BREEDING *VITIS RUPESTRIS* X *MUSCADINIA ROTUNDIFOLIA* ROOTSTOCKS TO CONTROL *XIPHINEMA INDEX* AND FANLEAF DEGENERATION.

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Abstract

Fanleaf degeneration, caused by grapevine fanleaf virus (GFLV) and vectored by the dagger nematode, *Xiphinema index*, remains one of the world's most serious grape diseases. *Muscadinia rotundifolia* has very strong resistance to *X. index* feeding and has been reported to resist GFLV. *Vitis rupestris* X *M. rotundifolia* seedlings were produced at the University of California, Davis and 200 F1 individuals were screened for *X. index* resistance. Seventy of them were found to be resistant and two seedling populations (8909 and 8913) were used for DNA marker screening and inheritance testing. One of 70 RAPD primers (OPA-12) was found to be linked with resistance. The OPA-12 product was cloned and sequenced to develop a more sensitive SCAR (sequence characterized amplified region) primer. About 30 of these resistant seedling selections are now in seven trials to evaluate field performance and resistance to fanleaf degeneration. Cytogenetic investigation of these F1 hybrids revealed 38 chromosomes, although abnormal segregation ratios for *X. index* resistance were obtained.

These *V. rupestris* X *M. rotundifolia* hybrids are unexpectedly fertile and we produced F2 generations of resistant X susceptible, resistant X resistant, backcrosses to both parents and a selfed resistant seedling to investigate the inheritance of resistance. More than 2,000 hybrid individuals have been tested for *X. index* resistance and normal Mendelian segregation ratios were obtained. The results, except those for the selfed group, suggest that resistance is controlled by a single dominant gene.

Construction of genetic linkage maps is a fundamental process in the detection of gene(s) controlling trait expression. One of the F2 seedling groups (resistant X susceptible) with 350 individuals was used for genetic linkage analysis. AFLP (amplified fragment length polymorphism) technology was used to search for markers in a 116 randomly selected seedlings from the group of 350 seedlings. Over 500 segregating AFLP markers generated 19 linkage groups for both parents. This map will form framework for introgression of many important resistance traits from *Muscadinia* into *Vitis*.

1. Introduction

The University of California, Davis effort to breed grape rootstocks resistant to fanleaf began with an evaluation of *Vitis* germplasm and identified *V. rufotomentosa* as highly resistant to *Xiphinema index* feeding (Kunde *et al.* 1968). This species and others were utilized as parents in the production of rootstock selections which began field

testing in 1979. This trial also included six *V. vinifera* X *Muscadinia rotundifolia* (VR) hybrids produced by Olmo in 1948 to study the cytogenetics of these genera and to introgress the disease and pest resistance of *M. rotundifolia* while maintaining the fruit quality of *V. vinifera* (Patel and Olmo 1955). Two rootstocks, O39-16 and O43-43, were released as a result of these trials (Walker et al. 1991).

At the time of their release, both O39-16 and O43-43 were considered to be fanleaf degeneration resistant. Since that time, however, both rootstocks have allowed movement of grapevine fanleaf virus (GFLV) into scions grafted on them. These stocks allow movement of GFLV into scions, but prevent GFLV's disruptive effect on fruit set (Walker *et al.* 1994). In 1990, a new rootstock breeding program began at UC Davis. O43-43 had recently collapsed to phylloxera, the continued phylloxera resistance of O39-16 was in doubt, and uncertainties regarding the long-term use and fanleaf resistance of these rootstocks led to renewed fanleaf degeneration breeding efforts. The report presented here summarizes these efforts.

2. Materials and Methods

Seedlings from crosses using two *V. rupestris* females and six *Muscadinia rotundifolia* cultivars as males were evaluated for resistance to *X. index*. This resistance screen also included seedlings from crosses of *V. berlandieri* X *V. rufotomentosa*. Table 1 presents the parents and the number of seedlings tested.

Five replicate cuttings from each seedling were inoculated with 100 viruliferous nematodes. The cuttings were rooted and planted in 500 cm^3 plastic pots with a sand:clay loam soil mix. Previous experiments optimized this evaluation system and determined that nematode populations peaked after 4 to 6 months. These experiments also determined that gall numbers were faster and easier to count than nematode numbers and that these parameters were highly correlated (R^2 = 0.876). All the inoculations were conducted with *V. rupestris* 'St. George' and *M. rotundifolia* 'Trayshed' as susceptible and resistant controls, respectively. The inoculated cuttings were evaluated after 5 months.

Polymerase chain reaction (PCR) with randomly amplified polymorphic DNA (RAPD) primers was used to find RAPD markers associated with *X. index* resistance. Seedling populations 8909 and 8913 were chosen for this analysis because they segregated for resistance and had relatively high numbers of seedlings. DNA extracted from the seedling progeny was screened for RAPD markers using 79 decamer primers from Operon Technology Inc. (random primer kits A, G, H, I and U) or the University of British Columbia (UBC 237, 238 and 239). These primers were used to produce 334 markers. OPA-12 produced a band that was very tightly linked to resistance. This marker band was sequenced and used to produce a sequence characterized amplified region (SCAR) primer. This SCAR primer was used to demonstrate the true hybrid nature of the seedlings, and produce a single amplified band in resistant seedlings.

An F2 generation of seedlings was produced from crosses of resistant X susceptible siblings (Table 2). These were screened for *X. index* resistance by inoculating 100 nematodes directly into seedling pots. After 4 months the seedlings were evaluated for resistance by scoring the number of root galls. One hundred and sixteen seedlings were

randomly selected for use in development of a preliminary genetic linkage map with amplified fragment length polymorphism (AFLP) markers.

3. Results and Discussion

Table 1 presents the ratios of susceptible to resistance seedlings within the various seedling populations. About 60 *V. rupestris* X *M. rotundifolia* seedlings were highly resistant to *X. index* feeding as were 21 of the *V. berlandieri* X *V. rufotomentosa* seedlings. Because of the genetically wide nature of the intergeneric crosses, and probable weak homology between chromosomes of *Vitis* and *Muscadinia*, Mendelian inheritance was not be expected (Patel and Olmo 1955). The inheritance of RAPD markers further supported the loss of *M. rotundifolia* chromosomes in the progeny populations. About 40% of the *M. rotundifolia* associated bands were lost in the seedling progeny. The progeny of some groups (8916 and 8925) were almost all resistant, while progeny in others (8911, 8912) were all susceptible.

An examination of 334 RAPD markers in the progeny populations 8909 and 8913 found three (OP-A12, OP-U1 and UBC-237) that were linked to the resistance gene(s). Among these, OPA-12 is an excellent candidate for evaluating *X. index* resistance in future, especially in F2 or backcross generations.

Rootstock trials to test the utility of about 30 of the *X. index* resistant selections in field sites infested with fanleaf degeneration are now in place in six areas of California: Rutherford, Napa County; Healdsburg, Sonoma County; Santa Maria, Santa Barbara County; Ripon, San Joaquin County; Lodi, San Joaquin County; and Bakersfield, Kern County, with 'Cabernet Sauvignon', 'Chardonnay' (3 trials), 'Viognier' and 'Flame Seedless' scions respectively. *Vitis rupestris* 'St. George' rootstock was included between every experimental rootstock selection to ensure that the populations of *X. index* were as high as possible. At least 5 years of yield and pruning weight evaluations, ELISA tests for GFLV in scions, and *X. index* counts in the root systems will be necessary before these selections can be promoted as fanleaf resistant rootstocks.

These first generation *V. rupestris* X *M. rotundifolia* seedlings were unexpectedly fertile and cytogenetic evaluations found 38 chromosomes in all progeny. This fertility allowed the production of an F2 generation of seedlings (Table 2). These seedlings were evaluated for resistance with *X. index* by inoculating with 100 nematodes. Feeding damage was observed within 2 to 3 weeks and the inoculated seedlings were evaluated after 4 months. Seedlings were scored susceptible if any galls were found on their roots. Resistance:suseptibility ratios (Table 2) suggest that resistance is inherited as a single major gene. However, it is more likely that many genes are involved and that resistance to *X. index* in *M. rotundifolia* based progeny is not an evolved resistance, but rather the result of this species inability to host this nematode.

Evidence for weak homology between *Vitis* and *Muscadinia* chromosomes was also found in the F2 seedling populations. The X^2 ratios for the backcrosses to the susceptible *V. rupestris* parents did not support the expected results. The results of selfing 8911-01, a resistant and the only hermaphroditic seedling, was expected to produce a 3:1 ratio of resistant to susceptible. The results did not support this expectation, and were likely due to the large loss of seedlings carrying deleterious recessive alleles, or damaging

chromosome configurations. Even more peculiar results were found when a susceptible seedling was backcrossed to the resistant *M. rotundifolia* parent 'Cowart'. Not only was this cross difficult to make, resulting in only 10 seedlings, but if resistance is controlled by a single dominant gene (homozygous resistant in this case), then all the progeny would be expected to be resistant. One susceptible seedling was detected.

One hundred and sixteen randomly selected seedlings from a cross between 8909-15 (resistant) X 8909-17 (susceptible) were used to develop a genetic linkage map with amplified fragment length polymorphism (AFLP) DNA markers. The double pseudotestcross method was used with JoinMap software to produce the linkage map from over 500 AFLP and 25 RAPD markers. Nineteen linkage groups were expected and were found. *Xiphinema index* resistance was located on the first linkage group.

Efforts are underway using GISH (genomic in situ hybridization) to locate genes associated with *X. index* resistance on the hybrid chromosomes. Preliminary results with genomic DNA probes from *V. rupestris* and *M. rotundifolia* suggest that more of the *M. rotundifolia* chromosomes are being discarded due to poor homology in the F2 generation. This is supported by the relatively poor hybridization of the *M. rotundifolia* probes with the hybrid chromosomes, when compared with the strong labeling with *V. rupestris* probes.

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Table 1. Results of a *Xiphinema index* resistance screen with crosses of *Vitis* X *Muscadinia*. Column 1 lists the female parents, the male parents are listed across the top of the table. The ratios are number of resistant : susceptible seedlings and the 4 digit number below is the seedling population number.

	M. rotundifolia 'Carols'	*M. rotundifolia* 'Dixie'	*M. rotundifolia* 'Cowart'	*M. rotundifolia* 'Magnolia'	*M. rotundifolia* 'Trayshed'	*M. rotundifolia* 'Southland'	j23-163*
V. riparia	0:1 8901	0:1 8904					
V. rupestris 'A. de Serres'	2:4 8908	0:2 8910	6:17 8909	1:7 8911	5:32 8913	0:7 8912	
V. rupestris 'Wichita Refuge'	1:0 8914	24:0 8916	3:1 8915	8:1 8917	3:0 8918	5:4 8919	
V. solonis	0:1 8920		1:3 8921			0:2 8924	
j23-172*							21:3 8925

* = The j23 seedling population is a cross of *V. berlandieri* X *V. rufotomentosa.*

Table 2. Results of screening the F2 generation of *Vitis rupestris* X *Muscadinia rotundifolia* seedling populations for *Xiphinema index* resistance. The numbers of resistant (Resist) and susceptible (Suscp) are presented along with their hypothesize segregation ratios and X^2 values. See Table 1 for pedigrees of the F1 seedling parents.

Cross	Resist	Suscp	Ratio Expected	X^2
8909-15 x 8909-17 (RxS sibling cross)	120	135	1:1 (Rr:rr)	0.88
8909-02 x 8909-24 (RxS sibling cross)	127	113	1:1 (Rr:rr)	0.82
8909-04 x 8909-23 (RxR sibling cross)	158	54	3:1 (Rr:Rr)	0.025
8909-04 x 8909-24 (RxS sibling cross)	78	72	1:1 (Rr:rr)	0.24
8909-15 x 8909-01 (RxS sibling cross)	34	41	1:1 (Rr:rr)	0.65
8909-1 x 8909-02 (SxR sibling cross)	27	21	1:1 (Rr:rr)	0.75
8913-39 x 8913-02 (SxR sibling cross)	30	38	1:1 (Rr:rr)	0.94
8913-39 x 8913-21 (SxR sibling cross)	45	47	1:1 (Rr:rr)	0.78
8911-01 (R selfed)	289	234	3:1 (Rr:Rr)	109.4
A. de Serres x 8913-02 (SxR backcross)	44	77	1:1 (Rr:rr)	9
A. de Serres x 8913-38 (SxR backcross)	32	82	1:1 (Rr:rr)	21.9
A. de Serres x 8913-21 (SxR backcross)	19	44	1:1 (Rr:rr)	10
Wichita Refuge x 8916-04 (SxR backcross)	33	27	1:1 (Rr:rr)	0.6
Cowart x 8915-06 (RxS backcross)	9	1	All R	