Tree Genetics & Genomes (2011) 7:363–372
DOI 10.1007/s11295-010-0337-3

ORIGINAL PAPER

Genetic studies on resistance to Valsa canker in apple: genetic variance and breeding values estimated from intra- and inter-specific hybrid progeny populations

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Received: 8 February 2010 / Revised: 23 August 2010 / Accepted: 1 September 2010 / Published online: 2 October 2010

Abstract *Malus sieboldii* Rehd. exhibits high levels of resistance to Valsa canker caused by *Valsa ceratosperma* (Tode ex Fr.) Maire while cultivated apples (*Malus domestica* Borkh.) are susceptible to the disease. In this study, progenies from 23 full-sib families derived from both inter- and intra-specific hybridization among 16 *Malus* genotypes as parents were assessed for resistance to *V. ceratosperma* (Vc) for two seasons using an excised shoot assay to determine the pattern of inheritance of the resistance and to also estimate the variance components, narrow-sense heritability, and breeding values of parental genotypes. Generally, *M. sieboldii*×*M. domestica* and its reciprocal crosses had more resistant progenies to Vc than intra-specific crosses of *M. domestica*. Resistance to Vc expressed as the relative lesion length among progenies showed continuous variation irrespective of cross, suggesting the quantitative nature of the resistance to the three virulent isolates of Vc that were tested. Resistance to Vc using the progeny population was analyzed using a mixed linear model based on restricted maximum likelihood. The parental effect (general combining ability (GCA)) was significant while the interaction effect between parents (specific combining ability (SCA)) was relatively small and non-significant. The ratio of SCA/GCA variance was about 32%, suggesting that additive genetic variance had a major contribution to the total genetic variance for resistance to Vc. There was a positive correlation (r=0.49, p<0.01) between mid-parental GCA and SCA predictions among 23 full-sib families for the resistance. Narrow-sense heritability estimated by sib analysis was moderate $\left(\hat{h}^2 = 0.29\right)$. The predicted breeding values (BV) of the 16 parents indicated that *M. sieboldii* "Sanashi 63" and "Hayanarisanashi 1" would be useful for breeding for high levels of resistance to Vc.

Keywords Apple · Breeding value · Heritability · General combining ability · Resistance · *Valsa ceratosperma*

Communicated by R. Burdon

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Introduction

Valsa canker, caused by *Valsa ceratosperma* (Tode ex Fr.) Maire (syn. *Valsa mali* Miyabe et Yamada), is one of the most important diseases of apple (*Malus*) especially in eastern Asia (Sakuma 1990; Uhm and Sohn 1995). The causal fungus infects trees through wounds at the pruning ends with infected trunks and shoots appearing in the spring while the canker develops rapidly in the late spring and early summer. Infection produces localized cankers followed by death of twigs, limbs, or an entire tree. Due to the ability of the mycelium of the causal fungus to thoroughly invade healthy tissues of bark, phloem, and xylem, and the perennial nature of the canker, spray applications of

fungicides are not successful for the control of Valsa canker.

Genetic resistance would seem to be a better means to control the canker than preventative sprays. Several genotypes belonging to *Malus sieboldii* Rehd. are resistant under field conditions, while all cultivated apples are susceptible to the disease (Liu et al. 1990). Since inter-specific hybridizations between cultivated apples and various wild *Malus* species, including *M. sieboldii*, are known to be successful (Korban 1986), resistant genotypes of *M. sieboldii* would appear to be of much value as resistant gene sources for Valsa canker. However, only a few reports on the variability and genetic control level of the disease have been published. This is due to a lack of standardized inoculation and evaluation methods that are reproducible, low cost and that require minimal labor.

Knowledge of the genetic basis for resistance to Valsa canker is important to inform breeding strategy. The development of an artificial inoculation method to evaluate the resistance in the laboratory (Bessho et al. 1994; Suzaki et al. 1997) now allows labor-saving assessment that will facilitate genetic studies using large datasets. We previously attempted to obtain genetic information based on inheritance of Valsa canker with this approach and the results suggested that the resistance in *M. sieboldii* to be quantitative (Abe et al. 2000). However, due to the small size of the hybrid population, we were unable to estimate genetic parameters.

The objective of the present study was to estimate the genetic variance components necessary to determine the mode of inheritance of Valsa canker resistance to inform breeding strategy development. Furthermore, we intended to rank parents, especially genotypes belonging to *M. sieboldii* in our breeding population, their combining ability.

Materials and methods

Plant materials

This study was carried out using F1 progenies from the apple breeding program at the Apple Research Center, National Institute of Fruit Tree Science (NIFTS), Morioka, Iwate. Sixteen apple cultivars/genotypes were used as parents (Tables 2 and 3). The population for the progeny test consisted of 23 full-sib families with seven to 75 individuals per family (Table 1). Seventeen families (population 1) came from crosses between genotypes of *M. domestica* made for the production of commercial cultivars and, therefore, had no specific mating design. As a result, the frequency of appearance of a genotype in crosses varied from 0.04 to 0.17 among parental genotypes. Six families (population 2) came from inter-specific crosses between *M. domestica* and *M. sieboldii*. Seedling progenies were planted in 1994 to 1996 in the orchard; trees within a family were planted side by side at a spacing of 0.5 m within a row with 3 m between rows. Parental plants were grown in neighboring fields.

Preparation of twigs for the excised shoot assay

Sixteen parental genotypes and a total of 492 offspring were inoculated with a virulent isolate VC96-A of *V. ceratosperma*. The parental genotypes and a total of 128 offspring derived from three crosses, "Jonathan"×*M. sieboldii* "Mo 15," *M. sieboldii* "Hayanarisanashi 1"×"Megumi", and *M. sieboldii* "Sanashi 63"×"Jonathan", were also inoculated with two other isolates of AVC-12 and AVC-55. "Fuji" was used as a susceptible control. Five dormant 1-year-old shoots from each genotype/progeny were collected from trees in the fields at the Apple Research Center (Morioka) in December or January. After a mild rinse of the shoot surface with

Table 1 Summary of the inoculation test using intra- and inter-specific hybrid populations in this study

Population	Type of cross	No. of families	No. of progeny used in		Progeny evaluated (pooled No.)	Percentage of *R* progeny[c]
			1998	1999		
1[a]	*Malus domestica*×*M. domestica*	17	80	266	285	0
2[b]	*Malus sieboldii*×*M. domestica*	3	32	99	104	24.0
2[b]	*M. domestica*×*M. sieboldii*	3	81	85	103	9.7
Total		23	193	450	492	7.1

R resistant

[a] Intra-specific hybrid population

[b] Inter-specific hybrid population

[c] RL<0.60

running water, shoots were dried with a laboratory towel. One 12-cm segment was then cut from the middle of each shoot and used in the excised shoot assay. Five twigs per genotype/progeny were subjected to the inoculation test.

Inoculum, inoculation, incubation, and disease assessment

The monoconidial isolates of VC96-A, originating from an infected apple tree in Iwate Prefecture, Japan, and AVC-12 and AVC-55, originating from an infected apple tree in Aomori Prefecture, Japan, were used. The isolates were multiplied for 14 days on a PDA medium at 25°C in darkness. The preparation of the inoculum and method of inoculation followed the method developed by Suzaki et al. (1997) with minor modifications based on Abe et al. (2007). After a 14-day incubation, fungal mycelium was added to a 100 ml volume of sterilized water and then mildly homogenized for about 30 s. The mixture after homogenization was used as an inoculum for the excised shoot assay. The distal cut end of twig segments was subjected to a scorch treatment with a flat iron to kill the tissue there and to ensure easy invasion by the hyphae of the inoculum into the twigs. After the scorch treatment, the five twigs were tied up and placed vertically into a plastic box so that the proximal cut end of each bundle of twigs was about 1 cm deep in the water in a plastic box. A droplet of 10 μl of the inoculum was dripped onto the distal end of each twig at the scorch site and the plastic box was immediately covered with a vinyl film to retain the humidity around the inoculated twigs. The twigs were kept in darkness at 25°C for 10 days. The lesion length of the necrosis caused by the inoculum was measured at 10 days post-inoculation for the excised shoot assay.

Data analysis

Since many families in population 1 have parents that are more or less genetically related to one another, while some others are not inbred, the families are different regarding their inbreeding level. To evaluate the relationship between the inbreeding coefficient (F) and inbreeding depression (ID) for resistance to Valsa canker, the inbreeding coefficient of each family (F_X) in population 1 was calculated according to Falconer and Mackay (1996) with slight modification on the assumption that the earliest recorded ancestors of each family were unrelated using the formula:

$$F_X = \sum_A [(1/2)^n (1 + F_A)], \quad (1)$$

where n is the number of genotypes (cultivars) in any path of relationship counting the parents of X and the common ancestor A. F_A is the inbreeding coefficient of the common ancestor A. For the families in population 1, ID was estimated according to Durel et al. (1996) as follows:

$$\mathrm{ID}(\%) = (FM_0 - FM_i) \times 100/FM_0, \quad (2)$$

where FM_0 is the overall family mean for $F=0$ in population 1, and FM_i is the family mean for $F=i$.

Statistical analysis

Differences among genotypes and among isolates of *V. ceratosperma* and the interaction between genotypes and isolates were analyzed by a two-way analysis of variance (ANOVA) for lesion length from 16 parental genotypes inoculated with three virulent isolates of *V. ceratosperma*. The following model was fitted to the data:

$$Y_{ijk} = \mu + G_i + Iso_j + (GIso)_{ij} + e_{ijk}, \quad (3)$$

where Y_{ijk} is the lesion length of the ith genotype inoculated with the jth isolate; μ is the overall mean; G_i is the effect of the ith genotype; Iso_j is the effect of the jth isolate; $GIso_{ij}$ is the interaction between the ith genotype and the jth isolate; and e_{ijk} is the residual term indicating the within-genotype deviation resulting from the effects of the kth shoot collected from the ith genotype.

Prior to estimating genetic parameters on the overall progeny data, the following linear model was fitted to the progeny data of population 1; this was done primarily to test the significance of the effect of inbreeding levels. A result of non-significance means that it is possible to estimate genetic parameters without incorporating the F effect into a model.

$$Y_{ijkl} = \mu + \mathrm{Y}r_i + IB_j + Fy_k(IB_j) + IND_l(Fy_k) + e_{ijklm}, \quad (4)$$

where Y_{ijkl} is the lesion length of the lth offspring from an intra-specific cross between the genotypes of *M. domestica* in the ith year; μ is the overall mean; Yr_i is the fixed effect of the ith year; inbreeding $(IB)_j$ is the random effect of the IB level of each family; $Fy_k(IB_j)$ is the random effects of the kth family nested in the jth IB; $IND_l(Fy_k)$ is the random effect of the lth individual tree (offspring) within kth family; and e_{ijkl} is the residual term indicating the within-tree deviation resulting from the effects of excised shoots of the lth offspring.

For population 2, the following linear model was fitted to the progeny data to test the significance of the effect of cross-types between *M. domestica*×*M. sieboldii* and its reciprocal:

$$Y_{ijkl} = \mu + Yr_i + CT_j + Fy_k(CT_j) + IND_l(Fy_k) + e_{ijklm}, \quad (5)$$

where Y_{ijkl} is the lesion length of the lth offspring from an inter-specific cross between *M. domestica* and *M. sieboldii* or its reciprocal in the ith year; μ is the overall mean; Yr_i is

the fixed effect of the *i*th year; CT_j is the random effect of cross-type (*M. domestica*×*M. sieboldii* (MD×MS) or *M. sieboldii*×*M. domestica* (MS×MD)) of each family; $Fy_k(CT_j)$ is the random effects of the *k*th family nested in the *j*th cross-type; $IND_l(Fy_k)$ is the random effect of the *l*th individual tree (offspring) within the family; and e_{ijkl} is the residual term indicating the within-tree deviation of the *l*th offspring.

Since the effects of inbreeding level in population 1 and the cross-type in population 2 were non-significant (Table 5), the pooled progeny data was then fitted to the following linear model to test the significance of the genetic group:

$$Y_{ijkl} = \mu + Yr_i + GG_j + Fy_k(GG_j) + IND_l(Fy_k) + e_{ijklm}, \quad (6)$$

where Y_{ijkl} is the lesion length of the *l*th progeny from an inter-specific cross between *M. domestica* and *M. sieboldii* or its reciprocal in the *i*th year; μ is the overall mean; Yr_i is the fixed effect of the *i*th year; GG_j is the random effect of the genetic group (MD×MD or MD×MS and MS×MD) of each family; $Fy_k(GG_j)$ is the random effects of the *k*th family nested in the *j*th genetic group; $IND_l(Fy_k)$ is the random effect of the *l*th individual tree (offspring) within the *k*th family; and e_{ijkl} is the residual term. The statistical analyses for each population described above were carried out using SAS PROC GLM.

To estimate the breeding value of the parental genotypes used in the study, the progeny test data was further analyzed by fitting the following mixed linear model (Xiang and Li 2001; Iwanami et al. 2008):

$$Y_{ijkl} = \mu + Yr_i + G_{j-m} + G_{k-f} + S_{jk} + e_{ijkl}, \quad (7)$$

where Y_{ijkl} is the lesion length of the *l*th offspring from a cross between the *j*th male parent and the *k*th female parent in the *i*th year; μ is the overall mean; $G_{j\text{-}m}$ and $G_{k\text{-}f}$ are the random effects of the *j*th male parent and the *k*th female parent, respectively, which are analogous to the parental effect (i.e., the general combining ability (GCA)) of the *j*th and *k*th parents; S_{jk} is the random effect of the *j*th and *k*th parent interactions, which is analogous to the interaction effect between parents (i.e., the specific combining ability (SCA)); and e_{ijkl} is the residual term indicating the within-family deviation resulting from the effects of the *l*th offspring of family *jk*. The estimation of the variance components of the random effect in this model was based on the restricted maximum likelihood (REML) algorithm in SAS (SAS Institute, Cary, NC) using the MIXED procedure. REML is well suited for unbalanced designs to estimate variance components and can give the variance of male GCA ($\sigma^2_{GCA\text{-}m}$) and female GCA ($\sigma^2_{GCA\text{-}f}$), the variance of SCA (σ_{SCA}^2), and the within-family variance (σ^2). Breeding value is generally expressed as twice GCA, which is defined without distinction between males and females in diallel mating designs. We therefore constructed dummy variables for each parent using the IML procedures in SAS (Xiang and Li 2001) so that $\sigma^2_{GCA\text{-}f}=\sigma^2_{GCA\text{-}m}=\sigma_{GCA}^2$, and then the variance component for GCA effect was estimated.

The narrow-sense heritability (h^2) of lesion length was estimated using the above results of sib analysis. Because the covariance of half-sibs was $COV_{(HS)}=1/4\ V_A=\sigma_{GCA}^2$ (where V_A=additive genetic variance; Xiang and Li 2001), narrow-sense heritability was estimated using the following equation:

$$\widehat{h}^2 = \widehat{V}_A/\widehat{V}_P = 4\widehat{\sigma}^2_{GCA}/\left(2\widehat{\sigma}^2_{GCA} + \widehat{\sigma}^2_{SCA} + \widehat{\sigma}^2\right)$$

(where V_P=phenotypic variance). Additive genetic variance estimated in an inbred population, however, is $(1+F)\ V_A$, where F is the inbreeding coefficient (Falconer and Mackay 1996; Kumar 2004). Since the F values were different among the families used in the study, the mean of the F values across families in populations 1 and 2 were calculated and regarded as an approximate of both populations. Using the F value, correct heritability on the slightly inbred population was calculated (Kumar 2004). The standard error of the heritability was estimated according to Falconer and Mackay (1996).

Results and discussion

Relationship between parental genotypes and pathogen isolates

The mean lesion lengths of parental genotypes in excised shoot assays caused by the inocula of *V. ceratosperma* isolates VC96-A, AVC-12, and AVC-55 had various values among genotypes across the isolates (Tables 2 and 3). Three genotypes belonging to *M. sieboldii* had low values of lesion length, ranging from 5.2 to 7.2 mm in "Sanashi 61", from 5.8 to 7.8 mm in "Mo 15",and from 5.4 to 10.8 mm in "Hayanarisanashi 1". Their values of lesion length (RL) relative to those of "Fuji" ranged from 0.25 to 0.56, showing a high level of resistance to *V. ceratosperma*. The mean lesion length of the other 13 genotypes belonging to *M. domestica* had higher values than those of *M. sieboldii*, 13.4 to 20.8 mm to VC96-A, 15.4 to 21.4 mm to AVC-12, and 15.2 to 24.0 mm to AVC-55. A two-way ANOVA indicated significant differences among genotypes ($p<0.001$) and among isolates ($p=0.038$), while no statistically significant interaction between genotypes and isolates was shown (Tables 2 and 3). The result of ANOVA suggests that screening tests using parental genotypes and progeny populations for the resistance to *V. ceratosperma* will be successful with a single isolate as inoculum.

Table 2 Lesion length on excised dormant shoots inoculated with three virulent isolates of *Valsa ceratosperma* in 16 apple genotypes

Genotype	Species	Lesion length (mm) caused by			Resistance level[b]
		VC96-A	AVC-12	AVC-55	
Fuji	*Malus domestica* Borkh.	16.2 (1)[a]	19.2 (1)	20.8 (1)	*S*
Tsugaru	*M. domestica* Borkh.	16.4 (1.01)	20.8 (1.08)	18.0 (0.87)	*S*
Jonathan	*M. domestica* Borkh.	15.6 (0.96)	17.6 (0.92)	20.0 (0.96)	*S*
Golden Delicious	*M. domestica* Borkh.	15.4 (0.95)	18.4 (0.96)	16.6 (0.80)	*S*
Akane	*M. domestica* Borkh.	13.4 (0.83)	16.6 (0.86)	15.2 (0.73)	*S*
Sansa	*M. domestica* Borkh.	13.8 (0.85)	16.4 (0.85)	19.0 (0.91)	*S*
Himekami	*M. domestica* Borkh.	15.8 (0.98)	18.6 (0.97)	–	*S*
Iwakami	*M. domestica* Borkh.	16.0 (0.99)	15.4 (0.80)	–	*S*
Senshu	*M. domestica* Borkh.	15.6 (0.96)	16.8 (0.88)	19.8 (0.95)	*S*
Starking Delicious	*M. domestica* Borkh.	20.8 (1.28)	21.4 (1.11)	24.0 (1.15)	*S*
Splendor	*M. domestica* Borkh.	16.0 (0.99)	19.4 (1.01)	18.8 (0.90)	*S*
Megumi	*M. domestica* Borkh.	14.6 (0.90)	16.6 (0.86)	17.2 (0.83)	*S*
Alps Otome	*M. domestica* Borkh.	18.6 (1.15)	20.8 (1.08)	24.6 (1.18)	*S*
Sanashi 63	*Malus sieboldii* Rehd.	7.2 (0.44)	8.2 (0.43)	5.2 (0.25)	*R*
Mo 15	*M. sieboldii* Rehd.	5.8 (0.36)	6.0 (0.31)	7.8 (0.38)	*R*
Hayanarisanashi 1	*M. sieboldii* Rehd.	7.0 (0.43)	10.8 (0.56)	5.4 (0.26)	*R*

R resistant, *MR* moderately resistant; *S* susceptible

[a] Relative lesion length values (RL value, mean lesion length of the respective genotype/mean length of Fuji) were presented in the parentheses

[b] RL<0.60 (*R*); 0.60≦RL≦0.70 (MR); RL>0.70 (*S*)

Inheritance pattern of resistance

Data on the lesion length of offspring showed a continuous variation across the families, with the family mean lesion length around the mean length of parents. The distribution pattern of the within-family offspring derived from inter-specific hybridization indicated the quantitative nature of the resistance in the genotypes of *M. sieboldii* tested in the study (Fig. 1). In crosses in which genotypes of *M. sieboldii* were used as parents, the distributions for the lesion length of progenies shifted toward lower values than those of progenies derived from crosses between genotypes of *M. domestica* (Fig. 1, Table 1).

Table 3 Result of analysis of variance for the lesion length on excised dormant shoots inoculated with three virulent isolates of *Valsa ceratosperma* in 16 apple genotypes

Source of variation	*df*	MS	*F*
G	15	331.77	7.86**
I	2	140.81	3.34*
G×*I*	30	3.38	0.08
Residual	182	42.19	

df degrees of freedom, *MS* mean square, *F* *F* static, *G* genotype, *I* isolate

*$p<0.05$; **$p<0.001$

Generally, families in population 2 had more offspring resistant to *V. ceratosperma* than those in population 1. On the basis of the RL value of 0.6 as the cut off to separate the resistant (*R*) and remaining offspring (Abe et al. 2007), *R* offspring were obtained with varying frequencies in the families derived from *M. sieboldii* as a parent; the overall frequency of *R* offspring in the families having *M. sieboldii* as a parent was 0.17, whereas no R offspring was produced in the families made by the intra-specific crosses of *M. domestica* (Table 1).

Relationship between the inbreeding level and resistance

The inbreeding level (IB) did not affect the family mean values of the lesion length (Table 4). There was no clear trend of inbreeding depression for the resistant trait with an increase of IB from $F=0$ to 0.25 in population 1. The result of ANOVA performed for the four levels of the inbreeding coefficient showed that the effect of IB was non-significant with family variation (Table 5). The estimated amount of contribution to the total variance by the factor IB was 0% (Table 6). The result of ANOVA in population 2 showed that the effect of cross-type (*M. domestica*×*M. sieboldii* or *M. sieboldii*×*M. domestica*) was also statistically non-significant, although the estimated percentage of contribution to the total variance by the factor cross-type was 11.5% (Tables 5 and 6).

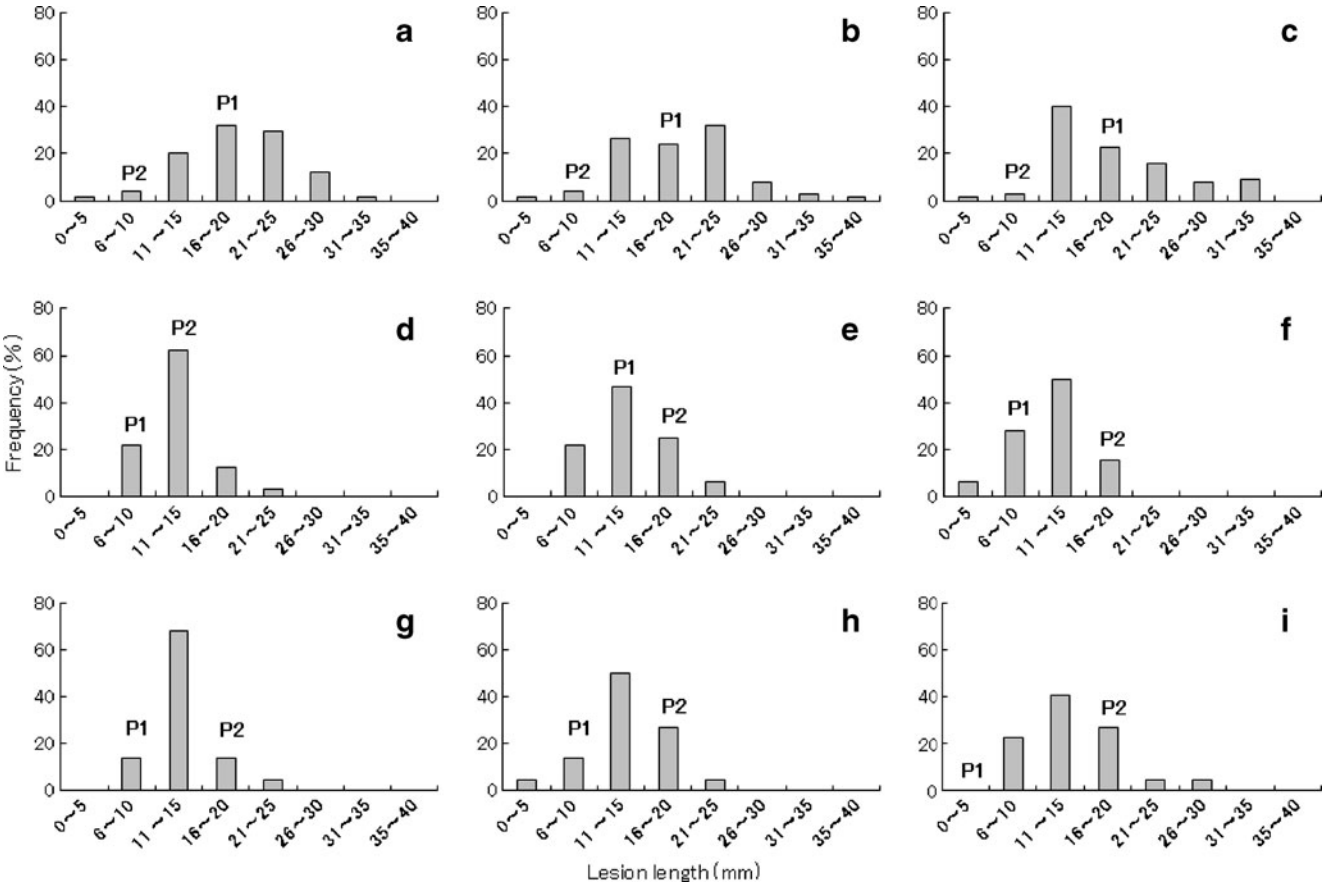

Fig. 1 Frequency distribution of lesion length in the F1 progeny derived from "Jonathan" and *Malus sieboldii* "Mo 15" (No. of progenies=75) inoculated with **a** *Valsa ceratosperma* isolate VC96-A; **b** isolate AVC-12; and **c** isolate AVC-55, in the F1 progeny derived from *M. sieboldii* "Hayanarisanashi 1" and "Megumi" (No. of progenies=32) inoculated with **d** isolate VC96-A; **e** isolate AVC-12; and **f** isolate AVC-55, and in the F1 progeny derived from *M. sieboldii* "Sanashi 63" and "Jonathan" (No. of progenies=22) inoculated with **g** isolate VC96-A; **h** isolate AVC-12; and **i** isolate AVC-55. *P1* and *P2* are the lesion lengths of the female and male parent, respectively

Due to insignificance of the factors by the IB and cross-type in each population, further ANOVA based on both populations with a pooled data set was performed to test only the effect of genetic group (intra- versus inter-specific hybrids); the effect was significant at the 5% probability level (Table 5). The result suggested that the inter-specific hybridization between *M. domestica* and *M. sieboldii* or its reciprocal was more effective to obtain resistant progenies than the intra-specific hybridization among *M. domestica* genotypes. The amount of the contribution to the total variance by the factor genetic group was about 5.1% (Table 6).

Table 4 Family mean values of the lesion length for the different *F* levels of the families in populations 1 and 2

Population	Inbreeding level[a]	FM
1	0	18.3
	0.06	18.5
	0.13	16.9
	0.25	18.7
2	0	13.5

FM family mean value of the lesion length (mm)

[a] Each value represent inbreeding coefficient (*F* value)

Variance component and heritability estimates by sib analysis

Further analysis of the variance of two populations combined for the resistance trait indicated that the parental effect (i.e., the GCA) was significant (Table 7). As contrasted with the GCA variance, the interaction effect between parents (i.e., the SCA) was relatively small and non-significant. The ratio of SCA/GCA variance was about 32%, suggesting that additive genetic variance gave a major contribution to the total genetic variance for the resistance.

Table 5 Result of ANOVA performed for resistance to Valsa canker using intra- and inter-specific hybrid populations

Source of variation	Population 1			Population 2			Whole population		
	df	MS	*F*	*df*	MS	*F*	*df*	MS	*F*
Genetic group	–	–	–	–	–	–	1	6,944.04	6.71*
Inbreeding level	3	489.71	0.83	–	–	–	–	–	–
Cross-type	–	–	–	1	7,375.26	5.68	–	–	–
Family	13	591.19	6.57**	4	1,299.35	12.80**	21	1,034.63	10.89**
Individual	268	89.98	1.61**	208	101.54	2.62**	476	95.03	2.00**
Year	1	56.47	1.01	1	19.13	0.49	1	16.40	0.35
Residual	1102	55.75		1056	38.79		2159	47.46	

*$p<0.05$; **$p<0.001$

Usually, additive genetic variance is found to be abundant and more important than non-additive genetic variance for previously unselected material and in the first few generations of selected materials in tree species (Namkoong et al. 1988), which may also be the case in apple breeding programs for resistance to *V. ceratosperma*. Since most fruit tree breeding programs are based on the premise that genetic gains will be cumulative over generations, an additive genetic effect is a major important source of variation to incorporate into breeding and production programs, while non-additive variance can only originate from the effects of gene pairs and would be considered difficult to handle in a tree breeding program (Yanchuk 1996). The presence of additive genetic variance in populations of fruit trees for disease-resistance traits is supported by several quantitative genetic studies, e.g., in the pathosystems of *Podosphaera leucotricha–M. domestica* (Bus et al. 2005), *Erwinia amylovora–Pyrus communis* (Bagnara et al. 1996; Bell and Janick 1977; Quamme et al. 1990), *Rubus* spp. (Stewart et al. 2005), and *Plasmopara viticola–Vitis* spp. (Brown et al. 1999).

The narrow-sense heritability ($\hat{h}^2$) estimated by sib analysis was moderate, 0.29 (Table 7). For a quantitative disease-resistance trait of fruit trees, it was consistent with the estimates found for fire blight (Luby et al. 2002) and powdery mildew (Oraguzie and Currie 2004) in apple, fire blight in blackberry (Stewart et al. 2005) and Anthracnose rot in blueberry (Polashock et al. 2005), but it was lower than those reported for perennial canker in peach (Chang et al. 1991), downy mildew in table grape (Brown et al. 1999), Alternaria late blight in pistachio (Chao et al. 2001) and scab in pecan (Thompson and Grauke 1994). While the narrow-sense h^2 estimate is not high, the value of 0.3–0.4 is still favorable and, as Durel et al. (1998) pointed out, should guarantee the efficiency of mass selection especially for the trait of interest in apple.

Relationship between GCA and SCA values

There was a positive relationship between mid-parental GCA and SCA predictions among 23 full-sib families for resistance to *V. ceratosperma* (Fig. 2). The GCA and SCA correlation was 0.492, and it was significant at the 1% probability level. This result of a significant positive correlation between mid-parental GCA and SCA is, to our knowledge, the first case in a fruit tree species. In forest tree

Table 6 Estimates of variance components for resistance to Valsa canker using intra- and inter-specific hybrid populations

Variance component	Population		
	1 (%)	2 (%)	Whole population (%)
σ_{GG}^2	–	–	3.52 (5.1)
σ_{IB}^2	0 (0)	–	–
σ_{CT}^2	–	7.47 (11.5)	–
σ_{Fy}^2	6.78 (9.8)	8.21 (12.6)	9.01 (13.1)
σ_{IND}^2	6.90 (9.9)	10.57 (16.3)	8.93 (13.0)
σ_e^2	55.75 (80.3)	38.79 (59.6)	47.46 (68.8)
Total	69.43 (100)	65.04 (100)	68.93 (100)

Table 7 Estimated variance components for apple Valsa canker resistance using 23 families in apple

Variance components		Estimates	*p*
Parental effect (GCA)	σ^2_{GCA}	5.26	0.04
Parental interaction effect (SCA)	σ^2_{SCA}	1.69	0.14
Within family	σ^2	56.46	<0.001
SCA/GCA (%)		32	
Narrow-sense heritability	h^2	0.29±0.097	

species, the same relationship has been reported by Wu and Matheson (2004) for the growth trait in radiata pine.

Determining the genetic cause of such a positive correlation is a substantial challenge. One possible explanation of the observed correlation, genetic background, and situation of parental genotypes could be a major contributing factor of the correlation. Linkage disequilibrium (LD) in a population produces a covariance between the additive and dominance gene effect, as described by Falconer and Mackay (1996); LD can generate a correlation between additive and non-additive gene action. LD can be caused by selection (Falconer and Mackay 1996), random genetic drift in populations of finite size (Barker 1979), or the mix of previously isolated and genetically different populations (i.e., from populations with different gene frequencies) (Barker 1979; Falconer and Mackay 1996). Most of the parental genotypes belonging to *M. domestica* used in our experiment were selected from numerous apple cultivars/genotypes for better fruit quality in recent apple breeding programs. Use of the parental genotypes, given intensive selection for fruit traits, is suspected to form a tentative LD in parental genotypes for genes associated with resistance to *V. ceratosperma* as well as for genes controlling fruit traits.

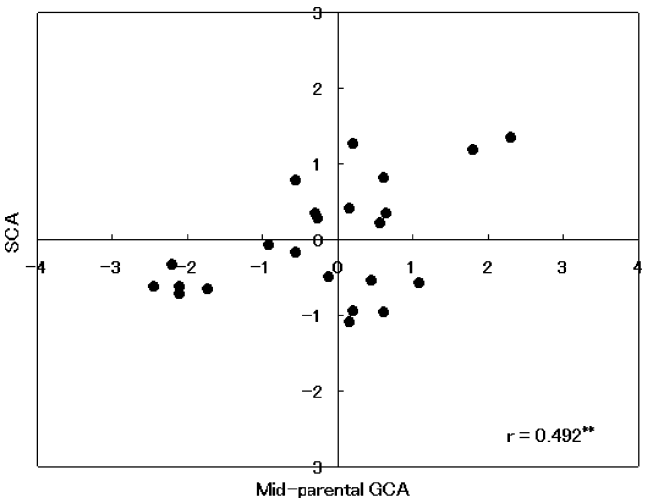

Fig. 2 The relationship between mid-parental general combining ability (*GCA*) and specific combining ability (*SCA*) predictions among 23 full-sib families for resistance to *Valsa ceratosperma*

This could be a reason for the positive correlation between GCA and SCA. The mixture of genetically different genotypes in a population of parents in our experiment is another possibility. Parental genotypes of *M. sieboldii* are regarded as genetically different from those of *M. domestica*, a finding that is supported by molecular research on genetic differentiation among *Malus* (apple) species (e.g., Hokanson et al. 2001) and by phylogenic studies on the genus *Malus* Mill. (Forte et al. 2002; Harris et al. 2002; Robinson et al. 2001). As a result, the use of genetically heterogeneous parents and the production of full-sib families containing several families of inter-specific-hybridization origin might be an alternative explanation for the positive correlation.

Regardless of the genetic cause of such a relationship between GCA and SCA, the positive correlation renders an advantage in the practical use of SCA variance, i.e., selection of the best GCA will capture some portion of SCA variance. It is of interest for practical breeding to predict how much extra genetic gain by SCA variance can be captured from selections on the basis of the estimated breeding values.

Predicted breeding values of parental genotypes

Parental BVs were predicted using data from full-sib apple families (Table 8). There was a marked difference among parental genotypes and, on the basis of the predicted BVs,

Table 8 Estimated breeding values for 16 parental genotypes based on best linear unbiased predictions (BLUPs)

Genotype	NPV	BV
Mo15	−2.039	1.4
Hayanarisanashi 1	−1.750	−6.411
Sanashi 63	−1.701	−7.77
Akane	−0.208	2.11
Sansa	−0.111	−1.3
Megumi	0.081	−2.4
Iwakami	0.081	2.170
Golden Delicious	0.274	−2.04
Jonathan	0.322	0.8
Senshu	0.322	3.28
Himekami	0.370	−1.49
Splendor	0.419	3.88
Fuji	0.467	0.417
Tsugaru	0.515	−0.864
Alps Otome	1.045	−0.21
Starking Delicious	1.575	8.415
	r^a=0.622**	

NPV normalized phenotypic value, *BV* breeding value

[a] Correlation coefficient between NPVs and BVs

the best parents for transmitting resistance to *V. ceratosperma* were *M. sieboldii* "Sanashi 63" (−7.768) and "Hayanarisanashi 1" (−6.411). Among the parents belonging to *M. domestica*, "Megumi" (−2.402) and "Golden Delicious" (−2.035) seemed to be good parents, while "Starking Delicious" (8.415), which was regarded as highly susceptible to *V. ceratosperma* (Abe et al. 2007; Bessho et al. 1994), was the poorest. Together with the data on the phenotypic values of the parental genotypes, a significant correlation (r=0.622) was observed between the normalized phenotypic values of parents and the BVs estimated by sib analysis.

Implications for breeding and cultivar development

The main purpose of our breeding program was the creation of new apple cultivars by intra-specific crossing among typical cultivars, selections, and breeding materials and the production of various breeding materials for increased disease resistance by inter-specific crossing between *M. domestica* and *M. sieboldii* for further breeding purposes in the apple breeding program at NIFTS. The primary objective of the sib analysis was to analyze the genetic variation available in a disease-resistance breeding program of apples at NIFTS, and the results are, for the most part, specific to this program. However, they indicate the extent and nature of the genetic variation available in a modern apple breeding program in which resistance to *V. ceratosperma* has been introgressed from *M. sieboldii*, and it is noteworthy that the methodology is of general relevance to apple breeding. Since many apple breeders worldwide are working with similar plant materials, such as "Golden Delicious", "(Red) Delicious", "Jonathan", "Gala", and "Fuji" (Laurens 1998; Kouassi et al. 2009), the results maybe generalized to current breeding populations of apple.

The breeding values shown in Table 8 indicate that *M. sieboldii* "Sanashi 63" and "Hayanarisanashi 1" were useful with respect to transmitting resistance to *V. ceratosperma*. The result will justify the use of such genotypes of *M. sieboldii* as a donor parent in a long-term apple breeding program to improve the resistance level to *V. ceratosperma*. To date, several accessions of *M. sieboldii* have been used to improve some disease-resistance traits in practical apple breeding programs, as they were found to possess the highest level of resistance to fire blight (Gardner et al. 1980), powdery mildew (Schuster 2000), and crown gall (Moriya et al. 2010).

The goal of apple breeding for disease resistance is to increase the resistance level while simultaneously improving or at least maintaining, original fruit quality. Our results indicate that it would be feasible to develop cultivars with increased resistance to Valsa canker. The experience with disease resistance in apple, e.g., apple scab, implies that continuous breeding is required (Crosby et al. 1992; Gessler et al. 2006). Further genetic information on Valsa canker resistance will facilitate resistance breeding and cultivar development in apple.

Acknowledgements We are grateful to Dr. Koichi Suzaki for providing the *Valsa ceratosperma* isolates used in this study. We also thank Dr. Hiroshi Iwanami for his help with statistical analysis.

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