ANALYSIS OF RESISTANCE TO VINE DECLINE DISEASE CAUSED BY MONOSPORASCUS CANNONBALLUS IN MELONS (*CUCUMIS MELO* L.)

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ABSTRACT

Vine decline disease (VDD) caused by the fungus *Monosporascus cannonballus*, is a major threat to melons (*Cucumis melo* L.) production worldwide. Resistance has been identified in some melon accessions, yet little is known abouts its genetic control and mode of action. Thus, the goals of this project were to determine the mode of inheritance and the type of gene action of the resistance found in the USDA accession: PI 124104 as well as to identify metabolites involved in it. The F_1, F_2, BC_1 and BC_2 populations from the cross of VDD-susceptible parent, TAM-Uvalde with a resistant VDD-USDA PI 124104. Generation means analysis indicated that additive and dominant effects were present in the inheritance of the VDD resistance trait. Broad-sense heritability estimate of this trait was high (0.74) and narrow-sense heritability estimate was moderate (0.47). Chi-square analysis indicates that the resistance is controlled by three independent genes. Additionally, mid parent-offspring regression showed that some hybrids developed with the variety USDA PI 124104 exhibited medium to high narrow sense heritability values (0.6 and 0.66). However, these results may be inflated due to the presence of heterosis. Several metabolites were differentially accumulated in resistant and susceptible genotypes in response to pathogen infection. Particularly, results shows that phthalic acid is constitutive and induced in VDD-resistant genotype in response to pathogen infection suggesting a putative role on VDD-resistance reaction. However, further research using forward, and reverse genetic approaches are needed to determine the role of phthalic acid in the resistance to *Monosporascus cannonballus*, in melons.

DEDICATION

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Contributors

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The data in Chapter II was analyzed by Professor Amir M.H Ibrahim at Texas A&M University.

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NOMENCLARURE

Ala	alanine
Asp	asparagine
B	formic acid nitrile
APT	after plants were transplanted into trays
BC_1	backcross 1
BC_2	backcross 2
BPT	before plants were transplanted into trays
CA	caffeic acid
CFU	colony forming units
cm^3	cubic centimeters
Cit	citrulline
ChA	chlorogenic acid
cwt	centum weight or quintal
d	additive component
g	grams
GA	gallic acid
GABA	ɤ-amino butyric acid
Gln	Glutamine
Gly	glycine
F_1	family 1
F_2	family 2
FA	ferulic acid
FW	fresh weight
h	dominant component

h^2_{BS}	broad sense heritability
HBA	hydroxy benzoic acid
h^2_{ns}	narrow sense heritability
HPLC	high liquid performance chromatography
Hypro	hydroxyproline
Kg	kilograms
Ile	isoleucine
ISR	immune systemic resistance
L	liters
LSD	least significant difference
Leu	leucine
m	mean and/or meter
mm	millimeters
MC	*Monosporascus cannonballus*
met	methionine
mg	milligram
min	minutes
N	normal
NPAAs	non proteic amino acid
PA	protocatechuic acid
PDA	potato dextrose agar
PI	plant introduction
P_1	parent 1
P_2	parent 2
Phe	phenylalanine
P-Cou	p-coumaric acid
RI	resistant inoculated

RN	resistant non-inoculated
ROS	reactive oxygen specie
RPM	revolutions per minutes
SAR	systemic acquired resistance
Ser	serine
SI	susceptible inoculated
SN	susceptible non-inoculated
TCA	trans-cinnamic acid
thr	threonine
v	volume
US	United States of America
Tyr	tyrosine
Val	valine
Var	variance
VBC_1	variance of backcross to the susceptible parent
VBC_2	variance of backcross to the resistant parent
VDD	vine decline disease
VF_1	variance of F1
VF_2	variance of F2
V8	tomato juice agar
Xg	times gravity
ß-ala	beta alanine
^0C	celsius
µg	micrograms
µL	microliters

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CHAPTER I
INTRODUCCION AND LITERATURE REVIEW

Melon (*Cucumis melo* L.) belongs to the *Cucurbitaceae* family. This family has 90 genera approximately and 750 species while there are approximately 55 other species of Cucumis, which are not sexually compatible with *Cucumis melo* (Kirkbride, 1993).

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 are monoecious, as are many modern cucurbit plants. Nevertheless, gynoecious and andromonoecious 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 due to the use of chemicals. This is especially true for the fungus *Monosporascus cannonballus*, which has often been controlled with methyl bromide and other highly toxic fumigants (Crosby, 2001; Martin and Miller, 1996)

Monosporascus cannonballus is a pyrenomycetes fungus, which is distinctive within the ascomycetes. It is homothallic and produces fertile perithecia in roots. It yields

one ascospore per ascus. It is dark brown or black when reaching maturity and resembles a cannonball. Also, it contains between 1 to 16 nuclei per spore, but usually it has 8 when mature. Its perithecia is spherical with a tiny neck and embedded in the root cortex. When it breaks, their spores are released into the soil. This fungus lacks conidial stage (Martin and Miller, 1996).

Vine decline diseases have been extensively studied and their symptoms documented by several authors. For instance, Martin and Miller (1996) reported that a rapid collapse of the vine takes place just before harvest, which results in fruits with sunburn, low sugar content and a premature abscission from the pedicle before ripening and consequently, they become unmarketable. 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.

Melons exhibit an immense variability throughout the world. Especially, in India and Africa. Thus, it can be exploited to improve traits and thus, the productivity of growers. Therefore, more studies are needed to know which traits can be incorporated into varieties of melons (Sebastian et al, 2010). Likewise, studies regarding metabolites produced by melons with respect to nutritional content and responses to pathogen attacks have been conducted in USA (Kasote et al, 2020a; Sign et al, 2020; Mallick and Masui, 1986). Nevertheless, more studies with regard to compounds produced by plants in response to pest and pathogen attack are needed to broaden this knowledge.

Melon breeding programs are becoming important to satisfy the needs of consumers because they seek variety in their diet. Thus, releasing new cultivars is key to

meeting their requirements, which are generally, guided by flavor, freshness, ripeness, and sweetness. Also, they prefer locally grown melons because there is a perception that domestically grown products are better. Additionally, melons are mostly consumed fresh (Heng and House, 2018; Lester, 2006; Boriss, Brunke and Kreith, 2006).

Melons suffered a drastic decline in their production and consumption in the past decade due to foodborne illness outbreaks. Moreover, the production of cantaloupes, which is the most popular melon, went from 60700 acres in 2018 to 40600 acres in 2020 and its production was 11.5 million of cwt in 2020 whereas 7000 acres of honeydews were planted in the same year with a production of 2.4 million of cwt. Additionally, the price of cantaloupes was $26.10 per cwt while the one of honeydews was $20.90 per cwt in 2020. Furthermore, their consumption in the US is estimated in 13 kg of melons per person each year and specifically, the consumption of cantaloupes and honeydews in 2017 was approximately 3.1 and 0.75 kg per person per year, respectively (Agricultural marketing resource center, 2018; USDA-ESMIS, 2018). In addition, the melon industry is focusing its efforts on improving harvesting and shipping techniques as well as developing sweeter hybrids to reverse the decline in their consumption (Boriss, Brunke and Kreith, 2006).

Metabolites studies in plants such as watermelons have been useful in identifying compounds that provide resistance to *Fusarium oxysporum.* For example, phenolics acids and free amino acids are reported to provide resistance (Liu et al, 2009). In the same vein, free amino acids are key for the synthesis of proteins and other functions related to metabolic pathways as well as signaling of transduction processes (Hildebrandt et al, 2015). Furthermore, amino acids act as a source of energy. Also, they are involved in

processes such as regulation of metabolism, reduction of blood sugar, and amelioration of vascular health.

Amino acids also act as neurotransmitters and antioxidants. For instance, arginine is involved in nitric oxide production by NO synthase (NOS), which is a vasodilator that improves cardiovascular health, sport performance and reduces the risk of stroke. Amino acids such as glutamate, aspartate, and ɤ-amino butyric acid (GABA) work as excitatory or inhibitory neurotransmitters in the central nervous system. Moreover, depression and mood disorders are related to their presence. Additionally, they are precursors for the gut microbiota to produce fatty acids, which have anti-inflammatory properties. For example, butyrate is synthetized by anaerobic bacteria from threonine, lysine, and glutamate. In addition, the biosynthesis of acetate and propionate require the presence of glycine, glutamate, and ornithine (Kasote et al 2020b). Additionally, the presence of amino acids such as ɤ-amino butyric acid (GABA), methionine, lysine and tryptophane has been linked to signaling processes and defensive responses of plants (Arçay et al, 2012; Busch and Fromm, 1999; Eskandari and Sharifnabi, 2019; Kuc, 1997; Burger and Chory, 2019; Hildebrandt et al, 2015; Navarova et al, 2012; Radwanski and Last, 1995).

Ascertaining the metabolites produced by melons plants under disease pressure could be useful in explaining the resistance observed in some genotypes (Crosby, 2000). In addition, it could also be useful in developing organic products to control diseases. Furthermore, little is known about the resistance observed in some genotypes against VDD.

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 USDA PI 124104 accession (Crosby, 2001). However, studies to elucidate the type of inheritance involved in the resistance are insufficient. Additionally, studies to profile metabolites produced by melons under disease pressure are needed.

The central hypothesis of this project is that VDD-resistance in USDA PI 124104 is genetically controlled and can be used to develop resistant cultivars. Furthermore, disease genetic control results in constitutive and induced metabolite changes responsible of defensive responses of the plant. Also, this study was undertaken with the following general objectives:

1- Elucidating the inheritance of the resistance to vine decline disease.

2- Profiling the metabolites produced by melons when affected by vine decline disease.

CHAPTER II

GENERATION MEANS ANALYSIS OF VINE DECLINE RESISTANCE IN MELONS

The worldwide production of melons in 2019 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). Nevertheless, the production of melons is affected by diseases such as vine declines, especially, the one caused by the fungus *Monosporascus cannonballus* (Martin and Miller, 1996).

Melons exhibit an enormous variability regarding traits related to disease resistance and it can be used to improved susceptible varieties (Kirkbride, 1993; Sebastian et al, 2010). Nonetheless, few studies have been conducted regarding the genetic of control of resistance observed in some varieties such as USDA PI 124104. Thus, understanding it could be useful in developing resistant varieties to VDD. Therefore, the central hypothesis of this study is that VDD-resistance in USDA PI 124104 is genetically controlled and can be used to develop resistant cultivars. The specific objectives of this study were to determine the type of genetic control of the resistance observed in the variety USDA PI 124104 and its heritability estimates as a basic information to elaborate plant breeding scheme strategies for trait introgressions.

Materials and Methods

Plant material

Two genotypes, a variety of Texas A&M university named TAM-Uvalde ♀ (Susceptible) and a USDA north central regional plant introduction identified as USDA PI 124104 ♂ (resistant) were chosen as parents for this study. The F_1, F_2 and reciprocal (TAM-Uvalde x 124104) BC backcrosses were obtained for this experiment.

Experimental design

An evaluation of the resistance was conducted between September and October 2019 at the Texas A&M HortTrec facility, in College Station, Texas (30^o 30' 56'' N; 96^o 26' 27'' W). 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.

Plants were grown under greenhouse conditions with an average temperature of 26 ^{o} C and 12 hours of light period. Trays of 36 holes were used, which hold a volume of 2376 cm^3 of media (37 seedlings/tray). Sterilized sand was used as medium. The sand was sterilized in an autoclave for 30 minutes and then cooled down at room temperature for 24 hours. Finally, it was re-sterilized following the same procedure previously described.

Inoculum production and inoculation

The pathogen (*Monosporascus cannonballus*) was isolated from infected roots of plants taken in Weslaco, Texas at the Texas A&M AgriLife Research Extension Center (26°07′26″ N;97°51''47″W). 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, which were incubated for 7 days at room

temperature. Once the isolate of pure culture was obtained, it was cut into pieces, and they were placed on V8 agar plates, which were also incubated at room temperature. 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 (15-10-15, 200 $mg.L^{-1}$, plus micronutrients). The plants were carefully extracted from the trays. The sand was flushed with tap water. Then, the roots were also washed with it.

Disease assessment

Individual plants were scored for vine decline disease symptoms on a scale of 1 to 5 as previously reported by Crosby (2001): 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 (Crosby, 2001).

Statistical and genetic analyses

Following confirmation of error variance homogeneity (P-Value = 0.1614) by performing and F test, data were analyzed (Gomez and Gomez, 1984). 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.

Individual scaling tests were computed following the methods reported by of Mather and Jinks (1971).

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 $A=2BC_1P_1-F_1-P_1$, $B= 2BC_1P_2-F_1-P_2$ 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.

Heritability estimates

Narrow sense heritability (h^2_{ns}) was estimated following the method proposed by Warner (1952); $h^2_{ns}=[2VF_2-(VB_1-VB_2)]/VF_2$, where VF_2, VB_1 and VB_2 are the variances of the F_2, BC_1, and BC_2 generations. The standard error for the narrow sense heritability was estimated as described by Ketata et al (1976). Broad-sense heritability (h^2_{BS}) was estimated as proposed by Burton (1951), which uses the F_1 data to estimate the environmental variance $h^2_{BS}= (VF_2-VF_1)/VF_2$ where VF_1 and VF_2 are the variables of the F_1 and F_2 generations.

Results and Discussion

The means, ranges, and variances of the P_1, P_2, F_1, F_2, BC_1, and BC_1 evaluated for vine decline resistance are displayed in Table 1. Parent USDA PI 124104 had mean score of 1.11/5 with no or low symptoms, which is indicative of its resistance to VDD. On the other hand, the susceptible parent TAM-Uvalde, had a mean of 3/5, which indicates its susceptibility to VDD. F_1 population had a mean score of 1.15/5 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 1.28/5. 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 epistasis are not present and simple autosomal inheritance was involved in the resistance against VDD.

The estimates of the genetic effects and their magnitude 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 sense heritability estimates (h^2_{ns}=0.47 ± 0.7) were less than broad sense heritability estimates (h^2_{bs}=0.79 ± 0.88) for the cross TAM-Uvalde x USDA PI 124104. Narrow sense heritability was low to moderate. 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.

Chi-square calculations

In examining the visual symptoms data (Table 4), it is notable that 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).

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. 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.

CHAPTER III

PARENT-OFFSPRING REGRESSION ANALYSIS OF VINE DECLINE DISEASE RESISTANCE IN MELONS

Parent-offspring regression is used to estimate narrow sense heritability values, which are central to elaborating plant breeding schemes strategies (Fehr, 1939). Also, broadening the knowledge of VDD resistance is important to develop resistant varieties. Therefore, implementing a different procedure to obtain narrow sense heritability estimates will generate more information, which could be useful for making comparisons and reaching conclusions regarding VDD resistance. Hence, it is hypothesized that parent offspring regression and generation means analysis procedures produce similar heritability estimates. Moreover, the specific objective of this study is to assess narrow sense heritability estimates using a different procedure from the one previously performed.

Materials and Methods

An experiment was conducted in summer 2021 in Weslaco, at the Texas A&M AgriLife Research and Extension Center, Weslaco, TX (26°09´17´´ N; 97°57´45´´W). Crosses between resistant and susceptible melon genotypes were evaluated for disease resistance symptoms under field conditions (Table 5). Four replications of 20-plant plots were direct seeded on black plastic mulch with subsurface drip irrigation. 10-10-10 fertilizer was applied at a rate of 0.06 $kg.m^{-1}$ of row, three times during the growing season. In addition, the soil in the field plot was highly infested with *Monosporascus cannonballus* after continuous crops of melons for over 30 years. Moreover, roots of infected melons grown in this field were used to isolate the pathogen used in chapter 2. Roots were

carefully pulled out of the soil when fruits were ready to be harvested and the disease symptoms were scored using the symptom severity scale proposed by Crosby (2001), which was described in chapter 2.

The pedigree information of the plant material used to evaluate vine decline disease under field conditions can be seen in table 5.

The plant material used in this experiment belongs to the melon breeding program of Texas A&M University, except for USDA PI 124104 and Ames 20608. They were obtained at the USDA North Central Regional Plant Introduction Station, located in Ames, Iowa

Mid-parent-offspring regression was performed to estimate narrow-sense heritability and an excel spreadsheet software (Microsoft, Redmond) was used (Falconer, 1989). The model is described as follows: $y = mx + b$; where y = offspring value; m= narrow sense heritability estimate; x = average parents value; b = linear regression coefficient (Fehr, 1939)

Results and Discussion

Mid-parent-offspring regression estimates for VDD resistance are presented in table 6.

These results indicate that hybrid M4 presented the highest narrow sense heritability value followed by hybrid M5 and M3.

The narrow sense heritability estimates obtained in this experiment may be biased because of the presence of heterosis. That is, they may have been inflated. Consequently,

they were higher than the ones obtained in chapter two. Additionally, yield could not be measured in this experiment because of the presence of powdery mildew, which caused a negative impact on the plants.

Similar parents-offspring regression studies regarding disease resistance have been conducted on peanuts and hybrids of casava. They produced similar narrow sense heritability estimates for disease resistance to casava mosaic disease, and early leaf spot in peanut (Anderson et al 1991; Njoku et al 2015).

Conclusions

The results of this study indicate that the resistance in the USDA PI 124104 can be used to incorporate resistance to VDD into susceptible varieties and that the genetic background of genotypes can result in differential levels of resistance obtained. Also, heterosis can be harnessed to combat VDD. However, elaborating plant breeding schemes strategies with heritability estimates derived from hybrids can lead to reaching wrong conclusions and consequently, valuable resources can be lost.

CHAPTER IV

METABOLITE PROFILE CHANGES IN RESPONSE TO VINE DECLINE DISEASE IN MELON

Studies regarding metabolites produced by melons when affected by VDD are needed. In fact, there has been studies focused on other diseases such as Fusarium in watermelons (Kasote et al 2020). Nonetheless, studies related to the production of metabolites in roots of melons when affected by soil borne diseases are insufficient.

Amino acids are essential molecules found in all living organisms. Additionally, some amino acids are precursors of molecules that are involved in plant immunity such as ethylene (Burger and Chory, 2019). Hence, ascertaining the amino acids produced by melons when affected by VDD may be useful for understanding the resistance observed in the variety USDA PI 124104.

Compounds such as hormones and phenolic acids have a role in defensive responses. For example, polyphenols are secondary metabolites present in plants that are involved in growth as well as reproduction. Also, they provide resistance to pathogens and predators. Moreover, phenolic acids are simple molecules, which are constituted of a benzene ring as well as a carboxylic group, and they present antimicrobial activities (Bravo, 1998).

Phenolic compounds have been linked to antimicrobial activities. For example, caffeic acid exhibits antibacterial effects as well as pyrogallic acid. However, gallic acid does have any anti-fungal activity against the fungus *Candida albicans* and *tropicalis*.

Nevertheless, gallic acid derivatives inhibit the growth of the fungus *Aspergillus niger* (Lima et al, 1996; Khatkar et al, 2017). Additionally, anti-fungal activities of some phenolic compounds such as gallic acid, trans-cinnamic acid, and tannins have been related to their synergistic effects (Carvalho et al, 2018; Hazir et al, 2017).

Phenolic acids have also been reported to be useful in chelating toxic elements such as boron, which causes abiotic stresses to plants (Reid et al 2004; Seneratna et al, 2003)

Chlorogenic acid is a precursor of caffeic acid. They both have a strong antioxidant activity (Sato et al, 2011). In the same vein, ferulic acid is a strong antioxidant compound and its presence is linked to terminating free radical chain reactions. (Itagaki et al, 2009).

P-coumaric acid possesses anti-microbial activity, too. For instance, it inhibits quorum sensing properties of bacteria and consequently, their growth is halted (Chen, 2020). In addition, fungi such as *aspergillius niger* degrades this compound, which suggests its anti-fungal activity (Lubbers et al, 2021; Kasote et al, 2020).

Phthalic acid esters are molecules ubiquitous to the environment. In addition, they are also used to make chemical products and most importantly, they possess allelopathic, antibacterial and insecticidal activity (Hung et al, 2021; Wu et al, 2015).

Phenolic acids are reported to promote spore germination of fungi. For example, it is known that phthalic, ferulic, and 4-hydrobenzoic promote spore germination of *fusarium oxysporum* in watermelon (Hao et al, 2010; Kasote et al, 2020a).

Hormones play a role in plant immunity, too. For instance, salicylic and jasmonic acid are involved in defensive mechanism such as systemic acquired resistance and immune systemic resistance (Burger and Chory, 2019). Nevertheless, the existence of these mechanisms in melons in unknown. Thus, this study may contribute to broadening the knowledge regarding plant immunity responses in melons.

It is hypothesized in this study that genetic controlled VDD-resistance in melon results on constitutive and induce, amino, phenolic acids, and plant hormone profile changes participating in resistance defensive mechanisms against *Monosporascus cannonballus* in melon. Therefore, the specific objective of this study was to identify constitutive and putative compounds involved in plant defensive responses in USDA PI 124104.

Materials and Methods

Location

An experiment was conducted between September and October 2021 at the Texas A&M HortTrec facility, in College Station, Texas (30º 30’ 56’’ N; 96º 26’ 27’’ W). Two genotypes were used for this study. A susceptible cultivar of Texas A&M university named TAM-Uvalde and a resistant USDA North-Central regional plant introduction accession USDA PI 124104.

Inoculum production

The pathogen, *Monosporascus cannonballus*, was isolated from infected roots taken in Weslaco, Texas at the Texas A&M AgriLife Research and Extension Center,

(26°07′26″ N;97°51°47″ W). Roots were washed under running water. After surface sterilization and rewashing with water, they were cut into pieces, placed on potato dextrose agar (PDA) plates, which were incubated for 7 days at room temperature. Once the isolate of pure culture was obtained, it was cut into pieces and they were placed on V8 agar plates, which were incubated at room temperature. When spores were observed, a mixture of sand and ground oat hulls, combined at a rate of 45 g of oat hulls to 500 cm^3 of sand was prepared. In 1 L flasks, 100 ml of water was combined with 500 cm^3 of this medium and autoclaved twice for 60 min with a 1-day interval. The medium was inoculated with three, 1 cm^2 pieces of colonized agar cut from a V8 culture. The flasks were kept at room temperature under 12 h of fluorescent light/day for 5 weeks at room temperature as previously described by Salari et al (2013). Finally, the inoculum yielded 1.22 10^7 colony forming units (CFU) of *Monosporascus cannonballus* per gram of sand medium.

Plant material and seedling development

A factorial design was used, which consisted of two genotypes (resistant vs susceptible) and two inoculation treatments (inoculated vs mock-inoculated control) for a total of 4 factorial treatments and 3 repetitions: RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus* (MC), RC, resistant (USDA PI 124104) mock-inoculated, SI Susceptible (TAM-Uvalde) inoculated with MC and SC susceptible (TAM-Uvalde) mock-inoculated.

Plants were grown under greenhouse conditions with an average temperature of 28 ^{o} C and 12 hours of light period. Seeds of melons were germinated in trays with sterilized

peat moss and 2-week-old seedlings were transplanted into trays of 36 cells, which hold a volume of 2376 cm^3 of medium (36 seedlings/tray).

Peat moss was used as a medium. It was sterilized in an autoclave for 30 minutes. Then, it was cooled down at room temperature for 24 hours. Lastly, it was re-sterilized following the same procedure previously described. Each cell in a tray was filled halfway up with peat most. Afterwards, 10 gr of inoculum was added. Finally, more peat most was added to each cell to fill it up completely.

Roots were sampled before being transplanted into the trays (BPT) for the 0 hours experiment. The rest of the plants were transplanted and taken out of the trays 24, 36, 72 hours and 6 weeks after being transplanted into trays (ABT) to sample their roots. They were washed to remove the medium. Then, they were bagged and refrigerated at -80 ^{o} C until proceeding to metabolite composition analysis.

Amino acids profiling

First extraction

Roots were ground in liquid nitrogen with a mortar and a pestle. 50 mg of them were put into a 15 ml tubes. Afterwards, 3 ml of solvent was added to the tubes. The content was homogenized for 2 min at 1000 RPM. Then, it was sonicated for 15 min and vortexed for 1 min. After vortexing the content, samples were centrifugated for 6 min at 8000 RPM at 10-15 ^{o} C. Finally, the supernatant was transferred into a 15 ml tubes (filtrate I).

Second extraction

The residue obtained during the first extraction, was mixed with 3 ml solvent, and homogenized for 2 min at 1000 RPM. Then, it was sonicated for 15 min and vortexed for 1 min. Afterwards, it was centrifuged for 6 min at 8000 RPM at 10-15 ^{o} C. Finally, the supernatant (filtrate II) was transferred into the previously labeled tube containing the filtrate I.

The final volume was recorded, a derivation step was performed and 700 µL were transferred into an amber color vial and then, 250 µL of dansyl chloride, 600 µL of buffer and 100 µL of internal standard were added to it. Afterwards, it was vortexed for 1 min and incubated for 30 min in water bath at 60 ^{o} C. Following incubation, 60 µL of 2N acetic acid were added and samples were vortexed for 30 sec to stop the reaction.

Lastly, the content was transferred from the amber vials to the centrifuge tubes. The tubes were centrifuged for 5 min at 10,000 rpm and the clear supernatant that was obtained was transferred into HPLC vial for HPLC-FLD analysis and the samples were kept at -80 ^{o} C before and after analysis (Kasote et al, 2020a).

Phenolic acids extraction

Root samples were macerated at room temperature in methanol–water (70:30) solution for 72 hours. After filtration and evaporation of the solvent an aqueous extract was obtained, which was subjected to extraction with n-butanol. The butanolic fraction (100 g) was subjected to RP-18 flash column chromatography. Some fractions were reprocessed on a silica gel flash column to ensure accuracy of the extraction and isolation of secondary metabolites, in this case phenolic acids (Hassine et al 2016).

Hormone analysis

Roots were ground in liquid nitrogen with a mortar and a pestle. 50 mg were transferred into 1.5 ml microfuge tubes, and 1 mL of isopropanol: water: acetic acid (80:19:1, v/v) was added. All samples were vortexed for 30 s, sonicated for 1 h at 4 ^{o}C, and centrifuged (10621Xg, 10 min). Supernatant was separated and used for UPLC/ESI-HR-QTOFMS quantitative analysis of plant hormones. The separation of plant hormones was achieved on the Eclipse Plus C18 RRHD (1.8 µm, 50 x 2.1 mm) column with flow rate of 0.15 mL min $^{-1}$. Binary mobile phase, 0.1% aqueous formic acid (A) and 0.1% formic acid acetonitrile (B), was used with the following gradient program: 0 min, 0% B; 11 min, 80% B; 15 min, 100% B; 16 min 0% B. Analyses were performed at the constant flow rate of 0.15 mL min $^{-1}$. The column temperature was kept constant at 30 ^{o}C. Mass spectra was acquired in a positive mode using the ESI interface with above-described operating parameters. Standards were used to optimize UPLC/ESI-HR-QTOFMS analysis conditions and to prepare calibrations curves (Kasote, 2019). The standards used were made by Sigma Aldrich company.

Statistical analysis

Statistical analyses were performed using Statistical Analysis System (SAS) PROC ANOVA (SAS Institute, 2020) and means were separated using LSD 5%.

Results and Discussion

Quantitative analysis of amino acids

0 hours

The means of amino acids concentrations are displayed in table 7. The concentration of glutamine, citrulline, serine, asparagine, glycine, ß-alanine, methionine, tyrosine, phenylalanine, isoleucine, leucine, hydroxy proline, and valine were significantly higher in TAM-Uvalde while the concentration of threonine and ɤ-amino butyric acid were significantly higher in USDA PI 124104.

24 hours

The means of the amino acid concentrations are displayed in table 8. The amino acids glutamine, citrulline, serine, glycine, ß-alanine, alanine, hydroxy proline, isoleucine, and valine presented significant differences among treatments due to the interaction variety x inoculation. The amino acids asparagine and methionine presented differences due to varieties. Lastly, the amino acids threonine and ɤ-amino butyric acid presented significant differences between treatments due to inoculations.

48 hours

The means of the amino acid concentrations are displayed in table 9. The amino acids serine, asparagine, threonine, glycine, ß-alanine, ɤ-amino butyric acid, and hydroxy proline presented significant differences among the treatments due to the interaction variety x inoculation. The amino acids glutamine and alanine presented significant differences between the treatments due to varieties. Finally, the amino acids citrulline and methionine presented significant differences between the treatments due to inoculations.

72 hours

The means of the amino acid concentrations are displayed in table 10. The amino acids glutamine, citrulline, threonine, glycine, ɤ-amino butyric acid, methionine, tyrosine, and hydroxy proline presented differences among the treatments due to the interaction variety x inoculation. Finally, the amino acids ß-alanine and alanine presented differences between the treatments due to varieties.

6 weeks

The means of the amino acid concentrations are displayed in table 11. The amino acids ß-alanine and hydroxy proline presented differences among the treatments due to the interaction variety x inoculation. Lastly, the amino acid methionine presented differences between the treatments due to varieties. In addition, the total amino acid content in response to VDD in the resistant and susceptible genotypes at each time point can be observed in figure 1.

It is commonly known that amino acids are part of all living organisms, and, in some cases, the concentration of amino acids was higher due to inoculations. Thus, they may have been produced by the pathogen. For example, Gong et al (2007) indicated that asparagine, which is an essential amino acid derived from citrulline, is needed by fungus *Coniothyrium minitans* for conidiation. In other words, it needs it to reach its reproductive stage. Likewise, Canonica et al (1979) reported that the fungus *Cochliobolus miyabeanus* requires methionine to produce metabolites derived from cochlioquinone. Therefore, it can be thought that the fungus *Monosporascus cannonballus* may have produced amino acids to carry out physiological functions. Also, the presence of the pathogen may have

triggered the production of these non-essential amino acids in the plants regardless of the variety used, which can be interpreted as a response of the plant, which was reflected in significant differences due to the inoculation. Hence, further research, for instance, gene expression studies may be useful in determining their origin (Joshi et al, 2019).

lower concentration of defensive compounds in resistant varieties has been previously reported. For example, Hanh, Bonhoof and Grisebach (1985) documented that the concentration of glyceollin, which is a phytoalexin, was higher in a susceptible variety of soybean 28 hours after being inoculated with the pathogen *Phytophthora magasperma f. sp. glycinea*. However, the resistant variety used in this experiment, exhibited a higher production of the same compound 8 hours after the inoculation.

Citrulline is commonly present in plants belonging to the *Cucurbitaceae* family. Moreover, its presence is associated with responses to abiotic stresses. Interestingly, citrulline is an amino acid that is not translocated long distances within the plant and is found in higher concentrations within fruits of the same family (Joshi et al, 2019). In addition, citrulline is a precursor of arginine that is a precursor of nitric oxide, which has been reported to be produced by plants as a defensive compound (Vitor et al, 2013). However, it was not present in the roots (Table 7 thru 11). Nevertheless, it is notable that citrulline was only present in treatments whose plants were inoculated, which suggests that it is not involved in resistance to VDD. However, citrulline may have been used as a signaling molecule. For example, molecules such as hydrogen sulfide and nitric oxide are important signaling molecules in plants. Therefore, citrulline may have been involved in the activation of a physiological process within the plant (Zang and Xie, 2021).

The concentration of amino acids is linked to several factors. For instance, genotype, environment, and cultural practices (Bernillon et al, 2013). Therefore, the presence of the pathogen along with a defense response of the plant to counter its attack may have caused an increase in the concentration of free amino acids over time such as ɤ-amino butyric acid (GABA) (Tables 8, 9 and 10).

Hypersensitive responses of plants have been related to the presence of Ca, which is involved in the production of GABA (Kinnersley and Turano, 2000). Xu and Heath (1998) reported that the concentration of Ca in cells of resistant cowpea plants rises to generate a hypersensitive response to counter the attack of cowpea rust fungus, *Urumices vignae*. Thus, the surge in the concentration of GABA previously described may have been an indication of a similar response of the plants against the attack of *Monosporascus cannonballus*.

ɤ-amino butyric acid (GABA) is a 4 carbon non-protein amino acid that is involved in several functions of plants. For instance, it regulates pH and osmotic potential as well as the growth of pollen tubes and more remarkably, it prevents the accumulation of reactive oxygen species when plants are under stress. Hence, the spike in the concentration of this amino acid may have been related to the stress underwent by the plant due to the attack of the pathogen. Similarly, Kinnersley and Turano (2000) documented that the production of GABA is linked to low pH concentrations. Thus, the use of peat most as a medium, may have contributed to its production.

ɤ-amino butyric acid is reported as a signaling molecule because it is produced rapidly when the plant is wounded or suffered mechanical damage (Akçay et al, 2012;

Busch and Fromm 1999; Kinnersley and Turano 2000). Therefore, its presence in in the inoculated treatments suggests that the pathogen penetrated the roots. Moreover, the higher concentrations of GABA in the variety TAM-Uvalde 48- and 72-hours APT (Tables 9 and 10) may be used as an indicator for susceptibility. Consequently, the production of this amino acid, in this case, could be used as a marker.

The presence of higher concentrations of GABA may be indicative of the synthesis of secondary metabolites that might have been used as defensive compounds. Kinnersley and Turano (2000) documented that GABA could be a potential source of carbon to replenish intermediaries in the Krebs cycle that are used to produce secondary metabolites with antimicrobial properties such as phytoalexins, coumesterol and coumarin. Furthermore, its presence has been related to increased tolerance to heat stress in mung beans plants (Priya et al, 2019).

Amino acids such as valine, threonine, methionine, phenylalanine, isoleucine, and leucine are commonly found in melons. Moreover, they are linked to characteristics related to quality. For example, fruit aroma, nutritional value, and health-promoting properties (Singh et al 2020).

Current knowledge indicates that the catabolism of glycine and serine is energetically expensive. Hence, they usually remain in the plant without any change. On the other hand, glutamine is metabolized to aspartate and glutamate, which is a precursor of GABA (Hildebrandt et al, 2015).

It is notable that some amino acids such as lysine and tryptophan were not present in the roots (Table 7 thru 11). Lysine is required for the synthesis of L-pipecolate, which is a regulator of inducible plant immunity and tryptophan is a precursor of auxins and secondary metabolites such as, phytoalexins, glucosinolates and alkaloids (Hildebrandt et al, 2015; Navarova et al, 2012; Radwanski and Last, 1995).

Phenolic acids

0 hours

The means of the phenolic acid concentrations are displayed in table 12. Caffeic, gallic and chlorogenic acids were significantly higher in TAM-Uvalde while phthalic acid was significantly higher in USDA PI 124104.

24 hours

The means of the phenolic acid concentrations are displayed in table 13. Hydroxy benzoic, protocatechuic, trans-cinnamic, p-coumaric and chlorogenic acids presented significant differences among the treatments due to the interaction variety x inoculation. Caffeic acid presented differences between the treatments due to inoculations. Lastly, phthalic acid presented significant differences between the treatments due to varieties.

48 hours

The means of the phenolic acid concentrations are displayed in table 14. Hydroxy benzoic, caffeic, protocatechuic, gallic, p-coumaric, ferulic and chlorogenic acids presented significant differences due to the interaction variety x inoculation. Phthalic acid

presented significant differences between the treatments due to varieties. Finally, trans-cinnamic acid presented significant differences between the treatments due to inoculations.

72 hours

The means of the phenolic acid concentrations are displayed in table 15. Hydroxy benzoic, phthalic, gallic, p-coumaric, ferulic and chlorogenic acids presented significant differences among the treatments due to the interaction variety x inoculation. Protocatechuic acid presented differences between the treatments due to varieties. Finally, trans-cinnamic acid presented differences between the treatments due to inoculations.

6 weeks

The means of the phenolic acid concentrations are displayed in table 16. Hydroxy benzoic, phthalic, gallic, trans-cinnamic and ferulic acids presented significant differences among the treatments due to the interaction variety x inoculation. P-coumaric acid presented significant differences between treatments due to varieties. Lastly, Protocatechuic and chlorogenic acids presented significant differences between treatments due to inoculations.

The influence of the pathogen *Monosporascus cannonballus* inoculation in VDD-susceptible, TAM-Uvalde and VDD-resistance USDA PI 124104 on the production of phenolic acids in roots was examined for 0 to 6-weeks after inoculation. In the absence of the pathogen (0 hours), the VDD-resistant genotype USDA PI 124104 had almost twice the content of phthalic acid (7039.54 µg/g) as compared with VDD-susceptible TAM-

Uvalde (3378.27 µg/g) (Table 12), indicating a constitutive difference between genotypes. However, after inoculation there are significant differences between varieties. TAM-Uvalde had the highest value with 7152.23 µg/g while USDA PI 124104 had a value of 1584.56 µg/g, which indicates that this compound was induced in the susceptible variety. Afterwards, at 48 hours APT, there were significant differences between varieties. TAM-Uvalde had the highest value with 9063.86 µg/g while USDA PI 124104 had a value of 8433.55 µg/g, which indicates that this compound was induced in both varieties. At 72 hours APT, significant interaction ($p<0.0012$) was observed between genotypes in response to pathogen inoculation (Table 15), while the pathogen infection induces phthalic acid accumulation in VDD-resistant genotype by doubling its content (9193.4 µg/g) compared to the susceptible genotype (3705.3 µg/g) where pathogen infection results on a 40% reduction in phthalic acid. Lastly, 6 weeks APT, the interaction genotype x inoculation was also significant ($p<0.0011$). treatment SI had the highest value with 4879.5 µg/g, followed by RI with 4174.11 µg/g. Therefore, it is possible that phthalic acid can participate on VDD resistance in melon by either higher constitutive content or by accumulation induction in response to the pathogen.

Phthalic acid was constitutively present in a higher concentration in the roots of the variety USDA PI 124104, which may have provided resistance to VDD. For instance, higher concentrations of constitutive compounds such as tannins have been documented to provide resistance to venturia shoot light in European aspen trees (*Populus tremula* L.) in some genotypes. Moreover, the presence of constitutive defensive compounds is advantageous because the plant can devote limited resources to growth and reproduction

(Bandau et al, 2021)

The existence of phthalic acid in melon roots may be associated with allelopathic properties, which are helpful for establishing dominance in their environment. (Huiyong et al, 2014). Hence, its presence in high concentration in the roots before inoculating the pathogen may be indicative of its production with the objective of controlling competition.

Several types of phthalic acids have been documented to have antimicrobial activities. For instance, Di(2-ethylhexyl) phthalate and di-n-butyl phthalate. These compounds inhibit mycelium growth and spore germination of fungi (Habib and Karim, 2009; Li et al 2021; Liang et al, 2020).

Phthalic acid has been reported to be present in 60 plant species belonging to 38 families (Liang et al, 2020). However, little is known about its role in melon roots and this study reports its presence in them. Nonetheless, further research is needed to pinpoint the type of phthalic acid found in the roots and its function.

Benzoic acid is a precursor of salicylic acid. Thus, its presence, in high quantities, may be an indication of the production of this hormone, which is linked to plant immunity (Ribnicky, Shulaev and Raskin, 1998). Moreover, trans-cinnamic acid, which was also present in the roots, may enhance the production of this hormone (Araniti et al, 2018).

Hormones

0 hours

The means of the hormone concentrations of the varieties are displayed in table 17. Salicylic and jasmonic acid were significantly higher in TAM-Uvalde.

24 hours

The means of the hormone concentrations are displayed in table 18. Jasmonic and abscisic acid presented significant differences among the treatments due to the interaction variety x inoculation. Lastly, salicylic acid presented significant differences between the treatments due to inoculations.

48 hours

The means of the hormone concentrations are displayed in table 19. Abscisic acid presented differences among the treatments due to the interaction variety x inoculation. Jasmonic and salicylic acid presented significant differences due to varieties. Lastly, kinetin and gibberellin acid presented significant differences due to inoculations.

72 hours

The means of the hormone concentrations are displayed in table 20. Jasmonic acid presented significant differences among the treatments due to the interaction variety x inoculation. Lastly, salicylic acid presented significant differences between the treatments due to inoculations.

6 weeks

The means of the hormone concentrations are displayed in table 21. Salicylic acid presented significant differences among the treatments due to the interaction variety x inoculation. Finally, abscisic acid presented significant differences between the treatments due to inoculations.

It is important to note that abscisic, indole acetic, gibberellic, jasmonic and salicylic acids as well as kinetin, melatonin, methyl jasmonate and zeatin were targeted in this analysis. However, only salicylic, jasmonic, abscisic, gibberellic acids and kinetin were found in the roots.

The presence of salicylic acid is usually associated with a defensive response of the plant against biotrophic pathogens, which is called systemic acquired resistance (SAR). On the other hand, jasmonic acid is associated with immune systemic resistance (ISR), which is activated against necrophitic pathogens and generally, both defensive mechanisms are antagonistic. However, the presence of both hormones has been documented. For example, some biotrophic pathogens stimulate the production of jasmonic acid to reduce the production of salicylic acid (Burger and Chory, 2019). Thus, the existence of both hormones in the roots suggests that the fungus *Monosporascus cannonballus* exploits this mechanism. In addition, such mechanisms of defense have not been reported in melons. Hence, this study infers their existence.

Ethylene is a hormone related to plant immunity and it works together with jasmonic acid. Nevertheless, it was not measured in this experiment. However,

methionine, which is its precursor was present in the roots (Table 8, 10 and 11). Thus, these results suggest the existence of this hormone in the roots (Burger and Chory, 2019; Ton et al, 2002).

The existence of salicylic and jasmonic acid before inoculating the pathogen (0 hours) may have been caused by an unknown environmental challenge (Table 17). Hence, more research is needed to clarify it. Moreover, the concentration of jasmonic acid decreases while the concentration of salicylic acid increases 24, 48, 72 hours and 6 weeks APT (Tables 18 thru 21). These results show the antagonistic relationship between the defensive mechanisms in which both hormones are involved.

Gibberellic acid was present in the roots, as well. It is widely known that this hormone is related to cell division and elongation. Additionally, it is also produced by fungi. For example, it has been reported to be produced by fungi that attack rice (Hedden and Sponsel, 2015). Hence, this hormone may have been produced by the fungus *Monosporascus cannonballus*.

Abscisic acid can be synthesized in the roots and enters the xylem to reach leaves and regulate stomata aperture. Thus, its presence in the roots is not likely to be involved in plant immunity (Srivastava, 2001)

Conclusions

Phthalic acid is constitutive and induced in VDD-resistant genotype in response to pathogen infection suggesting that it can putatively participate on VDD resistance reaction. However, further research using forward, and reverse genetic approaches are

needed to determine its role in the resistance to *Monosporascus cannonballus* in melons. In addition, the presence of ɤ-amino butyric acid seems to be associated with susceptibility since it is upregulated in response to pathogen infection in the susceptible but not in the resistant genotype.

CHAPTER V

CONCLUSIONS

The results of the generation means analysis show the existence of dominance and additive effects in the inheritance of VDD resistance. However, dominance effects could have caused a greater impact, which was reflected in a moderate narrow sense heritability estimate. Additionally, factors such as maternal effect or epistatic interactions did not play a significant role in the inheritance of this trait. In addition, three genes control the resistance, in the variety USDA PI 124104, which will be useful in developing resistant varieties using conventional phenotypic selection on visual assessment of root damage and traditional backcrossing methods.

The narrow sense heritability estimate calculated in generation means analysis was lower than the one of mid-parent-offspring regression. However, the latter was higher, possibly, due to the presence of heterosis. In addition, this study proves that resistance in USDA PI 124104 can be exploited to develop resistant plant material to VDD.

The existence of higher concentrations of ɤ-amino butyric acid in in the variety, TAM-Uvalde, could be interpreted as an indicator for susceptibility. Therefore, the production of this amino acid in roots when plants are affected by VDD might be used as a marker. Finally, phthalic acid was present in the roots before and after inoculation, which indicates its constitutive and induced presence. Thus, it may have had a role in the resistance observed in the variety USDA PI 124104. However, further research is needed to clarify its role.

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APENDIX

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**
P_1 (TAM-Uvalde)	5	3	3	0
P_2(124104)	9	1.1111	1-2	0.1111
F_1	37	1.1578	1-5	0.4608
F_2	138	2.2463	1-5	2.2162
$BC_1P_1(F_1xP_1)$	11	1.8181	1-5	1.9636
$BC_1P_2(F_1xP_2)$	14	1.2857	1-5	1.1428

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

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

NS = Not significant at **α** = 0.05. **A**=$2BC_1$-P_1-F_1; **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.

Table 3**:** 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 **α** = 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 resistance. The F_2 population from the cross of susceptible and resistant genotypes were evaluated for segregation ratios using Chi-Square test.

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

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

Table 5**:** Pedigree information of populations used to evaluate vine decline disease resistance under field conditions

Female Parent	Male Parent	Off-Spring
Dulce	USDA PI 124104	M1 (Parent)
Ames 20608	Dulce	M2 (Parent)
M1	M2	M3
M7	M8	M4
M6	M1	M5
212210	TAM-Uvalde	M6 (Parent)
TAM-Uvalde x MF126 (1405 PMR x USDA PI 124104)	1405 (Deltex x Perlita F12)	M7(Parent)
Dulce	USDA PI 124104	M8 (Parent)

Table 6**:** Mid parent offspring regression narrow sense heritability estimate for VDD resistance in melons.

Narrow sense heritability estimates			
Population	**M3**	**M4**	**M5**
VDD Resistance	0.166 ± 0.4	0.66 ± 0.81	0.60 ± 0.77

Narrow sense heritability ± standard error; **M3**= M1xM2; **M4**= M7xM8; **M5**=M6xM1

Table 7: Root amino acids composition in VDD-resistant and susceptible melon genotypes before inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences ($P<0.05$) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for varieties (green). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

0 Hours					
Class of Metabolites	Compound	RI	RC	SI	SC
Amino Acids (µg/g Fresh Weight)	**Arginine**	0	0	0	0
	Glutamine	0 b	0 b	7.15 a	7.15 a
	Citrulline	0 b	0 b	0.44 a	0.44 a
	Serine	0 b	0 b	0.53 a	0.53 a
	Asparagine	42.83 b	42.83 b	362.25 a	362.25 a
	Threonine	27.66 a	27.6 a	70.19 b	70.19 b
	Glycine	0 a	0 a	0.16 a	0.16 a
	ß-Alanine	14.85 b	14.85 b	19.5 a	19.5 a
	Alanine	48.72 a	48.72 a	18.28 b	18.28 b
	ɤ-aminobutyric acid	11.85 b	11.85 b	19.5 a	19.5 a
	Methionine	11.97 b	11.97 b	27.97 a	27.97 a
	Tyrosine	0.08 b	0.08 b	4.52 a	4.52 a
	Phenylalanine	0 b	0 b	0.86 a	0.86 a
	Isoleucine	0 b	0 b	1.68 a	1.68 a
	Leucine	0 b	0 b	1.26 a	1.26 a
	Hydroxy Proline	0 b	0 b	0.11 a	0.11 a
	Valine	0 b	0 b	0.4 a	0.4 a
	Proline	0	0	0	0
	Histidine	0	0	0	0

Treatments RI and SI were not inoculated

Table 8: Root amino acid composition in VDD-resistant and susceptible melon genotypes 24 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink), varieties (green) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

24 Hours					
Class of Metabolites	Compound	RI	RC	SI	SC
Amino Acids (µg/g Fresh Weight)	**Arginine**	0	0	0	0
	Glutamine	169.2 a	11.4 c	69.5 b	0 d
	Citrulline	9.59 a	0 b	0 b	0 b
	Serine	1.2 a	0 b	0 b	0 b
	Asparagine	661.01 a	661.01 a	260.4 b	260.4 b
	Threonine	58.62 b	11.02 a	58.62 b	11.02 a
	Glycine	1.75 a	0 b	0 b	0 b
	ß-Alanine	0 b	0 b	0 b	2.23 a
	Alanine	307.86 a	14.01 c	27.97 b	13.5 c
	ɤ-aminobutyric acid	26.23 b	4.85 a	26.23 b	4.65 a
	Methionine	8.53 a	8.53 a	29.67 b	29.67 b
	Tyrosine	0	0	0	0
	Phenylalanine	0	0	0	0
	Isoleucine	0 b	0 b	0 b	0.98 a
	Leucine	0	0	0	0
	Hydroxy Proline	0 b	0 b	4.76 a	0 b
	Valine	0 b	0 b	1.34 a	0 b
	Proline	0	0	0	0
	Histidine	0	0	0	0

Table 9: Roots amino acid composition in VDD-resistant and susceptible melon genotypes 48 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink), varieties (green) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

48 Hours					
Class of Metabolites	**Compound**	**RI**	**RC**	**SI**	**SC**
Amino Acids (µg/g Fresh Weight)	**Arginine**	0	0	0	0
	Glutamine	0.5 a	0.5 a	0 b	0 b
	Citrulline	45.22 a	0 b	45.22 a	0 b
	Serine	0 d	27.09 a	12.38 ab	2.08 c
	Asparagine	72.4 c	89.90 b	290 a	0 d
	Threonine	23 a	0 b	0 b	0 b
	Glycine	697.1 a	161.2 b	0 c	0 c
	ß-Alanine	22.75 b	122.8 a	120.5 a	5.99 c
	Alanine	563 a	563 a	90.05 b	90.05 b
	ɤ-aminobutyric acid	0 c	0 c	189.2 a	4.72 b
	Methionine	0 b	11.82 a	0 b	11.82 a
	Tyrosine	0	0	0	0
	Phenylalanine	0	0	0	0
	Isoleucine	0	0	0	0
	Leucine	0	0	0	0
	Hydroxy Proline	0 b	0 b	33.64 a	0 b
	Valine	0	0	0	0
	Proline	0	0	0	0
	Histidine	0	0	0	0

Table 10: Roots amino acid composition in VDD-resistant and susceptible melon genotypes 72 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink) and varieties (green). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

72 Hours					
Class of Metabolites	**Compound**	**RI**	**RC**	**SI**	**SC**
Amino Acids (µg/g Fresh Weight)	**Arginine**	0	0	0	0
	Glutamine	59.48 a	0 b	0 b	0 b
	Citrulline	0 b	0 b	77.91 a	0 b
	Serine	0	0	0	0
	Asparagine	0	0	0	0
	Threonine	31.08 a	3.15 b	2.05 b	0 c
	Glycine	0 b	0 b	32.85 a	0 b
	ß-Alanine	104.5 b	104.5 b	206.9 a	206.9 a
	Alanine	41.34 b	41.34 b	83.01 a	83.01 a
	ɤ-aminobutyric acid	59.29 c	60.48 c	91.52 a	84.19 b
	Methionine	18.67 c	32.04 b	54.04 a	38.81 b
	Tyrosine	0 b	0 b	0 b	0.38 a
	Phenylalanine	0	0	0	0
	Isoleucine	0	0	0	0
	Leucine	0	0	0	0
	Hydroxy Proline	0 b	0 b	42.66 a	0 b
	Valine	0	0	0	0
	Proline	0	0	0	0
	Histidine	0	0	0	0

Table 11: Roots amino acid composition in VDD-resistant and susceptible melon genotypes levels in plants 6 weeks after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences ($P<0.05$) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink) and varieties (green). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

6 Weeks					
Class of Metabolites	**Compound**	**RI**	**RC**	**SI**	**SC**
Amino Acids (µg/g Fresh Weight)	**Arginine**	0	0	0	0
	Glutamine	0	0	0	0
	Citrulline	0	0	0	0
	Serine	0	0	0	0
	Asparagine	0	0	0	0
	Threonine	0	0	0	0
	Glycine	0	0	0	0
	ß-Alanine	248.3 a	0 b	0 b	0 b
	Alanine	144.4 a	173.71 a	190.4 a	167 a
	ɤ-aminobutyric acid	38.56 a	31.71 a	37.31 a	37.64 a
	Methionine	24.7 b	24.7 b	45.88 a	45.88 a
	Tyrosine	0	0	0	0
	Phenylalanine	0	0	0	0
	Isoleucine	0	0	0	0
	Leucine	0	0	0	0
	Hydroxy Proline	0 b	0 b	2.58 a	0 b
	Valine	0	0	0	0
	Proline	0	0	0	0
	Histidine	0	0	0	0

Table 12**:** Roots phenolic acid composition in VDD-resistant and susceptible melon genotypes before inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for varieties (green). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

0 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Phenolic acids (µg/g. Fresh weight)	**Hydroxy benzoic acid**	3190.76 a	3190.76 a	3016.85 a	3016.85 a
	Caffeic acid	246.67 b	246.67 b	739.5 a	739.5a
	Protocatechuic acid	1025.85 a	1025.85 a	1052.91 a	1052.91 a
	Phthalic acid	7039.54 a	7039.54 a	3378.27 b	3378.27 b
	Gallic acid	0 b	0 b	5979.44 a	5979.44 a
	Trans-cinnamic acid	0 a	0 a	0 a	0 a
	P-Coumaric acid	0 a	0 a	0 a	0 a
	Ferulic acid	0 a	0 a	0 a	0 a
	Chlorogenic Acid	310.38 a	310.38 a	130.83 b	130.83 b

Treatments RI and SI were not inoculated

Table 13**:** Roots amino phenolic acid composition in VDD-resistant and susceptible melon genotypes 24 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink), blue (inoculation), and varieties (green). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus,* (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

24 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Phenolic acids (µg/g. Fresh Weight)	**Hydroxy benzoic acid**	3403.4 b	3017.5 c	3006.37 c	7792.88 a
	Caffeic acid	1603.75 a	0 b	1603.75 a	0 b
	Protocatechuic acid	1143.8 c	378.1 d	2355.65 a	1812.33 b
	Phthalic acid	1584.56 b	1584.56 b	7152.23 a	7152.23 a
	Gallic acid	956.3 d	3903.4 c	4393.71 b	20630.39 a
	Trans-cinnamic acid	1087.3 b	744.5 c	1181.69 b	5720.85 a
	P-Coumaric acid	295.55 c	26.07 d	532.95 a	383.05 b
	Ferulic acid	1811.5 b	1811.5 b	2346.71 a	2346.71 a
	Chlorogenic Acid	37.75 a	0 b	0 b	0 b

Table 14: Roots phenolic acid composition in VDD-resistant and susceptible melon genotypes 48 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences ($P<0.05$) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink), varieties (green) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

48 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Phenolic acids (µg/g. Fresh Weight)	**Hydroxy benzoic acid**	5087.88 d	7146.33 c	16342.89 a	8614.5 b
	Caffeic acid	0 b	0 b	993.66 a	0 b
	Protocatechuic acid	637.22 c	2057 b	12644 a	0 d
	Phthalic acid	8433.55 b	8433.55 b	9063.86 a	9063.86 a
	Gallic acid	34796.44 a	23537.78 c	22015.67 d	33797 b
	Trans-cinnamic acid	3380.39 a	1710.56 b	3380.39 a	1710.56 b
	P-Coumaric acid	5438.11 c	10293.2 b	15475.6 a	2766 d
	Ferulic acid	34796.44 a	23537.78 c	22015.67 d	33797 b
	Chlorogenic Acid	3027.55 d	8356.22 b	5334.28 c	9777 a

Table 15**:** Roots phenolic acid composition in VDD-resistant and susceptible melon genotypes 72 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink), varieties (green) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

72 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Phenolic acids (µg/g. Fresh Weight)	**Hydroxy benzoic acid**	5377.4 a	4587.4 b	5228.9 a	3738.6 c
	Caffeic acid	0 a	0 a	0 a	0 a
	Protocatechuic acid	611.25 b	611.25 b	1472.7 a	1472.7 a
	Phthalic acid	9193.4 a	4471.83 b	3705.3 c	4774.2 b
	Gallic acid	1630.95 a	0 c	318.37 b	0 c
	Trans-cinnamic acid	2065.01 a	1861.93 b	2065.01 a	1861.93 b
	P-Coumaric acid	1263.3 a	984.15 c	1091.9 b	1171.6 b
	Ferulic acid	5744 b	6140.37 a	2787.1 d	4349.6 c
	Chlorogenic Acid	0 d	54.82 c	70.23 b	613.09 a

Table 16**:** Roots phenolic acid composition in VDD-resistant and susceptible melon genotype 6 weeks after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety & inoculation (pink), varieties (green) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

6 weeks					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Phenolic acids (µg/g. Fresh Weight)	**Hydroxy benzoic acid**	4274.6 a	3566.4 c	3932.5 b	15684 d
	Caffeic acid	0 a	0 a	0 a	0 a
	Protocatechuic acid	1618.6 a	998.8 b	1618.6 a	998.8 b
	Phthalic acid	4174.11 b	3862.5 c	4879.5 a	2492.7 d
	Gallic acid	0 c	142.3 b	813.5 a	0 c
	Trans-cinnamic acid	2818.44 b	3032.3 a	2674.2 b	1613.9 c
	P-Coumaric acid	4073.56 a	4073.56 a	844.26 b	844.26 b
	Ferulic acid	2028.11 b	4634 a	4107.5 a	2325.9 b
	Chlorogenic Acid	441.21 a	0 b	441.21 a	0 b

Table 17**:** Roots hormone composition in VDD-resistant and susceptible melon genotypes before inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for varieties (green). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

0 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Hormones (µg/g. Fresh Weight)	**Abscisic acid**	0.12 a	0.12 a	0.32 a	0.32 a
	Jasmonic acid	1.88 b	1.88 b	3.52 a	3.52 a
	Gibberellic acid	2.47 a	2.47 a	2.14 a	2.14 a
	Salicylic acid	1.76 b	1.76 b	2.43 a	2.43 a
	Kinetin	1.34 a	1.34 a	2.12 a	2.12 a

RI and SI were not inoculated

Table 18**:** Roots hormone composition in VDD-resistant and susceptible melon genotypes 24 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for the interaction variety x inoculation (pink) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

24 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Hormones (µg/g. Fresh Weight)	**Abscisic acid**	0 a	0 a	0 a	0 a
	Jasmonic acid	1.08 a	0 c	0.33 b	0.53 b
	Gibberellic acid	0 a	0 a	0 a	0 a
	Salicylic acid	2.65 a	1.67 b	2.65 a	1.67 b
	Kinetin	0 b	0 b	0.75 a	0 b

Table 19**:** Roots hormone composition in VDD-resistant and susceptible melon genotypes 48 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety x inoculation (pink) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

48 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Hormones (µg/g. Fresh Weight)	**Abscisic acid**	0.25 b	0 c	0 c	0.51 a
	Jasmonic acid	3.56 a	3.56 a	1.06 b	1.06 b
	Gibberellic acid	4.16 a	0 b	4.16 a	0 b
	Salicylic acid	0.43 b	0.43 b	3.37 a	3.37 a
	Kinetin	1.87 a	0 b	1.87 a	0 b

Table 20**:** Roots hormone composition in VDD-resistant and susceptible melon genotypes 72 hours after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety x inoculation (pink) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

72 Hours					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Hormones (µg/g. Fresh Weight)	**Abscisic acid**	0 a	0 a	0 a	0 a
	Jasmonic acid	1.92 a	0 b	0 b	0 b
	Gibberellic acid	0 a	0 a	0 a	0 a
	Salicylic acid	3.29 a	2.24 b	3.29 a	2.24 b
	Kinetin	0 a	0 a	0 a	0 a

Table 21**:** Roots hormone composition in VDD-resistant and susceptible melon genotypes 6 weeks after inoculating plants. Varieties TAM-Uvalde (Susceptible) and USDA PI 124104 (Resistant). The significant differences (P<0.05) among the treatments are shown by different letters, based on a post hoc least significant difference test. Different colors indicate means separation for interaction variety x inoculation (pink) and inoculation (blue). RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus*, (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.

6 Weeks					
Class of Metabolite	**Compound**	**RI**	**RC**	**SI**	**SC**
Hormones (µg/g. Fresh Weight)	**Abscisic acid**	1.57 b	2.79 a	1.57 b	2.79 a
	Jasmonic acid	0.32 a	0.92 a	0.89 a	0.32 a
	Gibberellic acid	0 a	0 a	0 a	0 a
	Salicylic acid	3.26 a	2 c	2.76 b	2.94 b
	Kinetin	0 a	0 a	0 a	0 a

Figure 1: Time course. Total amino acid content in response to VDD in melon resistant and susceptible genotypes. RI, resistant (USDA PI 124104) inoculated with *Monosporascus cannonballus,* (MC); RC, resistant (USDA PI 124104) mock-inoculated; SI, susceptible (TAM-Uvalde) inoculated with MC; and SC, susceptible (TAM-Uvalde) mock-inoculated.