducing a low-chilling requirement in the Japanese peach breeding programs. When they were grown in Japanese climate conditions, poor fruit quality was characterized, for example unpleasant flavor for table use. ‘Sakuhime’ (former name: Momo Tsukuba 127, resulting from 296-16  $\times$  332-16) recently released (Yaegaki *et al.* 2017) was exemplified by excellent fruit quality, large fruit size, and a low-chilling requirement (Sawamura *et al.* 2017), requiring over three generations in the breeding. Sherman and Lyrene (1981) suggested that there was no relationship between the chilling requirement and the degree of susceptibility to bacterial spot. The suggestion is consistent with that ‘Sakuhime’ has a low-chilling requirement and a high susceptibility to bacterial spot. It was shown that new predominant cultivars like ‘Sakuhime’ could be developed with excellent fruit quality by repeated crossing over a few generations among Brazilian and Japanese cultivars/selections.

DNA marker-assisted selection has been remarkably developed in many woody fruit crops (Luby and Shaw 2001).

So far, the large plant size of fruit trees limits the number of seedling trees that can be planted in selection fields, thereby hindering tree fruit breeding in contrast with the breeding of annual crops. By contrast, marker-assisted selection enables breeders to increase tremendously the number of seedlings that breeders raise, selected plants from which are thereafter planted in the selection field if it is available to obtain a large number of cross-seedlings by crossing. Marker-assisted selection is available with very young and small plants. DNA markers associated with the resistance gene present in the Brazilian cultivars/selections genome will be developed through DNA marker mapping and QTL analysis in future studies. Such technologies will promote breeding by using the backcrosses of Brazilian cultivars/selections with low LLVs with Japanese cultivars/selections to produce new cultivars that combine high bacterial spot resistance and high fruit quality in Japan.

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