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Evaluation of powdery mildew-resistance of grape germplasm and rapid amplified polymorphic DNA markers associated with the resistant trait in Chinese wild *Vitis*

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ABSTRACT. The resistance of wild *Vitis* germplasm, including Chinese and American wild *Vitis* and *Vitis vinifera* cultivars, to powdery mildew (*Uncinula necator* Burr.) was evaluated for two consecutive years under natural conditions. Most of the Chinese and North American species displayed a resistant phenotype, whereas all of the European species were highly susceptible. The Alachua and Conquistador accessions of *Vitis rotundifolia* species, which originated in North America, were immune to the disease, while Baihe-35-1, one of the accessions of *Vitis pseudoreticulata*, showed

the strongest resistance among all Chinese accessions evaluated. Three rapid amplified polymorphic DNA (RAPD) markers, OPW02-1756, OPO11-964, and OPY13-661, were obtained after screening 520 random primers among various germplasm, and these markers were found to be associated with powdery mildew resistance in Baihe-35-1 and in some Chinese species, but not in any European species. Analysis of F_1 and F_2 progenies of a cross between resistant Baihe-35-1 and susceptible Carignane (*V. vinifera*) revealed that the three RAPD markers were linked to the powdery resistant trait in Baihe-35-1 plants. Potential applications of the identified RAPD markers for gene mapping, marker-assisted selection, and breeding were investigated in 168 F_2 progenies of the same cross. Characterization of the resistant phenotype of the selected F_2 seedlings for breeding a new disease-resistant grape cultivar is in progress.

Key words: Grape germplasm; Chinese wild *Vitis*; Powdery mildew; Disease resistance; RAPD marker

INTRODUCTION

Grape powdery mildew is an important agronomical disease that is caused by the fungal species *Uncinula necator* Burr. The disease has been reported in grape production areas worldwide (Alleweldt et al., 1990; Roy and Ramming, 1990; He et al., 1991; Gee et al., 2000; Akkurt et al., 2007; Belhadj et al., 2008). Major commercial grape cultivars with high fruit quality are usually derived from the species *Vitis vinifera* L. through genetic crossing; however, this species is highly susceptible to infection by the powdery mildew pathogen (Boubals, 1961; Alleweldt et al., 1990; Pearson and Gadoury, 1991; Li and Zhang, 1992; Dalbó et al., 2001; Pauquet et al., 2001; Barker et al., 2005; Belhadj et al., 2008). In vineyards, the powdery mildew disease is primarily controlled by fungicide applications. However, heavy applications of fungicide pollute the environment, potentially affecting the safety of grape berries for consumption due to remaining fungicide residues. Frequently, fungicide application also increases selective pressures that likely facilitate the evolution of more resistant strains in the field. Therefore, the development and use of host resistant strains through genetic crosses and breeding would be an effective and more environmentally friendly strategy to comprehensively control powdery mildew disease.

In nature, highly resistant traits have evolved in various grape species derived from different parts of the world. For example, almost all grape species native to North America are, to various degrees, inherently resistant to powdery mildew disease (Boubals, 1961; Alleweldt et al., 1990; He and Luo, 1994; Reisch and Pratt, 1996; Staudt, 1997; Pauquet et al., 2001; Barker et al., 2005; Hoffmann et al., 2008). However, due to genomic variation in chromosome numbers, introgression of the resistant trait from *Vitis rotundifolia* into the high fruit quality cultivars derived from *V. vinifera* is extremely difficult (Lu et al., 2000). Moreover, *Vitis* species native to North America have relatively poor fruit quality. These genetic barriers impede the rapid development of new cultivars with high fruit quality and powdery mildew resistance. High levels of powdery mildew resistance have also been found

in some *Vitis* species native to China (He and Ren, 1990; He et al., 1991; Wang et al., 1995; Wang et al., 1998; Zhang et al., 2002; Wan et al., 2007), one of the major regions from which *Vitis* species originated (He et al., 1991). The major sources of powdery mildew resistance were found in wild Chinese *Vitis* species from across the country; however, most of these species are associated with poor fruit quality, an undesired trait for grape production. Unlike North American germplasm such as *Vitis labrusca*, these wild Chinese *Vitis* species have no foxy flavor and can be easily crossed with *V. vinifera* without genetic barriers, making the wild Chinese *Vitis* germplasm attractive candidates for genetic improvement of disease resistance in existing high fruit quality grape cultivars.

Despite numerous reports on the characterization of powdery mildew resistance in grape germplasm, the nature of resistance and linkage of genetic and molecular markers with resistance remains relatively unknown, especially in wild Chinese *Vitis* species. Selection for germplasm and hybrids based on phenotype is a time consuming process of plant cross breeding, whereas molecular markers allow for direct selection of favorable genotypes. As a valuable tool for genetic studies, random amplified polymorphic DNA (RAPD) technology has several distinct advantages: the procedure is simple, a very low amount of DNA is required for analysis, reaction costs are low, and the technology is faster and easier than amplified fragment length polymorphism (AFLP) or simple sequence repeat (SSR) analyses. Therefore, RAPD has been widely applied in grapevine genetic analyses (Weeden et al., 1994; Wang et al., 1996; Dalbó et al., 2001; Luo et al., 2002; Akkurt et al., 2007; Ercisli et al., 2008; Hoffmann et al., 2008). In this study, we comprehensively evaluated the powdery mildew resistant trait under natural conditions in wild Chinese *Vitis*, wild American *Vitis*, *V. vinifera*, and in interspecific hybrid F_1 and F_2 progenies derived from a cross between wild Chinese *Vitis pseudoreticulata* Baihe-35-1 and the *V. vinifera* cultivar Carignane. Furthermore, RAPD markers were developed and identified that were associated with the powdery mildew-resistant trait in *V. pseudoreticulata* Baihe-35-1. The identified RAPD markers were analyzed among the interspecific hybrid F_2 progenies in order to explore the application of the markers for marker-assistant selection breeding programs.

MATERIAL AND METHODS

Plant materials and powdery mildew-resistance identification

The grape materials used in this study included 23 accessions of nine wild Chinese *Vitis* species, 11 accessions of nine wild American *Vitis* species, 14 cultivars of *V. vinifera* (Table 1), seven individuals of the interspecific hybrid F_1 generation, and 207 individuals of the F_2 generation derived from Baihe-35-1 (*V. pseudoreticulata*) x Carignane (*V. vinifera*) (Table 2 to Table 6). Baihe-35-1, which served as one of the parents in the cross population, has been found to be the most resistant to powdery mildew among all native Chinese grape germplasms (Wang et al., 1995; Wang and He, 1997; Zhang et al., 2002). The F_1 progenies 6-12-1, 6-12-2, 6-12-3, 6-12-4, 6-12-5, 6-12-6, and 6-12-7 were derived from interspecific hybridization between Baihe-35-1 and Carignane in 1987. The 207 F_2 individuals resulted from self-crossing of each of the F_1 individuals 6-12-1, 6-12-2, 6-12-4, and 6-12-6 in 2001. These materials are maintained in the grape nursery of the Northwest Agriculture and Forestry University in China.

Table 1. Resistance to powdery mildew and demonstration of RAPD markers in grape resources.

Species	Accessions or varieties	Susceptibility index (SI)	Resistance level	Phenotype	OPW02-1756	OPO11-964	OPY13-661
V. pseudoreticulata	Baihe-35-1	7.23	3	R	+	+	+
W.T. Wang	Baihe-35-2	34.29	4	S	-	+	+
	Baihe-13	18.79	3	R	+	-	+
	Baihe-13-1	18.67	3	R	+	-	+
	Guangxi-1	16.36	3	R	-	-	+
	Guangxi-2	30.36	4	S	-	-	+
	Shangnan-1	28.93	4	S	-	-	-
	Shangnan-2	30.93	4	S	-	-	+
	Hunan-1	67.07	5	HS	-	-	+
V. davidii Foex.	Xuefeng	11.23	3	R	-	-	-
	Tangwei	10.50	3	R	-	+	+
V. ficifolia Bunge	Weinan-3	8.67	3	R		-	+
V. quinquangularis	Taishan-12	12.83	3	R		-	+
Rehd.	Shangnan-24	11.05	3	R	-	+	+
	83-4-96 (♀)	14.44	3	R	+	+	-
	Danfeng-2	15.51	3	R		+	+
V. amurensis Rupr.	Tonghua-3	21.00	3	R	-	-	+
	Zuoshan-2	23.69	3	R	+	+	+
	Heilongjiang Seedling	24.25	3	R		+	+
V. yeshanensis J.C. Chen	Yanshan-1	40.30	4	S	-	-	-
V. piasezkii Maxim.	Gansu-91	39.14	4	S	-	-	-
V. thunbergii Sieb. et Zucc.	Taishan-1	37.23	4	S	-	-	-
V. qinlingensis P.C. He	Pingli-5	32.98	4	S	-	-	-
V. rotundifolia Michx.	Alachua	0	1	IS	-	-	-
	Conquistador	0	1	IS	-	-	-
V. riparia Michx.		18.00	3	R	-	-	+
V. californica Benth.	Gold Hill#1	15.86	3	R	+	-	-
V. champini Planch.		11.86	3	R	+	-	-
V. rupestris Scheele	A. De Serres	6.15	3	R	-	-	-
V. berlandieri Planch.		8.00	3	R	-	-	-
V. labrusca L.	Y157 (Y18-95)	19.09	3	R	-	-	-
V. arizonica Engelm.		9.50	3	R	+	-	-
V. cinerea Engelm.		12.57	3	R	+	-	-
V. vinifera L.	Carignane	49.27	4	S	-	-	-
	Riesling	44.43	4	S	-	-	-
	May Purple	28.36	4	S	-	-	-
	Chein Blanc	42.50	4	S	-	-	-
	Muscat Rose	39.14	4	S	-	-	-
	Muscat Blanc	41.36	4	S	-	-	-
	Muscat Hamburg	57.43	5	HS	-	-	-
	Blue French	85.00	5	HS	-	-	-
	Ugni Blanc	71.78	5	HS	-	-	-
	Dalihong Seedless	52.39	5	HS	-	-	-
	Jingkejing	66.84	5	HS	-	-	-
	Black Rose	61.24	5	HS	-	-	-

IS = insusceptible, the resistance level is class 1; HR = high resistance, class 2; R = resistance, class 3; S = susceptible, class 4; HS = high susceptible, class 5; (+) = present for genes marker, (-) = absent for genes marker. (The same as below).

The presence of powdery mildew resistance was evaluated in all tested plants between July and August in 2004 and 2005, respectively, under field conditions, since powdery mildew symptoms become fully developed between July and August. A total of 100 leaves from each clone were randomly surveyed, and symptoms in each leaf sample were scored in eight categories (0, 1, 2, 3, 4, 5, 6, and 7), which corresponded to the estimated percentage of lesions in the entire leaf area (0, 0.1-5.0, 5.1-15.0, 15.1-30.0, 30.1-45.0, 45.1-65.0, 65.1-85.0, and above 85.0), respectively. A score of zero represents the most resistant phenotype, whereas a score of seven represents the most susceptible phenotype. The scores were converted to a susceptibility index:

SI = [(Sum of grade value x Number of leaves in that grade)/(Total leaf number x Highest grade value)] x 100. The average SI across the two years was used as the final SI of each clone. The resistance level of each clone was classed within five classes based on its SI: Insusceptible (IS), SI = 0, class 1; High resistance (HR), SI = 0.1-0.5, class 2; Resistance (R), SI = 5.1-25.0, class 3; Susceptible (S), SI = 25.1-50, class 4; Highly susceptible (HS), SI = 50.1-100, class 5.

RAPD analysis

Genomic DNA was isolated from young grapevine leaves using the CTAB method as described by Wang et al. (1996). The polymerase chain reaction (PCR) was run in a total 25 µL volume, containing 500 mM KCl, 100 mM Tris-HCl, pH 9.0, 1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.15 µM primer, 45 ng genomic DNA, and 1 U Taq DNA polymerase (Hua Mei Co. Luoyang, China). The amplification was performed in a DNA thermal cycler (Model PTC-100, Gene Co.) for pre-denaturation at 94°C for 5 min followed by 45 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C, with a final extension step at 72°C for 10 min. The amplification products were separated by electrophoresis on 1.2% agarose gels containing 0.5 µg/mL ethidium bromide in 1X TAE buffer (40 mM Tris-acetate, pH 8.0, 1 mM EDTA) at 120 V for 1 h. In all cases, the DNA marker was used as the size marker. The gel was examined and photographed on a UV-light box. All reactions were repeated at least three times.

Linkage analysis for RAPD markers linked to powdery mildew-resistant genes

To obtain specific primers, 520 random primers were initially screened with the resistant parent Baihe-35-1 and the susceptible parent Carignane as well as with their F_1 resistant (6-12-6) and susceptible (6-12-2) progenies. The linkage between the identified RAPD markers and powdery mildew resistance was roughly established through analysis of F_1 and F_2 (6-12-6 selfing) segregation populations of crosses between Baihe-35-1 and Carignane, and was then further verified in wild Chinese *Vitis*, *V. vinifera*, and wild American *Vitis* plants or germplasms. The map distances and associations were analyzed using data from 39 progenies of the 6-12-6 selfing population with the Joinmap version 4.0 software.

Cloning and sequencing of RAPD products

The amplified RAPD marker fragments were excised, purified using the DNA agarose gel cleanup kit (Tian Wei Shi Dai Biotechnology Co. Beijing, China), and cloned into the pGEM-T easy vector (Promega, USA). Several positive clones of each selected fragment were isolated and sequenced by the Shanghai Sangon Company. The marker sequences of a 1756-bp fragment amplified by the primer OPW02, a 964-bp fragment by the OPO11 primer, and a 661-bp fragment by the OPY13 primer were deposited in the GenBank database under the accession numbers AY491396, DQ493890, and DQ493892, respectively.

Marker detection in the F_2 generation of Baihe-35-1 x Carignane using the three specific primers

To verify the three RAPD markers in accordance with the resistance phenotype under

field conditions, RAPD analysis was performed with selfing progenies from F_1 plants 6-12-1, 6-12-2, and 6-12-4 using the three specific primers OPW02, OPO11, and OPY13, respectively. The method for RAPD analysis was identical to that described above.

RESULTS

Evaluation of powdery mildew resistance performance in Chinese grape germplasm

Various accessions or cultivars were evaluated for resistance and susceptibility to powdery mildew infection in two years under the same field conditions, and all results are summarized in Table 1. As expected, North American and European species were highly resistant and susceptible to powdery mildew infection under the same conditions, respectively, which is consistent with results of previous studies (Boubals, 1961; Olmo, 1971; Pearson and Gadoury, 1991; Li and Zhang, 1992; Wang et al., 1995; Staudt, 1997; Wang and He, 1997; Zhang et al., 2002). Some accessions of native Chinese *V. pseudoreticulata* are known to be particularly resistant to several common pathogens that infect grape plants in the Yangling area of China (He and Ren, 1990; He et al., 1991; Wang et al., 1995; Wang et al., 1998; Zhang et al., 2002; Wan et al., 2007). Of the nine accessions of *V. pseudoreticulata* evaluated, four showed resistance, while the rest were susceptible to powdery mildew infection. Of the resistant accessions, Baihe-35-1 displayed the strongest resistance with the lowest SI value (7.23), followed by Guangxi-1, Baihe-13-1, and Baihe-13, with SI values of 16.36, 18.67, and 18.79, respectively. Conversely, Hunan-1 was severely infected with a high SI value (67.07), which is comparable to that of *V. vinifera*, a species that is known to be the most susceptible to powdery mildew infection. We also found that all four accessions from *V. yeshanensis*, *V. piasezkii*, *V. thunbergii*, and *V. qinlingenesis* were susceptible, and all 10 accessions from the other four species, including *V. davidii*, *V. ficifolia*, *V. quinquangularis*, and *V. amurensis* showed resistance, although their SI values, an inverse indicator of resistance, were higher than that of Baihe-35-1 plants, indicating that the Baihe-35-1 line is the most resistant of all resistant wild Chinese *Vitis* lines evaluated.

Inheritance of the powdery mildew resistant trait in F_1 and F_2 progeny of an interspecific cross between the resistant Baihe-35-1 and the susceptible Carignane parental lines

To investigate the inheritance behavior of the identified resistant trait in the Baihe-35-1 line, Baihe-35-1 was crossed with the susceptible Carignane line, and resistance was evaluated in F_1 hybrid plants. Table 2 shows that only three (6-12-4, 6-12-5, and 6-12-6) of the seven hybrid plants displayed resistance, while the rest (6-12-1, 6-12-2, 6-12-3, and 6-12-7) were susceptible to powdery mildew infection, indicating that resistance in Baihe-35-1 is not a completely dominant trait. To further analyze the resistance pattern in F_2 plants, the resistant F_1 line 6-12-6 (SI = 23.92) was chosen for self-crossing, and 39 F_2 plants were obtained. Table 3 shows that 26 of the 39 lines were resistant, while the rest were susceptible, representing a roughly 2:1 ratio of resistance to susceptibility. This ratio deviates from the typical 3:1 ratio of a single dominant gene phenotype segregation pattern.

Furthermore, self-crossing in the susceptible F_1 lines 6-12-1 (SI = 55.15) and 6-12-2 (SI = 69.93) were also performed, yielding a total of 56 and 61 F_2 plants, respectively, which both showed

a roughly 1:1 ratio of resistance to susceptibility (32:24 and 33:28) (Tables 4, 5). For the resistant F_1 line 6-12-4 (SI = 24.38), 51 individuals were obtained from its selfing, and only one individual showed susceptibility, while the others were all resistant (Table 6). These results are not in accordance with the typical 3:1 ratio of a single dominant gene phenotype segregation pattern. Therefore,

Table 2. Resistance to powdery mildew and the demonstration of RAPD markers in F_1 individuals from Baihe-35-1 x Carignane.

Parents and F_1 individuals	Susceptibility index	Level of resistance	Phenotype	OPW02-1756	OPO11-964	OPY13-661
Baihe-35-1	7.23	3	R	+	+	+
Carignane	49.27	4	S	-	-	-
6-12-1	55.15	5	HS	-	-	-
6-12-2	69.93	5	HS	-	-	-
6-12-3	51.53	5	HS	-	+	-
6-12-4	24.38	3	R	+	+	-
6-12-5	22.44	3	R	+	+	+
6-12-6	23.92	3	R	+	+	+
6-12-7	44.56	4	S	-	+	+

Table 3. Resistance to powdery mildew and RAPD analysis results in the progenies from 6-12-6 selfing using the special primers.

Ancestor parents, parents and progenies	Susceptibility index	Level of resistance	Phenotype	OPW02-1756	OPO11-964	OPY13-661
Baihe-35-1	7.23	3	R	+	+	+
Carignane	49.27	4	S	-	-	-
6-12-6	23.92	3	R	+	+	+
6-1	19.91	3	R	+	+	+
6-2	19.40	3	R	+	+	+
6-4	23.66	3	R	+	+	+
6-5	35.34	4	S	+	+	+
6-6	19.68	3	R	+	+	+
6-9	29.50	4	S	+	-	-
6-10	7.05	3	R	+	+	+
6-11	20.08	3	R	+	+	+
6-12	11.31	3	R	+	+	+
6-13	14.51	3	R	+	+	-
6-14	13.88	3	R	+	+	+
6-15	12.60	3	R	+	+	-
6-16	18.86	3	R	-	+	+
6-17	35.89	4	S	-	-	-
6-18	24.35	3	R	+	-	+
6-19	7.90	3	R	+	+	+
6-20	32.01	4	S	+	-	+
6-21	19.43	3	R	-	-	-
6-22	11.29	3	R	-	-	-
6-24	35.68	4	S	-	+	-
6-25	25.81	4	S	-	-	-
6-26	40.67	4	S	-	-	-
6-27	17.32	3	R	+	-	+
6-28	25.43	4	S	+	+	+
6-29	15.62	3	R	+	+	+
6-30	37.88	4	S	+	+	+
6-31	20.61	3	R	+	+	+
6-32	16.67	3	R	+	-	+
6-33	25.99	4	S	+	-	+
6-34	22.18	3	R	+	+	+
6-38	31.29	4	S	+	-	+
6-39	5.61	3	R	+	+	+
6-40	5.00	2	HR	+	+	+
6-47	15.67	3	R	+	+	-
6-48	25.40	4	S	-	+	+
6-49	16.87	3	R	-	+	+
6-50	19.37	3	R	+	+	+
6-51	27.89	4	S	-	-	-
6-52	13.81	3	R	+	-	+

we suggest that resistance in Baihe-35-1 plants is controlled by multiple genes, including a major resistant gene and some minor resistant genes. Resistance in grapevine is mainly determined by the major resistant gene, and the minor resistant genes have accumulative action in hybrid progeny.

Table 4. RAPD detection results of offspring individuals from 6-12-1 selfing using the three special primers.

Ancestor parents, parents and progenies	Susceptibility index	Level of resistance	Phenotype	OPW02-1756	OPO11-964	OPY13-661
Baihe-35-1	7.23	3	R	+	+	+
Carignane	49.27	4	S	-	-	-
6-12-1	55.15	5	HS	-	-	-
1-1	9.04	3	R	+	+	-
1-3	5.25	3	R	+	-	-
1-4	30.04	4	S	+	-	-
1-5	12.92	3	R	-	+	-
1-6	15.92	3	R	+	-	-
1-7	14.83	3	R	+	-	-
1-8	48.04	4	S	+	-	-
1-9	9.29	3	R	-	-	-
1-11	14.68	3	R	+	+	-
1-12	23.21	3	R	+	+	-
1-13	8.10	3	R	+	+	-
1-14	13.54	3	R	+	-	-
1-15	28.97	4	S	-	-	-
1-16	20.69	3	R	+	-	-
1-17	8.29	3	R	+	-	-
1-18	25.23	4	S	-	-	-
1-19	40.24	4	S	+	-	-
1-20	27.85	4	S	+	-	-
1-21	40.21	4	S	-	-	-
1-22	13.17	3	R	+	+	-
1-23	8.45	3	R	+	+	
1-24	10.00	3	R	-	-	-
1-25	13.12	3	R	+	-	-
1-26	42.25	4	S	+	-	-
1-27	30.57	4	S	-	-	-
1-28	18.37	3	R	+	-	-
1-31	25.12	4	S	-	-	-
1-32	29.82	4	S	+	-	-
1-33	33.10	4	S	+	-	-
1-34	6.13	3	R	+	-	-
1-35	20.86	3	R	+	-	-
1-36	42.71	4	S	-	-	-
1-37	23.36	3	R	+	-	-
1-38	19.61	3	R	-	-	-
1-39	21.00	3	R	+	+	-
1-40	17.80	3	R	+	+	-
1-41	31.86	4	S	-	+	-
1-42	41.65	4	S	-	-	-
1-43	36.80	4	S	+	-	-
1-44	31.40	4	S	-	-	-
1-45	11.45	3	R	+	+	-
1-47	14.86	3	R	+	-	-
1-48	24.24	3	R	-	-	-
1-49	22.85	3	R	+	-	-
1-50	14.20	3	R	-	+	-
1-53	18.27	3	R	-	-	-
1-55	45.71	4	S	+	-	-
1-56	31.10	4	S	-	-	-
1-57	31.53	4	S	+	-	-
1-58	16.45	3	R	+	+	-
1-59	40.40	4	S	+	-	-
1-60	51.10	5	HS	-	-	-
1-61	15.20	3	R	+	+	-
1-62	28.00	4	S	+	-	-
1-63	61.82	5	HS	-	-	-
1-64	7.14	3	R	+	+	-

Table 5. RAPD detection results of offspring individuals from 6-12-2 selfing using the three special primers.

Ancestor parents, parents and progenies	Susceptibility index	Level of resistance	Phenotype	OPW02-1756	OPO11-964	OPY13-661
Baihe-35-1	7.23	3	R	+	+	+
Carignane	49.27	4	S	-	-	-
6-12-2	69.93	5	HS	-	-	-
2-1	48.61	4	S	-	-	-
2-2	46.29	4	S	-	-	-
2-3	35.55	4	S	-	-	-
2-4	46.69	4	S	+	-	+
2-5	37.68	4	S	-	-	-
2-7	62.34	5	HS	-	-	-
2-8	29.83	4	S	-	-	-
2-9	36.83	4	S	+	-	-
2-10	59.21	5	HS	+	-	-
2-13	48.41	4	S	-	-	-
2-15	48.79	4	S	-	-	-
2-16	13.50	3	R	+	+	-
2-17	7.02	3	R	+	+	-
2-18	21.00	3	R	+	-	+
2-19	30.06	4	S	+	-	+
2-20	34.05	4	S	-	-	-
2-21	42.87	4	S	-	+	-
2-22	7.42	3	R	-	+	-
2-23	53.90	5	HS	+	-	-
2-24	38.50	4	S	+	+	+
2-25	21.93	3	R	-	-	-
2-30	35.08	4	S	-	+	-
2-35	19.57	3	R	+	-	+
2-37	12.24	3	R	-	-	-
2-38	9.67	3	R	+	+	-
2-39	12.70	3	R	-	+	-
2-42	14.81	3	R	+	-	-
2-43	16.60	3	R	-	-	+
2-44	11.46	3	R	+	+	+
2-45	10.43	3	R	-	+	-
2-46	9.32	3	R	+	-	-
2-47	23.50	3	R	-	-	-
2-48	27.65	4	S	-	-	-
2-49	28.38	4	S	+	-	-
2-50	14.14	3	R	-	-	-
2-51	12.89	3	R	-	-	+
2-54	26.20	4	S	-	+	-
2-55	27.61	4	S	-	-	-
2-56	22.58	3	R	+	-	-
2-57	10.85	3	R	-	-	-
2-58	16.17	3	R	-	-	-
2-59	30.09	4	S	-	+	-
2-60	41.68	4	S	-	-	-
2-61	21.15	3	R	-	-	-
2-62	35.28	4	S	-	-	-
2-63	29.18	4	S	-	+	-
2-64	35.31	4	S	-	-	-
2-65	32.73	4	S	-	-	-
2-66	19.29	3	R	+	+	-
2-67	24.36	3	R	+	+	-
2-68	14.85	3	R	+	-	-
2-69	22.00	3	R	+	+	+
2-70	27.26	4	S	-	-	+
2-71	21.00	3	R	-	-	-
2-72	14.18	3	R	+	-	-
2-73	21.14	3	R	-	-	-
2-74	13.58	3	R	-	+	-
2-75	15.09	3	R	-	-	-
2-76	20.22	3	R	+	-	-
2-77	21.79	3	R	-	-	-
2-78	10.50	3	R	+	-	-

Table 6. RAPD detection results of offspring individuals from 6-12-4 selfing using the three special primers.

Ancestor parents, parents and progenies	Susceptibility index	Level of resistance	Phenotype	OPW02-1756	OPO11-964	OPY13-661
Baihe-35-1	7.23	3	R	+	+	+
Carignane	49.27	4	S	-	-	-
6-12-4	24.38	3	R	+	+	-
4-1	18.86	3	R	+	+	-
4-2	5.28	3	R	-	+	-
4-3	6.38	3	R	+	-	-
4-5	5.57	3	R	-	+	-
4-7	8.31	3	R	+	-	-
4-8	9.05	3	R	+	-	-
4-12	9.81	3	R	+	-	-
4-13	18.55	3	R	+	-	-
4-15	6.15	3	R	-	+	-
4-18	7.15	3	R	+	+	-
4-19	16.75	3	R	+	+	-
4-20	10.04	3	R	+	+	-
4-21	6.41	3	R	+	+	-
4-22	12.72	3	R	-	-	-
4-25	15.05	3	R	-	+	-
4-26	13.00	3	R	+	-	-
4-28	10.24	3	R	-	+	-
4-30	12.84	3	R	+	-	-
4-31	3.80	2	HR	-	-	-
4-32	8.57	3	R	-	+	-
4-33	7.00	3	R	-	+	-
4-34	6.79	3	R	+	+	-
4-41	9.41	3	R	-	+	-
4-42	8.76	3	R	-	+	-
4-44	8.86	3	R	+	+	-
4-46	5.68	3	R	+	+	-
4-48	5.89	3	R	-	+	-
4-49	6.72	3	R	+	+	-
4-50	11.51	3	R	+	-	+
4-51	15.81	3	R	-	+	+
4-52	11.28	3	R	+	-	+
4-53	7.64	3	R	+	+	+
4-54	10.98	3	R	+	-	-
4-55	10.61	3	R	-	+	-
4-56	25.57	4	S	-	+	-
4-57	10.33	3	R	+	+	-
4-58	5.98	3	R	-	+	-
4-59	6.27	3	R	+	+	-
4-60	5.71	3	R	-	-	+
4-61	6.47	3	R	-	+	-
4-62	6.75	3	R	+	+	-
4-64	5.36	3	R	-	+	-
4-65	10.36	3	R	-	-	-
4-72	9.20	3	R	-	-	-
4-75	20.44	3	R	+	-	-
4-76	14.44	3	R	+	+	+
4-78	6.96	3	R	+	+	-
4-81	4.73	2	HR	-	+	+
4-82	4.85	2	HR	+	+	-
4-83	7.40	3	R	-	-	-
4-84	18.00	3	R	+	-	-

Identification of RAPD markers associated with the powdery mildew-resistant trait in wild Chinese *Vitis*

Of the 520 random primers screened, at least three primers, OPW02

(5'-ACCCCGCCAA-3'), OPO11 (5'-GACAGGAGGT-3'), and OPY13 (5'-GGGTCTCG-GT-3'), were found to be associated with the resistant trait in Baihe-35-1, as well as in some other Chinese *Vitis* species. As shown in Table 1, these markers could amplify a specific DNA fragment size in Baihe-35-1 plants and in some other resistant wild Chinese *Vitis* species, but not in any of the four susceptible Chinese and European lines. This result suggested that these markers are specifically associated with powdery mildew resistance in wild Chinese *Vitis* Baihe-35-1 and some other accessions. In F_1 progenies derived from the cross between Baihe-35-1 and Carignane, the OPW02 primer amplified a specific 1756-bp band from three resistant lines (6-12-4, 6-12-5, and 6-12-6), but not in the susceptible lines (6-12-1, 6-12-2, 6-12-3, and 6-12-7) (Table 2, Figure 1). A similar association was also found using the OPO11-964 and OPY13-661 markers that could amplify DNA fragments in parental and F_1 resistant lines, as well as in some of the susceptible F_1 lines (Table 2, Figures 2, 3), indicating that all three RAPD markers tested are generally linked to the powdery resistant trait in the Baihe-35-1 accession or clones. DNA cloning and sequencing analyses showed that none of the three DNA fragments amplified by the individual RAPD analysis had any relationship to sequences of genes involved in disease resistance.

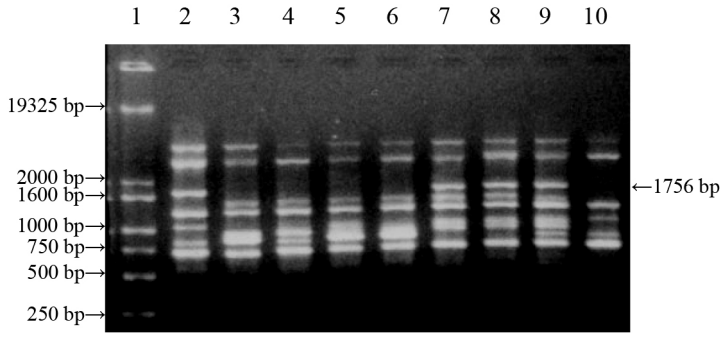

Figure 1. Schematics of RAPD products obtained from parents and F_1 individuals of Baihe-35-1 x Carignane using RAPD primer OPW02.

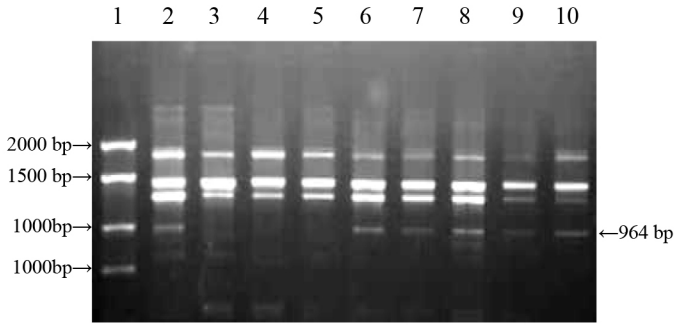

Figure 2. Schematics of RAPD amplification products from parents and F_1 individuals of combination Baihe-35-1 x Carignane using RAPD primer OPO11.

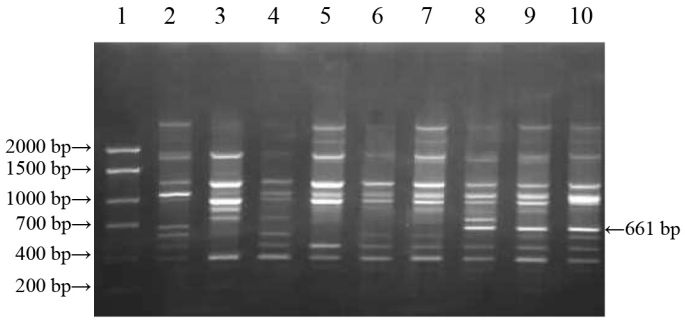

Figure 3. Schematics of RAPD amplification products from parents and F_1 individuals of combination Baihe-35-1 x Carignane using RAPD primer OPY13.

To further verify this linkage, 39 F_2 progenies from the self-crossing of the resistant 6-12-6 F_1 line were subjected to the same RAPD analyses as described above. We found that 22 of the 26 resistant plants, and 7 of the 13 susceptible lines gave the OPW02-1756 marker pattern, corresponding to the 1756-bp fragment (Table 3, Figure 4). Twenty-one of the 26 resistant plants, and 5 of the 13 susceptible lines yielded the OPO11-964 marker band corresponding to a 964-bp fragment (Table 3, Figure 5), and 22 of the 26 resistant plants, and 7 of the 13 susceptible lines generated the OPY13-661 marker band corresponding to a 661-bp fragment (Table 3, Figure 6). These results again strongly suggested that all three markers were linked to the resistant gene(s) in Baihe-35-1 plants. This suggestion was further confirmed by linkage analysis using the Joinmap program. The markers OPW02-1756 and OPY13-661 were found to link together and were located 22.2 cM away from the resistance gene on one side, while the marker OPO11-964 was located at a similar distance to the resistant gene, but on the opposite side.

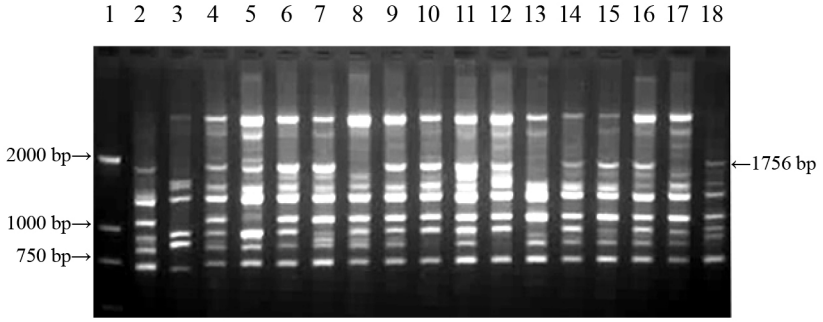

Figure 4. Schematics of RAPD amplification products from individuals of 6-12-6 self-pollination using RAPD primer OPW02.

Detection of the identified RAPD markers for powdery mildew resistance in interspecific hybrid F_2 progeny

The linkage of the identified three markers provides a great utility for selection and

breeding for powdery mildew resistance in progenies of genetic crossing programs. To test this utility, we self-crossed three F_1 lines, including 6-12-1, 6-12-2, and 6-12-4, and a total of 168 F_2 individuals were obtained. One hundred fifteen of the 168 individuals demonstrated resistance to powdery mildew under natural field conditions. We found that four of the 115 resistant individuals carried all three RAPD markers, 36 individuals carried two of the markers, and 53 possessed only one of the three markers (Tables 4, 5, and 6). This indicated that 93 resistant individuals possessed the RAPD markers, whereas the remaining 22 resistant individuals and 27 of the 53 susceptible individuals had none of the three markers. This pattern might have been due to chromosome exchange during pairing between male and female gametes.

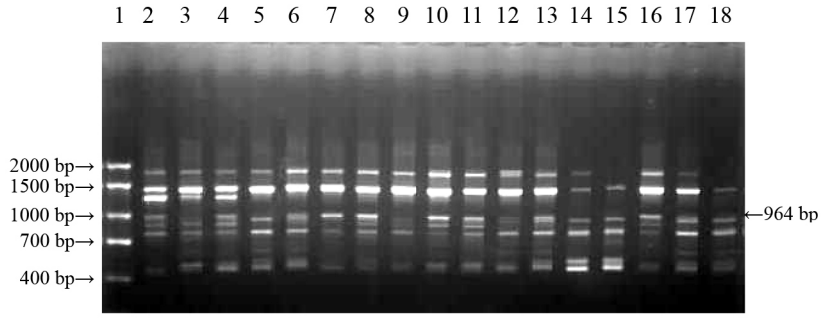

Figure 5. Schematics of RAPD amplification products from individuals of 6-12-6 self-pollination using RAPD primer OPO11.

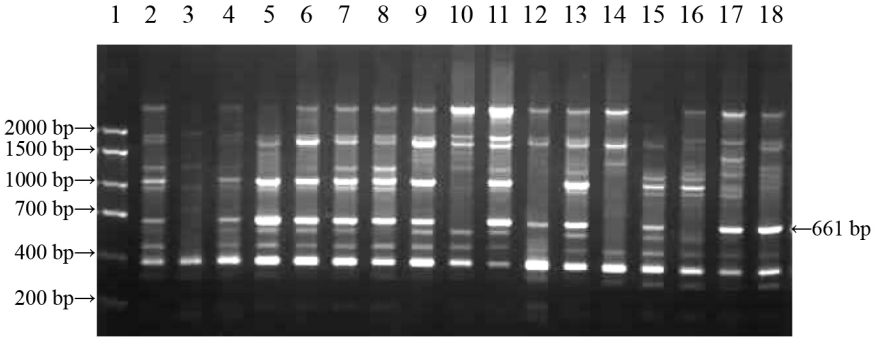

Figure 6. Schematics of RAPD amplification products from individuals of 6-12-6 self-pollination using RAPD primer OPY13.

DISCUSSION

Characterization of powdery mildew resistance in grape germplasm have been extensively reported, and powdery mildew-resistant traits were found to have independently evolved in North American and Chinese species, but not in European species (Boubals, 1961; Olmo, 1971; Bouquet, 1980; Roy and Ramming, 1990; He et al., 1991; Li and Zhang, 1992; Honrao et al., 1992; Wang et al., 1995; Staudt, 1997; Kozma, 2000; Zhang et al., 2002; Fischer

et al., 2004; Wan et al., 2007). Consistent with these findings, we also observed that only Chinese and North American, but not European, species showed resistance to powdery mildew disease to various degrees in plants grown in the same location over two consecutive years (Table 1), indicating that resistance persists in various climates, and is therefore likely determined by genetic factors. Interestingly, North American species appear to generally confer much stronger resistance to powdery mildew infection compared to Chinese species. Some North American species, such as *V. rotundifolia*, were completely resistant, as evidenced by a zero SI index value, whereas Baihe-35-1, the most strongly resistant line among Chinese species, remains susceptible to the infection to some extent with an SI index value as high as 7.23 (Table 1). Whether North American and Chinese species share similar or different resistance mechanisms remains unknown. The fact that three RAPD markers were found to be associated with resistant genes only in the Chinese species Baihe-35-1 suggests that the particular resistant genes or mechanisms evolved might be different between North American and Chinese species. However, the possibility that the diverged genome sequences of North American and Chinese species may contribute to differential detection of the three markers cannot be excluded. Hence, further molecular and genetic analyses would provide insight into understanding the evolution of resistance mechanisms in different *Vitis* species.

Previous studies have shown that *V. pseudoreticulata* distributed across eastern and southern China confers broad resistance to various fungal diseases, such as *Plasmopara viticola*, *Sphaceloma ampelinum, U. necator*, and *Glomerella cingulata* (He et al., 1991; Wang et al., 1995; Wang et al., 1998; Zhang et al., 2002; Wan et al., 2007). The present study also demonstrated that at least four of the nine accessions tested were barely infected by the powdery mildew under natural conditions, indicating that *V. pseudoreticulata* species have acquired resistance to the powdery mildew pathogen. We also found that not all of the *V. pseudoreticulata* accessions were resistant, and five of the nine were susceptible. In particular, Hunan-1, one of the nine accessions, was extremely susceptible at a level comparable to that of European cultivars such as Jingkejing or Black Rose (Table 1). This result is not completely consistent with those of Wan et al. (2007).

Powdery mildew resistance was shown to be controlled by genetic factors. Previous research showed that the resistant behavior was a qualitative genetic trait that was presumably controlled by a single dominant gene. For example, Filippenko and Shtin (1975) studied the inheritance of powdery mildew resistance in hybrids of *V. vinifera* x *V. amurensis*. Bouquet (1980) investigated powdery mildew resistance in the *Vitis* subgenus *Muscadine*. Resistance to powdery mildew in *Muscadinia rotundifolia* was mapped to the single dominant locus *Run1* (Barker et al., 2005; Dry et al., 2009), and was also mapped to a single dominant locus, *REN1* in the Central Asian *V. vinifera* variety 'Kishmish vatkana' (Hoffmann et al., 2008). Another study suggested that resistance in *V. amurensis* and *V. labrusca* species was controlled by a dominant gene (Kozma, 2000), while similar analyses showed that although resistance in Chinese *Vitis* species behaved in a dominant independent inheritance pattern, it was most likely controlled by a couple of genes (Wang and He, 1997). It is generally believed that powdery mildew resistance is controlled by quantitative genes (Boubals, 1961; Li and Zhang, 1992; Honrao et al., 1992; Eibach, 2000; Dalbó et al., 2001). The present analysis on resistance inheritance patterns in F_1 and F_2 progeny of the same cross favors a major dominant gene control mechanism since F_1 progenies of the Baihe-35-1 and Carigane cross displayed a close 1:1 ratio of resistance to susceptibility, and F_2 progenies of the same cross followed a roughly 2:1

ratio of resistance to susceptibility (Tables 2, 3), indicating that more than one gene is likely involved in the regulation of resistance in Baihe-35-1 plants.

Dominant inheritance of powdery mildew resistance in Baihe-35-1 plants would facilitate the development of genetic and molecular markers for gene mapping and association, as well as for marker-assisted selection and breeding. One of the most powerful mapping strategies is bulked segregant analysis (BSA) (Michelmore et al., 1991), which is an efficient method for mapping resistance genes in highly heterozygous fruit trees. The BSA method requires the construction of resistant and susceptible pools, respectively, based on the segregation of resistance genes in the F_2 generation. In the present study, construction of such pools was limited due to the low number of F_1 individuals. Therefore, we selected the F_1 resistant 6-12-6 and susceptible 6-12-2 lines, as well as both of their parental lines for primer selection. Three potential candidate RAPD markers, OPW02-1756, OPO11-964, and OPY13-661, that were potentially associated with powdery mildew resistance, were initially obtained through screening over 500 random primers. Analyses in parental, F_1, and F_2 progenies revealed that the markers were located approximately 22 cM away from the powdery mildew resistance gene in Baihe-35-1 plants on either side. Practical applications of these markers for marker-assisted selection during breeding were also investigated in several crosses. F_2 progenies associated with one, two, or all three markers were successfully identified (Tables 4, 5, 6), and these selected lines will expedite the breeding process, especially during the early stage of breeding.

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