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GENETIC ANALYSIS OF GRAPEVINE RESISTANCE TO PIERCE'S DISEASE THROUGH SCREENING THE F_1 PROGENY OF N18-6 × 'CABERNET SAUVIGNON'

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Abstract. **In order to understand the genetic basis of Pierce's Disease (PD) resistance, a segregating population with 184 F_1 progeny of N18-6 × 'Cabernet Sauvignon' was investigated in the experimental vineyard at Florida A&M University, Tallahassee, Fla. The population evaluated was used to identify gene(s) responsible for host resistance to *Xylella fastidiosa*, the causative agent of PD. A field evaluation was conducted utilizing 0 to 5 scales for severity of PD symptoms, where 0 = no PD symptoms, 5 = the most severe PD symptoms. A total of six evaluations were made in the summer and fall, 2002 and one in spring of 2003. Individual progenies started to show symptoms in late August, with severity ranging from 0-3. More seedlings developed PD symptoms in September, with majority of them showing PD symptoms ranging from 1 to 3, with the exception of two plants that showed 4 and 5. PD seemed to progress through late fall/early winter for some hybrids as evidenced by the increase of PD infected vines (91%). Some severely infected seedlings did not recover in the following spring. The resulting population is being used to tag and map the genes responsible for PD.**

Pierce's Disease is a bacterial disease that is detrimental to grapevines. It is caused by the bacterium *Xyllela fastidiosa* that lives in the water conducting tissues (xylem) of grapevines. There is impeded water movement in the xylem tissues of the infected vines and, depending on the severity of infection, the disease may cause vine death. The bacteria are spread from plant to plant by xylem feeding insects such as the sharpshooter subfamily leafhopper (Cicadellinae) and spitting bug (Cercorpidae) (Mullins et al., 1992). It takes approximately three to five months for a vine to show symptoms (Purcell, 2001). Since 1994, over one thousand acres of PD infected vineyards have been replaced in California at an estimated cost of over $30 million. Because of the favorable environment that exists for the development of PD in Florida and the southeast United States, PD is the limited factor to grow *V. vinefera* grapes in that region of the country (Purcell, 2001). The continued success of vineyards in Florida is dependent on improving PD resistance of the *Euvitis* species in order to combat the disease.

Mortensen (1968) reported the inheritance pattern of the PD resistance as trigenic dominant, where he stated that all three dominant genes are necessary for the disease resistance. The ultimate desire of any plant breeder is to identify progenies possessing appropriate combination of genes desired in a cultivar. However, many traits important to agriculture, such as disease and pest resistance are not easily recognized by morphology or convenient screening procedures. In the past, screening progeny was dependent on field evaluations performed by grape breeders. The incorporation of foreign genes into suitable genetic backgrounds often can be greatly assisted by the use of closely linked markers that are easily and reliably scored (Williams et al., 1990).

In this study, we used a F_1 population of 'N18-6' × 'Cabernet Sauvignon' to screen the segregated population for PD resistance. 'N18-6' is a hybrid that was developed at the Florida A&M University in the 1990s, which is highly resistant to PD while the 'Cabernet Sauvignon' is a red wine grape known worldwide, but highly susceptible to PD. The objective of this

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Table 1. PD scores among the F1 population of 'N18-6' × 'Cabernet Sauvignon'.

		PD scores					
Eva. Dates	Population size	0	1	2	3	4	5
July 16, 2002	185	0	0	0	0	0	0
Sept. 1, 2002	185	171	12	1	1	0	0
Sept. 25, 2002	184	154	25	4	1	0	0
Oct. 6, 2002	184	152	25	4	1	1	1
May 5, 2003	184	15	32	8	7	29	93

Table 2. Chi-square analysis for PD resistance in the F_1 population of 'N18-6' ($PD_1pd_1PD_2pd_2PD_3pd_3$) × 'Cabernet Sauvignon' ($pd_1pd_1pd_2pd_2pd_3pd_3$).

Phenotype	Genotype	Dominant Loci	PD	Plant #	Expected x^2	$x^2$0.01 df = 2
Resistant	$PD_1pd_1PD_2pd_2PD_3pd_3$	3	0	15	23	0.6576
Moderate	$PD_1pd_1PD_2pd_2pd_3pd_3$	2	1-4	76	69	0.2663
	$pd_1pd_1PD_2pd_2PD_3pd_3$	2				
	$PD_1pd_1pd_2pd_2PD_3pd_3$	2				
Susceptible	$PD_1pd_1pd_2pd_2pd_3pd_3$	1	5	93	92	0.0054
	$pd_1pd_1PD_2pd_2pd_3pd_3$	1				
	$pd_1pd_1pd_2pd_2PD_3pd_3$	1				
	$pd_1pd_1pd_2pd_2pd_3pd_3$	0				
Total				184	184	0.9293 < 9.21

$X^2 = 4.884 < X2$ at 0.01 = 9.21 with df = 2.

study was to determine the genetic basis for PD resistance and identify resistant genotypes within the F_1 generation.

Materials and Methods

One hundred and eighty four F_1 hybrids from 'N18-6' × 'Cabernet Sauvignon' were used for a field screening to determine the phenotypic expression of PD resistance versus susceptibility among the seedlings. This study was conducted at the Center for Viticulture and Small Fruits Research, Florida A&M University, Tallahassee, Fla. A modification of Hopkins (1987) scoring technique was used for the first season evaluation, based on visual observations. The evaluation was based on a scale, ranging from 0 to 5, where: 0 = no PD symptoms, 1 = minor symptoms up to 10% of leaves with marginal necrosis (MN), 2 = 11 to 30% of leaves with MN, 3 = 31 to 50% of leaves with MN, 4 = 51 to 75% of leaves with MN, 5 = over 76% of leaves with MN, or a dead vine. The disease became more progressive throughout the fall and winter and severe dead arms or trucks were observed in the spring, 2003. Thus, a new scoring technique was used: where 0 = no PD symptoms, 1 = any death of shoot tips, 2 = 10-30% dead shoots, 3 = 31-49% death of shoots, 4 = 50-74% of dead shoots, 5 = 75% dead up to half of the main trucks. The data collected was analyzed using the chi-square technique.

Results and Discussion

A total of four evaluations were made between July and Oct. 2002. When the first evaluation was done in mid-July, no PD symptoms were observed among the seedlings. Three plants (1.6%) showed symptoms in the second evaluation on 1 Sept. 2002, with PD symptoms ranging from 1 to 3. In successive evaluations, more vines developed PD symptoms. In the last evaluation of the season (6 Oct. 2002), about 23% of the vines showed PD symptoms ranging from 1 to 3 in severity with the exception of 2 plants that had PD severity in 4 and 5, respectively (Table 1).

The F_1 progeny of 'N-18-6' × 'Cabernet Sauvignon' population expressed increased symptoms during the fall and winter months of 2002. In the spring of 2003, only 15 plants were without PD symptoms, while 76 showed symptoms ranging from 1-4, and 93 vines fell into the most severe category 5. Based on the X^2 analysis, our result fits reasonably well into the three-gene theory for PD resistance proposed by Mortensen (1968). He reported that three gene loci are responsible in controlling PD resistance of grapes. Our result confirmed that the three independent dominant genes PD_1, PD_2 and PD_3, are all necessary to give a complete resistance, any two of these three dominant loci will give some degree of PD tolerance, while two and three recessive gene loci will result in susceptible plants (Table 2). The data also suggest that 'N18-6' is a heterozygous $PD_1pd_1PD_2pd_2PD_3pd_3$ and 'Cabernet Sauvignon' carries all three homozygous recessive gene loci $pd_1pd_1pd_2pd_2pd_3pd_3$ and appears to be highly PD susceptible.

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