

Generation Means Analysis of Vine Decline Resistance in Melons (*Cucumis melo* L.)

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ABSTRACT

*Vine decline disease (VDD) caused by the fungus *Monosporascus cannonballus*, is a threat to melons (*Cucumis melo* L) worldwide, yet little is known about the genetic control of its resistance. Our goal was to determine the mode of inheritance and the type of gene action of the resistance found in the variety USDA PI 124104. This resistant line was crossed with the susceptible "TAM-Uvalde" variety for segregation analysis. The Parental lines, F_1 , F_2 and backcrosses were inoculated and scored for disease severity. Generation means analysis indicated that additive and dominant effects were present in the inheritance of the VDD resistance trait. Narrow-sense heritability estimate of this trait was high. Chi-square analysis indicates that the resistance is controlled by three independent genes. Thus, selecting for resistance can be achieved using conventional phenotypic selection on visual assessments of root damage. Progress for selection for VDD resistance in early generations can be accomplished.*

Keywords: generation means analysis; narrow sense heritability; disease severity; vine decline disease

1. Introduction

The production of melons in 2019 in the world was 27,501,360 tons and was worth more than 1 billion US dollars. Thus, it constitutes a valuable source of revenues for growers. Due to its popularity, it is widely grown in many countries. Similarly, the production of this crop is important in Texas (FAO, 2018).

The genetic makeup of melons, their morphology as well as their reproductive biology facilitates the application of plant breeding procedures to develop improved varieties. For instance, melons plants are climbing herbaceous annual fruiting vegetables. They are also cross-pollinated diploid ($2n = 2x = 24$) species. Melons were originally monoecious, as are many modern cucurbit plants. Nevertheless, gynoecious and monoecious cultivars are found, as well. Male and female flowers are formed at different nodes, with the female flowers at higher nodes than the male (Kirkbride, 1993).

Melon growers confront many problems, especially, in Texas. Problems such as pests, lack of labor, competition for markets and diseases are factors that cause an impact on their production and therefore, their economic return. Among the diseases that affect the production of melons, vine declines are some of the most damaging and their control increases costs and pollutes the environment. This is especially true for the fungus *Monosporascus cannonballus*, which has often been controlled with methyl bromide and other highly toxic fumigants.

The symptoms of VDD have been documented by several authors. For example, according to Martin and Miller (1996) a rapid collapse of the vine takes place just before harvest, which results in fruits with sunburn, low sugar content, premature abscission from the pedicel before ripening and consequently, they become unmarketable. Moreover, such symptoms become more severe when the plant is under conditions that may generate stress. For instance, heavy fruit load, drought, heat, and heavy insect feeding.

Systematic efforts are, therefore, being made to identify sources of resistance and incorporate this trait into varieties of melons. Considerable genetic variability has been observed for disease resistance in melons and lines with high levels of resistance have been identified. For example, the variety USDA PI 124104.

However, studies to elucidate the inheritance and to identify the quantitative trait loci (QTL) involved in resistant genotypes are limited, making it difficult to identify appropriate breeding strategies to develop improved varieties. The central hypothesis of this project is that existing genetic variability in melons can be utilized to elucidate genes/QTL conditioning resistance and incorporate this trait into varieties of melons. (Crosby, 2001).

2. Material and Methods

2.1- Plant material

Two genotypes, a cultivar of Texas A&M university named TAM-Uvalde (susceptible) and a USDA north central regional plant introduction identified as 124104 (resistant) were chosen as parents for this study. TAM-Uvalde was used as female to produce the (TAM-Uvalde x 124104) F_1 , (TAM-Uvalde x 124104) F_2 , and BC backcrosses with male and female parents.

2.2-Experimental design

An evaluation of resistance was conducted between September and October 2019 at the Texas A&M HortTrec facility, in College Station, Texas. The experimental material consisted of six populations, P_1 (TAM-Uvalde), P_2 (USDA PI 124104), F_1 (TAM-Uvalde x 124104), F_2 (TAM-Uvalde x 124104), BC_1 (TAM-Uvalde x F_1) and BC_2 (124104 x F_1). The progeny derived from backcrossing the F_1 to the female parent were designated as BC_1 and those from backcrossing to the male parent as BC_2 .

The plant material used in this experiment belongs to the melon breeding program of Texas A&M University, except for the variety USDA PI 124104. It was obtained at the USDA North Central Regional Plant Introduction Station, located in Ames, Iowa and is originated in India.

The study included 9 plants of the resistant parent P_2 , 5 plants of the susceptible P_1 , 37 plants of the F_1 , 138 plants of the F_2 , 11 BC_1 and lastly, 14 BC_2 . Seeds for the parents were obtained from uniform seed lots.

Plants were grown under greenhouse conditions with an average temperature of 28 °C and 12 hours of light period and a RH of 74%. Trays of 38 holes were used, which hold a volume of 2376 cm³ of media (38 seedlings/tray). Sterilized sand was used as a medium. The sand was sterilized in an autoclave for 30 minutes and then cooled down at room temperature for 24 hours. Finally, it was sterilized one more time following the same procedure previously described.

2.3-Inoculum production

The pathogen (*Monosporascus cannonballus*) was isolated from infected roots of plants taken in Weslaco, Texas at the Texas A&M AgriLife Research Extension Center. They were washed under running water. After surface sterilization and rewashing with water, they were cut into pieces. Then, they were placed on potato dextrose agar (PDA) plates and incubated for 7 days at room temperature. Once the isolate of pure culture was obtained, it was cut into pieces. Then, they were placed on V8 agar plates and incubated at room temperature, as well. When spores were observed in the plates, the inoculum was prepared using sterilized distilled water. The concentration of inoculum was measured with a hemocytometer and adjusted to 2000 spores.ml⁻¹ prior to inoculation. For each soil inoculation, each cell in the tray was filled halfway up with sterilized sand and 3 ml of inoculation solution was added with a pipette. Then, cell trays were filled completely with more sterilized sand and disease severity was evaluated 6 weeks after sowing. Plants were hand-watered as needed with distilled water and supplied with nutrient solutions 4 times (15 N-10 P-15 K, 200 mg.L⁻¹, plus micronutrients). The plants were carefully extracted from the trays and the sand was flushed with tap water. Then, the roots were also washed with it.

2.4-Disease assessment

Individual plants were scored for vine decline disease symptoms on a scale of 1 to 5, as previously reported by Crosby(2001). Briefly, plants with no visible symptoms were scored as 1; 2= slight necrosis of fine roots, few tan lesions; 3= slight necrosis of all roots, moderate tan lesions; 4= severe necrosis of all roots; and 5= only tap root remaining, necrotic and completely tan to brown.

2.5-Statistical and Genetic Analysis

The variances of the non-segregating populations (F_1 , P_1 and P_2) was 0 (Table 1). Thus, they were homogeneous, which also indicates that the environmental variance (V_E) was 0. Statistical analyses were performed using Statistical Analysis System (SAS) PROC GLM (SAS Institute, 2020), whose model was VDD resistance = $m + a + d$; where m is the mid parent value, d is the additive component and h is the dominant component.

The 3-parameters model (mean, additive, and dominance effects) was first tested using the scaling tests of Ketata et al. (1976) and Mather and Jinks (1971) with $A=2BC_1-F_1-P_1$, $B=2BC_2-F_1-P_2$ and $C=4F_2-2F_1-P_1-P_2$ to test the fitness of our data to the additive-dominance model. A t test was used to detect if A, B and C were significantly different from 0. The observed means of the 6 generations were used to estimate m (mean), d (additive component) and h (dominant component). The model was declared adequate when t tests and chi-square tests were non-significant.

2.5.1-Heritability estimates

Narrow sense heritability (h_{ns}^2) was estimated following the method proposed by Warner (1952); $h_{ns}^2=[2VF_2-(VB_1-VB_2)]/VF_2$, where VF_2 , VB_1 and VB_2 are the variances of the F_2 , BC_1 , and BC_2 generations. Broad-sense heritability (h_{BS}^2) was estimated as proposed by Burton (1951) and uses the F_1 data to estimate the environmental variance $h_{BS}^2=(VF_2-VF_1)/VF_2$ where VF_1 and VF_2 are the variables of the F_1 and F_2 generations.

3- Results and Discussion

The means, ranges, and variances of the P_1 , P_2 , F_1 , F_2 , BC_1 , and BC_2 evaluated for vine decline resistance are displayed in Table 1. Parent USDA PI 124104 (P_1) had mean score of 1/5 with no symptoms, which is indicative of its resistance to VDD. On the other hand, the susceptible parent TAM-Uvalde (P_2), had a mean of 3/5, which indicates its susceptibility to VDD. F_1 population had a mean score of 1/5 indicating a dominant inheritance of the genes controlling resistance to VDD. The segregating F_2 population presented a mean score of 2.24/5, which is indicative of a segregating population regarding VDD resistance. BC_1 presented a mean scored of 1.81/5 and BC_2 presented a mean score of 1/5. BC_2 showed a higher degree of resistance compared to BC_1 , which is an indicator for a higher degree of resistance of the variety USDA PI 124104 (P_1).

Individual scaling test (A, B and C) were used to test the fitness of the three-parameter model (mean, additive, and dominance). Such a model is used to explain the variability observed among the progeny from crosses (Ketata et al., 1976). Based on the individual scaling tests results, the model fitted the data in the TAM-Uvalde x USDA PI 124104 cross for vine decline symptoms scores (Table 2) since no significant effects were observed. They also indicate that maternal effects as well as other epistatic interactions are not present and simple autosomal inheritance was involved in the resistance against VDD.

The estimates of the genetic effects are displayed in Table 3. The three parameters model showed that additive (d) and dominance (h) effects were highly significant ($P<0.01$) for vine decline resistance for the cross TAM-Uvalde x USDA PI 124104, indicating that they significantly contributed to the inheritance of this trait.

Narrow and broad sense heritability estimates were approximately the same ($h_{ns}^2=1.1$; $h_{BS}^2=1$). Narrow sense heritability was high, which indicates that resistance to VDD is highly heritable and genetic gain regarding this trait can be achieved in a short period of time. Narrow sense heritability estimates are important in elaborating plant breeding schemes strategies.

Previous generation means analysis studies conducted on melons reported similar broad sense heritability values for traits such as average fruit and branch number per plant, average weight per fruit, and days to anthesis. Furthermore, traits such as fruit weight per plant exhibited similar narrow sense heritability values (Zalapa, Staub and McCreight, 2006). Moreover, fruits of plants affected by VDD do not reach maturity, present sun damage and a decrease in quality (Martin and Miller, 1996). Thus, resistant plants to VDD could exhibit better yield and fruit quality. However, more studies regarding VDD resistance and yield as well as fruit quality are needed.

3.1-Chi Square calculations

Visual symptoms data can be observed in table 4. Notably, the resistant plants of the F_2 population add up to 90 whereas the rest of the plants add up to 48. This proportion fits the phenotypic ratio of three independent genes providing resistance in a F_2 segregating population. VDD damage was scored using the scale previously described (Crosby, 2001).

4- Conclusions

The results of the generation means analysis indicate additive and dominant effects are involved in the inheritance of VDD resistance and epistatic interactions are not significant. In addition, three major genes present in the variety USDA PI 124104 are conferring resistance in melon plants to VDD. Simple inheritance can facilitate the work to develop new resistant varieties using conventional phenotypic selection on visual assessment of root damage and traditional backcrossing methods.

5-References

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Table 1: Number of individuals, means, ranges and variance of families evaluated for vine decline resistance inheritance in melons.

Symptom Scores				
Population	N	Mean	Range	Var
P1 (TAM-Uvalde)	5	3	3	0
P2(124104)	8	1	1	0
F1	37	1	1	0
F2	138	2.2463	1-5	2.2162
BC1(F1xP1)	11	1.81	1-5	1.96
BC2(F1xP2)	14	1	1	0

Symptom rating on a scale of 1 to 5; 1 = No symptoms; 2=slight necrosis of fine roots and few tan lesions.

3=slight necrosis of roots, moderate tan lesions;4=severe necrosis of all roots; 5=Necrotic plant

N= number of plants evaluated for vine decline disease resistance in each generation

Var= variances

Table 2: Scaling test for vine decline disease resistance in melons

Trait	Cross	Scaling Test		
		A	B	C
Symptom rating	TAM-Uvalde x USDA PI 124104	NS	NS	NS

NS = Not significant at $\alpha = 0.05$. $A=2BC_1-P_1-F_1=0$; $B= 2BC_2 -P_2- F_1 = 0$

$C= 4F_2-2 F_1-P_1-P_2 = 0$. Mean values of $P_1, P_2, F_1, F_2, BC_1, BC_2$.

Table3:Analysis of variance test for vine decline disease resistance in melon

Source	DF	SS	Mean Square & S.E	F value	P>F
m	1	230.1481	236.2934±0.2328	133.23	<.0001
h	1	14.6963	18.8211±0.30032	10.61	0.0013**
d	1	16.9346	20.3941±0.3834	11.5	0.0008**

** Highly significant $\alpha = 0.01$

m=mean; **d**=additive component; **h**=dominant component; **DF**= degrees of freedom; **SS**= sum of squares; **S.E**= standard error.

Table 4: Three independent genes are involved in vine decline disease resistance in melons. The F₂ population from the cross of susceptible and resistant genotypes was evaluated for segregation ratios using Chi-Square test.

	Observed	Expected	*Pr.ob > Chi-square
Disease present	48	35	0.167693NS
No-Disease present	90	103	
Total	138	138	

α = 0.05; NS= not significant; *probability not equal to hypothesized value (two side chi squared)