IMPROVING WATERMELON PRODUCTIVITY AND ANTHRACNOSE RESISTANCE IN SOUTH TEXAS

A Thesis

by

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ABSTRACT

In 2018, the production value of watermelon in the United States was $657 million out of 2.0 Mt harvested. That same year, Texas was second largest watermelon producing states with 0.40 Mt harvested. The first objective of this study was to evaluate the yield potential of genotypes in south Texas, while the second objective was to improve our understanding of how anthracnose race 2 affects watermelon.

In 2018, there were 34 genotypes tested at three locations (College Station, Uvalde, and Weslaco, TX). In 2019, 20 of the previous 34 genotypes were evaluated. The germplasm was evaluated for total yield, soluble solid, rind thickness, fruit length, circumference and weight. In the first year, TAM 2, 'Sunshade', 'Chubby Gray', and 'Big Crimson', had high total yield, 31 to 29 Mg ha^{-1}. During the second year, 'Sunshade' showed high total yield, 36 Mg ha^{-1}, once again. Path analysis indicated that fruit weight and total fruit could indirectly select for total yield, with direct effects of 0.93 and 0.92, respectively. Correlation to total yield showed fruit weight (r^2 = 0.48) to have a higher correlation than fruit number (r^2 = 0.17). Study allowed us to gain a better understanding of watermelon, identify potential parents for population development, and to further the watermelon breeding program.

The second objective was to improve the understanding of anthracnose, couple of studies were conducted; inoculation procedure optimization, germplasm screening, and population genetic study. The procedure optimization looked at different anthracnose race 2 inoculum concentrations and potential DAI to rate seedlings for disease severity. The results indicated that 100,000 spore mL^{-1} was an adequate inoculum concentration to screen watermelon seedlings and 8 DAI was an optimal day to rate seedlings. The germplasm screening evaluated previously screened genotypes

for anthracnose race 2 resistance. The AU-series developed in Alabama showed resistance, as did PI 189225 and 271778. In the population genetic study, four different F_2 populations were evaluated for anthracnose race 2 resistance, with the goal of looking at the mode of inheritance.

DEDICATION

I dedicate this work to my parents, Christina Covarrubias and Jesus M. Correa, who from an early age instilled hard work and determination. Although they were not able to help with reading assignments or tricky math problems, they were able to provide a roof over our heads, food, and love. They left family and friends in Mexico to give their children and themselves a better future and life. If it were not for their decision to immigrate to the United States, there is a high chance that I would not be the person that I am today. To my family and friends, who have been there to listen and help.

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Contributors

This work was supervised by a thesis committee consisting of Dr. Subas Malla, primary advisor (Department of Horticultural Sciences), Dr. Kevin M. Crosby, co-advisor (Department of Horticultural Sciences), and Dr. Seth C. Murray, committee member (Department of Soil and Crop Sciences).

The watermelon seeds that were used for the germplasm evaluation in Chapter 1, were obtained from Dr. Cecilia McGregor, Associate Professor at University of Gerogia. The data acquired from Weslaco, TX during the 2018 and 2019 season found in Chapter 2 was thanks to Dr. Carlos A. Avila, Assistant Professor in the Department of Horticultural Science. Dr. Avila grew and managed the plants throughout the 2018 and 2019 growing season. The anthracnose race 2 inoculations in Chapter 3 were thanks to Dr. Anthony P. Keinath, Professor at Clemson University in the Department of Plant and Environmental Sciences. Dr. Keinath provide the anthracnose race 2 isolates that were used to inoculate and test the resistance of seedlings.

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NOMENCLATURE

CST	College Station, Texas
UVD	Uvalde, Texas
WES	Weslaco, Texas
TY	Total yield (Mg per hectare)
TF	Total fruit count (thousand fruit per hectare)
FW	Fruit weight (kg)
FL	Fruit length (cm)
FD	Fruit diameter (cm)
SC	Total soluble solids (°brix)
RT	Rind thickness (cm)
ANOVA	Analysis of variance
GxE	Genotype by environment interaction
PVP	Plant Varietal Protection
USDA	United States Department of Agriculture
DAI	Day after inoculation
PLL	Percentage of Leaf Lesion
PCL	Percentage of Cotyledon Lesion
SHL	Severity of Hypocotyl Lesion
SPL	Severity of Petiole Lesion
INDX	Disease index created at NCSU
AUDPC	Area under the disease progress curve

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CHAPTER I

1. INTRODUCTION: WATERMELON PRODUCTION IN TEXAS

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai], considered a staple summertime fruit in the United States is actually classified as a vegetable by commercial companies and the research community. Despite this misclassification, watermelon is an important crop to both consumers, who enjoy it, and growers, who make a living off of it. Watermelon is a refreshing fruit that is mostly made up of water, approximately 92% of the overall weight. It is a low calorie, nutritional fruit that contains, citrulline, lycopene, vitamin A, B6, and C (Collins et al., 2007; Hayashi et al., 2005; Perkins-Veazie et al., 2006). Although the watermelon flesh is commonly enjoyed as a sliced dessert, or in salads and smoothies, the rind is also consumed as pickled rind (Wehner, 2008). Watermelon is grown throughout the world with the largest country producer of watermelon for the past decade being China (FAO, 2018).

In 2018, the production value of watermelon in the United States was $657 million out of 1.98 Mt harvested. That same year, Texas was one of the top five watermelon producing states with 0.40 Mt harvested. In spite Texas' high production, it lagged behind other top producing states in productivity (43 Mg ha^{-1}) (NASS, 2019). In Texas, we observed regions that practice relay cropping of watermelon and wheat, where the wheat is killed off and the watermelon is planted afterwards within the standing straw. There are also abiotic (wind stress) and biotic stresses (various diseases) that may contribute to the lower yields (Hall and Holloway, 2006; Larry, 2017). Lastly, a difference in cultivars used in the various states, where states with high productivity grow hybrids and triploids, while in Texas some producers grow old, open-pollinated cultivars (Crosby,

2017). The lower productivity can be attributable to the environment, pests, or varieties grown in Texas.

In Texas during the 2018 season, watermelon was the most important vegetable crop with a production value of $143 million, while potato was the second most important at $94 million (NASS, 2019). Due to Texas being such as large state, 696,241 km2, different regions have their own micro-climate. The eastern part of the state is known for a more humid climate, while the southern region is known to have a drier climate (Nielsen-Gammon, 2011; Webb, 1950). Although this different, watermelons are grown in practically every region of the state, from the Rio Grande Valley up to the Plains of the Panhandle (Anciso et al., 2012). Several growers can trace back their watermelon operations to the early 1900's, "Greg Green Farms in Henderson started in 1915 and is thought to be the first farm to commercially grow watermelon in Texas" (Lane, 2016). There exist several festivals throughout the state, which celebrate watermelons, such as the 'Melon Fest', 'Watermelon Thump', and 'What-A-Melon-Festival' (Parish, 2018; Reinhard, 2015; Tuder, 2015). Two Texas seed companies have been developing watermelon varieties since the early 1900's, Willhite Seed Company (Poolville, TX) and Coffey Seed Company (Plainview, TX). In the late 1960's Willhite and Coffey merged and kept the name of Willhite Seed Company. Now known as Willhite Seed Inc., it is owner of several expired plant variety protections (PVP) varieties, which include 'Legacy', 'Chubby Gray', 'Big Crimson', and 'Yellow Crimson'. As well, Willhite released 17 varieties to the United States Department of Agricultural-Agricultural Research Service (USDA-ARS) germplasm collection (Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, USDA-ARS, Griffin, GA). Unfortunately due to a severe seed borne disease, bacterial fruit blotch, Willhite had to cutback and become a "retail only"

company (Hinsley, 2013). This has led to a vacuum in watermelon germplasm development that is suitable for Texas and small farm owners.

Growers in Texas have to combat several diseases that affect watermelon production. The prominent diseases include; fusarium wilt (*Fusarium oxysporum f. sp. niveum*), gummy stem blight (*Didymella bryoniae*), anthracnose (*Colletotrichum orbiculare*), powdery mildew (*Sphaerotheca fuliginea*), downy mildew (*Pseudoperonospora cubensis*), bacterial fruit blotch (*Acidovarax avenae* subsp. *citrulli*), watermelon mosaic virus, squash leaf curl virus, and papaya ring spot (Anciso et al., 2012; Hall and Holloway, 2006; Keinath, 2018; Wehner, 2008). In Texas, diseases that affect watermelon production if not controlled, include fusarium wilt, gummy stem blight and anthracnose. They are a severe issue in East Texas, where anthracnose has the potential of destroying 100% of the crop if not managed and fusarium wilt has an 85% probability (Hall and Holloway, 2006). These diseases are important in other top watermelon producing states and were considered of top research priorities for the Watermelon Research and Development Group (WRDG) (Kousik et al., 2016). The WRDG classified anthracnose as a top ten priority and was placed into Group 2, which included bacterial fruit blotch. This makes it a disease of importance in watermelon production, it has historically been an issue in the majority of the eastern U.S. (Dutta, 1958; Gardner, 1918; Sowell Jr et al., 1980). Currently, anthracnose race 2 is a major issue and is affecting many of the southeastern states (Gay, 2017; Keinath, 2018). A potential method to manage these diseases is to grow resistant watermelons, but unfortunately there does not exist resistant cultivars with adequate yield and fruit quality.

The goal of this thesis work is to evaluate a diverse set of germplasm at three distinct locations, (Weslaco, TX, Uvalde, TX and College Station, TX) for yield, its components, and the various quality traits. These location are in regions of major watermelon production (Winter

Garden and Rio Grande Valley) and one location (College Station, TX) has been used in a large multi-state germplasm evaluation study (Anciso et al., 2012; Dia et al., 2016a). The goal is to identify potential parents, from our pool of germplasm, suited for germplasm development in Texas. This work also aimed at better understanding anthracnose, specifically race 2, which no resistant commercial cultivar is currently available. The ultimate goal of this thesis work and program is to develop watermelon varieties adequate to Texas with resistance to anthracnose race 2 and other diseases mentioned previously.

CHAPTER 2

2. WATERMELON GERMPLASM EVALUATION IN SOUTH TEXAS

2.1 Introduction

The warm season vegetable crop of watermelon is grown throughout the southeastern U.S., it belongs to the Cucurbitaceae family that includes cucumber (*Cucumis sativus*), melons (*Cucumis melo* L.), squash (*Cucurbita*), and several other species. Close relatives of watermelon include, *Citrullus amarus, Citrullus colocynthis* , *Citrullus lanatus* var. *citroides*, which have been used as a source of diseases resistance and expand genetic diversity of cultivated watermelon (Wehner, 2008). Watermelon is believed to have originated in Africa, the exact region of domestication is still in question (Rubatzky and Yamaguchi, 2012). Watermelon made its way to Southeast Asia then to Japan, followed by Europe, and lastly to the Americas in the 1500's. Africa is known as the primary center of diversity, with China being a second center of diversity (Wehner, 2008). Knowledge on the region of origin and domestication aids in the understanding of the environmental constraints that were imposed on the crop before humans began selecting for traits of their own interest and where to find new genetic sources of improvement.

The warm weather of the south United States is optimal for watermelon production, where day temperatures between 32 to 38°C are optimal conditions for watermelon production. A majority of watermelon production begins as transplants from protected conditions of a greenhouse to the field, the seedlings are transplanted after three to four weeks. Once transplanted into the field, plants begin to vine seven to ten days afterwards, at the same time the plant initiates branching. Watermelon plants are made up of trailing vines that contain two leaves, a tendril, and a flower at each node. After vining it takes about five to fourteen days for flowering. Watermelons

are monoecious with four to twelve staminate flowers before a single pistillate flower, ratio favors staminate flowers over pistillate flower (4-12 staminate: 1 pistillate). Bees are the common pollinators used in commercial production of watermelon. After pollination and fertilization, it takes approximately 30 to 45 days for fruit maturity. Watermelon's full growing cycle is short, usually 80 to 100 days. At harvest, there are three common methods of determining fruit maturity, drying of the nearest tendril, yellowing of the fruit's ground spot, and a hollow sound when tapping the watermelon. Growers are looking for optimal varieties that have high productivity and allow them to harvest multiple times.

The productivity or yield is an important trait that growers are interested in due to its impact on their finances and well-being. In agriculture, yield is the production of a commodity per a given area. In 2017, the average fresh yield for watermelon in the U.S. was 47.2 Mg ha^{-1}. However, most of this is water and at an average dry matter content of 8%, the dry matter yield of watermelon would be 3.8 Mg ha^{-1}. Yield is calculated from the marketable fruit, individual fruits that are not bottlenecked, undersized, or deformed. Those individuals that contain the blemishes mentioned are known as cull fruit, which decrease profits and is an important trait for breeders. Important yield component traits include, marketable yield, fruit count, early fruit number, cull fruit number, and fruit size. Fruit size and fruit number are significantly positively correlated with yield (Kumar and Wehner, 2011). The narrow-sense and broad-sense heritability for yield, were investigated and found to be low to intermediate (Kumar and Wehner, 2011). It was suggested that selecting for yield and its components would not be possible via single-plant hills, due to environmental effects and low heritability. It is however possible to adequately select for high yielding individuals if they were in later generations, tested at multiple locations, and in replications (Kumar and Wehner, 2011, 2013). In Dia et al. (2016a), a genotype by environmental interaction was identified for yield

and its components in 40 watermelon genotypes grown for three years and at eight location in the southern United States, including College Station, TX. Kang's stability analysis for those individuals was conducted and found that 'Stone Mountain', 'Stars-N-Stripes' (F_1), 'Calhoun Gray', 'Big Crimson', and 'Regency' were the most stable high yielding individuals (Dia et al., 2016a). Dia et al. (2016a) grouped genotypes into three categorize according to yield and stability, the fruit size differed across the categorize from large to small to mini, indicating fruit size as being an important component.

Watermelons are grouped into distinct size categories: mini (1.5 to 4.0 kg), icebox (4.0-5.5 kg), small (5.0-8.0 kg), medium (8.0-11.0 kg), large (11.0-14.0 kg), and giant (>14.0 kg). Currently, consumers prefer icebox, small, or medium sized watermelons and have been moving away from the large and giant sized watermelons since the early 2000's (Gusmini and Wehner, 2007; Sandlin et al., 2012). Heritability for fruit size was found to be low to intermediate (Gusmini and Wehner, 2007; Kumar and Wehner, 2011). They determined that fruit size can be changed within a few years of high selection intensity, but due to a small number of effective factors regulating size, the trait may be fixed within a few generations of selection. Instead, it would be easier to introgress specific traits into individuals with a fruit weight of interest via pedigree or backcross breeding. Although yield and its components are important for breeders, they are also concerned about fruit quality traits, such as sugar content, fruit shape, rind pattern, flesh color, and nutritional factors.

For consumers and therefore for breeder, one of the most important traits in watermelons is sweetness or total soluble solids, which is measured as degree Brix (°Brix). According to the U.S. Standards for Grades of Watermelons, any watermelon with ≥ 8° Brix at the center of the flesh is sufficiently ripe and considered "good internal quality" by the USDA and those ≥ 10° Brix

are “very good internal quality” (USDA, 2006). In watermelons, the total soluble solid content can be divided into three different sugars, fructose, glucose, and sucrose (Elmstrom and Davis, 1981; Kader, 2008; Kyriacou et al., 2016). A gene (*ClTST2*) was identified to be associated with sugar accumulation in the watermelons flesh, the specific gene was found to be a vacuolar Tonoplast Sugar Transporter gene (Ren et al., 2018). A broad-sense heritability was calculated using 96 recombinant inbred lines and the parents, PI 296341 and 97103, for °Brix, fructose, glucose and sucrose resulted in 0.95, 0.88, 0.86 and 0.66 heritability, respectively (Ren et al., 2018). Unfortunately, total soluble solids can vary on the year and location. In an experiment looking at mean vs. stability of 40 genotypes in three years, and at eight locations within the United States, there was only one consistent genotype in the top six stable and high performing group, which was ‘Quetzali’ (Dia et al., 2016b). Among environmental and GxE variation is the potential that total soluble solids can fluctuate due to management practices. Water is cut-off a couple days or a week before maturity, allowing sugars to accumulate. Even though total soluble solids can vary by year, location, and management the majority of commercial varieties will contain > 8° Brix satisfying the minimum requirement for USDA standards, with many above 10° Brix.

Fruit shape is another watermelon quality trait with specific USDA standards. A watermelon is classified as U.S. No. 1 Grade if it is “fairly well formed”, while a U.S. No. 2 Grade must be “not badly misshapen” (USDA, 2006). Anything that falls below the U.S. No. 2 Grade is considered a cull fruit. The major fruit shape categories are round, oval, blocky, or oblong (Maynard, 2001; Wehner, 2008). Oblong fruit shape is controlled by a single, incomplete dominant gene (*O*), where oblong fruits are homozygous dominant (*OO*), blocky are heterozygous (*Oo*) and round are homozygous recessive (*oo*). A candidate gene (*ClFS1*) has been identified via genome wide association study (GWAS) and bulk segregant analysis (BSA) and found to be responsible

in dictating fruit shape (Dou et al., 2018). Watermelon rind pattern is a more complex trait, where it was first proposed that three alleles at a single locus determined striped and solid green (dark and light rind). The allelic series described by Weetman (1937) is *G* allele for dark green, *g* allele for light green, and g_s allele for strips. The g_s is dominant to *g* and recessive to *G*. Recently, Lou and Wehner (2016) identified additional alleles for rind pattern. They suggested that fruit rind pattern was controlled by the following: *G* (solid medium or dark green), g_W (wide stripe), g_M (medium stripe), g_N (narrow stripe), and *g* (solid light green or gray). Dominance for the alleles is as follows, $G > g_W > g_M > g_N > g$. There are other rind patterns which are unique and uncommon, such as yellow polka dots on a dark green rind, intermittent strips, and golden yellow color that develops at maturity (Barham, 1956; Gusmini and Wehner, 2006).

Although some of the traits mentioned previously are qualitative, traits such as yield, its components, and sugar content are quantitative traits that can be influenced by the environment. The observed phenotypes for these traits are composed of the individual's genotype, the environment, and interaction between the two. To adequately determine how much influence environment and genotype by environment (GxE) interaction has on the phenotype, multi-environmental trials must be conducted (Comstock, 1977). In most instances these trials are conducted in the major producing regions of that specific crop, such as the work done by Dia et al. (2016a) on watermelons. These trials should involve multiple years as well, which would allow access to the variation that may exist in an environment year to year (Ågren and Schemske, 2012). The genotype by environment interaction can highly influence a breeding program, from identifying the most adequate testing locations, the allocation of resources within a program, to determining what individuals and breeding strategy to employ (de Leon et al., 2016).

Path analysis is a method to determine the influence of independent factors on dependent factor, as well as calculating correlation between pairs of independent factors (Wright, 1921, 1934). This is among the best available methods of identify how much certain traits contribute to specific traits of interest, for example yield components on yield. The direct effects are the total correlations from the specific independent factors on the dependent factors, while the indirect effects are the simple correlations between the independent factors (Cramer et al., 1999). The method to obtain the total correlation is to take the simple correlation of the independent factors and regress all independent factors on each dependent factor separately. This leads to the direct effects in the form of partial regression coefficients (path coefficient) (Li, 1975). Indirect effects are calculated by multiplying the path coefficients by the simple correlations of each factor. Lastly, the total correlation is calculated by adding the direct and indirect effects. There are various software packages to conduct path analysis (Cramer et al., 1999; De Mendiburu, 2014). The direct and indirect effects obtained from path analysis are specific to the population being used to obtain the dependent and independent factors (Stage et al., 2004). A diverse population would best represent how much an independent trait influences a dependent trait for specific crops (Cooper et al., 2012).

2.2 Material and Methods

2.2a Location

During the spring of 2018, genotypes were evaluated at three locations College Station, TX (30.6280° N, 96.3344° W; CST), Uvalde, TX (29.2097° N, 99.7862° W; UVD) and Weslaco, TX (26.1595° N, 97.9908° W; WES). While during the spring of 2019, genotypes were evaluated at only two locations UVD and WES. In UVD and CST, there are heavy soils with UVD having a

silty clay loam soil (sand = 28%, clay = 47%, silt = 25%, pH = 8.2). The soil at WES is a lighter soil, sandy clay loam soil (sand = 63%, clay = 25%, silt = 12%, pH = 7.9).

2.2b Plant Material

A total of 68 diploid genotypes were to be evaluated in 2018, which were provided by Dr. K. Crosby (Texas A&M University), Dr. C. McGregor (University of Georgia), and USDA-ARS National Germplasm System through the Germplasm Resource Information Network (GRIN). Those provided by Dr. Crosby included early and late generation progeny developed at Texas A&M, while those provided by Dr. McGregor were heirloom cultivars. In 2018 issues with germination, seedling health, and seed availability led to a decrease in genotypes successfully evaluated at the different locations; 62 in CST, 45 in WES, and 51 in UVD. There were a total of 34 common genotypes between the three locations. In 2019, there were 20 diploid genotypes evaluated at two locations, chosen from the previous 34 genotypes, to encompass the largest possible of variation between the genotypes for total yield.

2.2c Plant growth

For a majority of the locations and years seeds were sown into polystyrene trays (34 cm x 67 cm), with 128 square cells of 3.3 cm length, 6.3 cm depth, and 42 mL volume (Hortiblock, Beaver Plastics Ltd., Edmonton, Canada), containing Sungro Metro-Mix (Agawam, MA). They were placed in a greenhouse at 35°C day / 22°C night temperatures. Seedlings were irrigated every two to three days depending on soil dryness. After approximately four weeks, seedlings were transplanted into the field. The seedlings were transplanted on April 1st in WES 2018, April 21st and 23rd in CST 2018. The plants in UVD 2018 were direct seeded on April 14th because seedlings

in the greenhouse were killed by bacterial blight due to *Pseudomonas sp.* prior to transplanting. In 2019, seedlings in WES were transplanted on March 14th, while in UVD they were transplanted on April 5th.

Growing practices recommended by Anciso et al. (2012) were followed for the two years and all locations. Plastic mulch rows were 3 m center-to-center, and plots were 7.3 m long with 0.91 m of space between individual plots. Within the plots, the plants were 0.91 m from each other, with a total of 8 plants per plot. Plots were in a randomize complete block design with two replications at each location. Seedlings were irrigated and fertilized via drip tape. They were irrigated once a week for approximately 4 to 6 hours and were fertilized at different growth stages as recommended by Southeastern Vegetable Extension Workers (2018). The amount of nitrogen, phosphorus, and potassium (NPK) applied to the field varied depending on the soil analysis, it ranged from 10:8:1 to 90:50:30 lb acre^{-1}. Throughout the season the plants were sprayed for any diseases or pest, such as downy mildew, thrips, aphids, and cucumber beetles.

2.2d Harvest and Processing

In 2018 at each location, there were two days dedicated to harvest, the first was to harvest the first replication and a second to harvest the second replication. At WES fruits were harvested on June 7th and 8th; at CST 2018, fruits were harvested on July 3rd and 12th;; and at UVD fruits were harvested on July 20th and 23rd. In 2019 at WES, fruits were harvested on June 6th and 7th, while in UVD fruits were harvested from June 19th through July 10th. There were approximately four harvests during this time frame. For the single harvest, plots were assessed for maturity if 80% of the plots were mature then the fruits were harvested. Maturity was assessed by a dried tendril, yellow ground spot, and hollow sound. The number of plants at the end of the season were

counted before harvest to asses yield on a plant basis. All the fruits from the plot were harvested and weighted for total weight per plot, which was used to calculate the total yield (Mg per hectare; TY) of the plot. The fruits were separated into marketable and cull fruit in all locations and both years, except for WES in 2018, where total fruit (thousand per hectare) was taken. There were three marketable fruits set aside in CST, UVD, and WES that represented the overall plot. These chosen fruits were mature fruits that had an overall similar fruit size to the rest of the fruits in the plot.

The fruits were assessed for fruit length (cm; FL), fruit diameter (cm; FD), weight (kg; FW), rind thickness (cm; RT), and total soluble solids (°brix; SC). Fruit appearance was recorded during the 2018 and 2019 growing season, such as flesh color, rind pattern, and fruit shape. Fruit length was measured from the blossom end to the stem end, while fruit diameter was measured across the fruit between the blossom end and stem end. SC, and RT were measured with a refractometer (Sper Scientific Direct, U.S.A), and caliper (Electron Microscopy Sciences, U.S.A.), respectively. SC was taken from flesh located at the center of the fruit. RT was measured from the flesh to the outer rind of the fruit. The flesh color, rind pattern, and fruit shape were visually determined based off of literature and personal observations.

2.2e Statistical Analysis

The data was evaluated as two groups, 34 common genotypes in 2018 and 20 common genotypes across two locations and two years. Models were developed using REML, and the analysis of variances (ANOVA) were obtained to identify whether genotype by environment interactions were statistically significant ($P < 0.05$). The least square means (lsmeans) of the germlines were derived via 'Standard Least Squares' function on JMP® Pro 14. The model was

developed considering genotype, location, year and replication as random with year and location nested within replication. The direct and indirect effects of all the traits on TY were determined via path analysis using the lsmeans. The path analysis and correlation were conducted through R software (v. 1.1.383) using 'Agricolae' package (De Mendiburu, 2014; R Core Team, 2014). Least significant difference (LSD) was conducted on all the traits, except fruit appearance traits, measured via JMP® Pro 14 to statistically compare the germlines (JMP®, 2017). The genotypes were categorized and grouped according to fruit appearance traits, such as flesh color, rind pattern, and fruit shape.

2.3 Results and Discussion

2.3a Analysis of Variance

In the 2018 three location analysis of variance (Tables 2.1), showed a significant ($P < 0.01$) effect of genotype for FW, FL, FD, SC, and RT. While for the 2018 and 2019 two location combined analysis, there was a significant ($P < 0.01$) effect of genotype for FW, FL, FD, and SC but not RT (Table 2.2). The statistical differences between the genotypes for those traits is indicative of having a repeatable measurement and having diverse germplasm. There may have not been a significant effect of genotype for TY or TF due to high variation in the data, leading to large confidence intervals. The lsmeans for the 34 genotypes had a larger range for TY than the range of the 20 genotypes. This indicates that the 2018 environment provided a better differential than 2019 environment because the 20 genotypes were selected due to their difference seen in 2018.

Source	DF	TY	TF	FW	FL	FD	SC	RT
Location	2	5105.70	33.4	95.9	112.7	393.3	93.2	4.9
Genotype	33	180.30	12.4	9.7**	164.2***	19.1***	12.6***	0.3***
Genotype x Location	66	94.70	6.2	2.0	6.9	3.2	1.0	0.1
Replication[Location]	3	160.80	3.7	3.5	10.2	8.5	1.5	0.3

Table 2.1 Analysis of variance for 34 genotypes. Were evaluated in 2018 at three locations (CST, UVD, and WES). The mean square of the following traits is presented; TY = total yield (Mg ha$_{-1}$), TF = total fruit count (one thousand fruit ha$_{-1}$), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm). Blank squares are not significant at $P = 0.05$. * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

Source	DF	TY	TF	FW	FL	FD	SC	RT
Year	1	5088.2	29.7	148.2	326.3	73.2	2.0	3.8
Location	1	86.2	141.3	1.5	17.2	75.7	9.9*	0.1
Genotype	19	290.9	23.0	22.0**	316**	41.8**	13.7**	0.5
Genotype x Year	19	149.4	5.6	3.9	5.8	3.6	0.8	0.1
Genotype x Location	19	192.0	13.6*	1.3	6.8	2.1	0.2	0.2*
Year x Location	1	9394.7	14.2	182.6	225.2	499.1	73.3	3.7
Genotype x Year x Location	19	117.5	4.4*	2.8	10.5	1.8	0.6	0.1
Replication[Year,Location]	4	63.8	1.6***	5.0	12.8	7.3	0.5	0.1

Table 2.2 Analysis of variance for 20 genotypes. Were evaluated in 2018 and 2019 at two locations (UVD, and WES). The mean square of the following traits is presented; ; TY = total yield (Mg ha$_{-1}$), TF = total fruit count (one thousand fruit ha$_{-1}$), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm). Blank squares are not significant at $P = 0.05$. * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

Ultimately, a majority of the traits showed no significant GxE effect, except for TF at $P < 0.05$ (Table 2.1 and 2.2). This indicated that there was no difference in watermelon production attributable between WES and UVD over the years and genotypes evaluated. This suggest that improved progeny can be developed and tested in UVD, and this progeny should perform similarly in WES, which is a large watermelon producing region in Texas. Contrasting our results, a previous study identified significant ($P < 0.01$) genotype by environment effects for various yield and quality parameters across 8 locations; Kinston, NC, Clinton, NC, Charleston, SC, Cordele, GA, Quincy, FL, Lane, OK, College Station, TX, and Woodland, CA (Dia et al., 2016a). The similar environmental conditions between WES and UVD, as well as field management, could potential indicate why no significant GxE interaction was observed. As for the study conducted by Dia et al. (2016a), it encompassed 40 genotypes, three years, and eight different locations throughout the United States. Of the eight locations evaluated, College Station, TX had the second lowest marketable yield with a grand mean of 29.2 Mg ha^{-1} across the three years. Texas had the second highest percentage of cull fruit at 12.5%, which could have influenced the lower marketable yield. The second highest marketable yield, 81.2 Mg ha^{-1}, from Georgia had the lowest percentage of cull fruit, 4.8%. The total yield from two year and two locations in Texas had a grand mean of 33.8 Mg ha^{-1}, which would be decrease if marketable yield would have been recorded. Although, no GxE interaction was observed, it would be advised to conduct advance progeny field trials in WES, due to the importance that the region plays in Texas watermelon production

2.3b Genotype Performance

In 2018, the highest TY genotype was TAM 2, produced by the Texas A&M program, which had a TY of 31.39 Mg ha^{-1} (Table 2.3). The lowest TY came from ZWRM 111 at 20.52 Mg ha^{-1}, which is a genotype from Asia, and is most likely not adapted to the environmental conditions of Texas. The rest of the top five highest total yielding genotypes across three locations, were 'Sunshade', 'Chubby Gray', 'Big Crimson', and 'Crimson Diamond' (Table 2.3). The TY for these genotypes ranged from 30.94 to 28.79 Mg ha^{-1}, which were developed by commercial companies. The genotypes were released through the Plant Varietal Protection Act (PVP), and their protection has now expired. In our evaluation these genotypes performed better than their predecessors, such as 'Crimson Sweet' and 'Charleston Gray'. Both 'Chubby Gray' and 'Sunshade', are germlines derived from 'Charleston Gray'. 'Sunshade' is a mutant from Charleston Gray that has broad leaves instead of lobed leaves. 'Chubby Gray' is derived from a cross between 'Yellow Flesh Tendersweet' and 'Charleston Gray' with a unique blocky fruit shape. While 'Big Crimson' and 'Crimson Diamond' are derived from reciprocal cross between 'Crimson Sweet' and 'Black Diamond'. Interestingly, 'Chubby Gray', 'Crimson Diamond' and 'Big Crimson' were developed in Texas by Coffey Seed Company, which is now a part of Willhite Seed Inc, so these genotypes were developed and selected under similar environmental conditions to that of this experiment.

The following year, where less genotypes were tested, the top three TY genotypes were 'Crimson Diamond', TAM 2, and 'Sunshade', they ranged from 36.19 to 35.63 Mg ha^{-1} (Table 2.4). There was an increase in TY the following year compared to that of the previous year, which can be seen by the grand mean and the higher range of the top TY genotypes. The highest TY genotypes from 2018 and 2019 were in the top five highest yielding the previous year. 'Chubby

Gray' was the only genotype in the top five highest TY from the previous year that did not perform as well, compared with the following year. Despite the higher TY in 2019, there were other genotypes that performed better than 'Chubby Gray' during the 2019 season.

The five lowest TY in 2018 for three locations, were ZWRM 111 (PI 593390), 'Sugar Baby', 'Tastigold', Wm-21 (PI 381711), and Handuel (PI 612468) (Table 2.3). Their total yield ranged from 22.63 to 20.52 Mg ha^{-1}, with all having a lower FW than the median and mean FW, 5.2 and 5.4 kg respectively. Some of the lower TY genotypes had a lower TF than the median and mean, which were 5.35 and 5.83 thousand fruit ha^{-1}. Several of these genotypes, ZWRM 111, Handuel, and Wm-21, were originally obtained from Asia, so potentially these genotypes are not well adapted to the dry and hot environment of south Texas. There were other genotypes that had foreign origins, such as V-CI-9 (PI 512375) from Spain, Lakri Kumda (PI 183399) from India, and two from China, ZWRM 50 (PI 593359) and Grif 1732 (PI 629101). The majority of these foreign genotypes are below the mean TY. These genotypes were primarily selected due to their high SC and early maturity, indicated by the USDA GRIN database.

There was a lower TY observed in our study compared to other studies that have evaluated germplasm (Dia et al., 2016a; Gusmini and Wehner, 2005). This could potentially be due to a single harvest, test plot size, and/or management practices. In watermelon production, there are usually two or three harvest dates at the end of the season, while a single harvest was conducted for the majority of the locations and years, only exception was UVD 2019. The genotypes' FW in our study were considerably lower than that observed in previous studies (Dia et al., 2016a; Gusmini and Wehner, 2005). A comparison between the yields obtained from the previous studies was done by ranking the genotypes in common, allowing a generalization of the germplasm.

In 2018 three locations evaluation, there were a total of ten common germlines between our evaluation and that conducted by Gusmini and Wehner (2005), which evaluated a total of 80 genotypes two years and two locations. 'Chubby Gray', one of the top TY genotypes in our evaluation (Table 2.3), which ranked in the top five, was a moderate performing individual in their study, ranking 56th out of 80 genotypes. 'Big Crimson' and 'Calhoun Gray' were two high TY genotypes in our study, ranked 4th and 9th out of 34 respectively and genotypes were also found to perform well in the evaluation of Gusmini and Wehner (2005), ranked 9th and 10th out of 81 genotypes. A common poor performing genotype for TY, in both evaluations, was 'Sugar Baby', ranked 75th out of 80 and 31st out 34 genotypes in our study. A large scale multi-environment watermelon germplasm evaluation conducted by Dia et al. (2016a) also showed similarities. They identified 'Big Crimson' to have the highest marketable yield and 'Sugar Baby' to have one of the lowest marketable yield, out of 40 genotypes evaluated. Several commercial hybrids, 'Starbrite', 'Stars-N-Stripes', and 'Fiesta', were in the top marketable yielding category, potentially showing improvement from heterosis and parents used to create the hybrid. The common high and low TY genotypes mentioned, were found to be stable genotypes according to multiple stability parameters such as, regression coefficient, deviation from regression, and Shukla's stability variance (Dia et al., 2016a). Most likely, due to the stability of these genotypes, we were able to obtain some similarities between the genotypes evaluated.

In the 2018 evaluation with three locations (Table 2.3), the sweetest genotype was ZWRM 111 with 11.2 °brix, followed by 'Crimson Sweet' (11.0 °brix) and TAM 4 (10.9 °brix). It was noted that the sweetest genotypes in general had a lower TY, with high TY genotypes usually having a moderate level of SC. A potential explanation for this may be that the sweetest genotypes in general were smaller, lower FW, so the fruit is able to concentrate a higher portion of

carbohydrates compared to larger fruits with higher TY. In our study, a couple of the sweetest genotypes have Asian origin, for example ZWRM 111 was obtained from China and Handuel from South Korea. These sweet genotypes may have low TY due to not being adapted to the Texas environment. Dia et al. (2016b) found 'Crimson Sweet' to be the sweetest genotype (12.1 °brix) in a study evaluating the fruit quality of 40 genotypes across multiple-environments. They also identified that the sweetest genotypes were usually the poorer yielding genotypes. The genotypes with the lowest SC were TAM 2, Wm-21, TAM 9, and TAM 14, with °brix ranging from 6.3 to 7.5, which are lower than the standards set by the USDA (USDA, 2006). These individuals from the program were developed from crosses to plant introductions (PI) individuals with disease resistance and lower °brix.

2.3c Fruit Appearance

Fruit appearance, such as rind pattern, flesh color, and fruit shape, were recorded on 34 genotypes (Table 2.3). Fruits were classified into eight different rind pattern categorizes; narrow striped, medium striped, wide striped, gray, light green, green, dark green, and intermit. The most common rind pattern was gray with 24% of the germplasm having that pattern. The least common rind pattern was intermit at 3%, followed by green rind pattern at 6%. The fruit's flesh color was categorized into six different groups; coral red, scarlet red, pink, orange, yellow, and white. The most common flesh color was coral red with 44% of the germplasm or 15 genotypes and was followed by scarlet red at 21% of the germplasm or 7 genotypes. The least common flesh colors were white and yellow at 3% (1) and 9% (3) of the germplasm. The fruit shapes were separated into four groups; round, oval, blocky, and oblong. The most common fruit shape was round with 18 genotypes or 53% of the germplasm, followed by blocky at 21%. There were only 4 genotypes

with oblong fruit shape or 9% of the germplasm. A combination of all three fruit appearance traits was looked at. The most common combinations, with 3 germlines each, were fruits with coral red flesh, narrow stripe rind pattern and round fruit shape, and fruits with coral red flesh, gray rind pattern and oblong fruit shape.

#	Genotype	TY	TF	FW	FL	FD	TSS	RT	Flesh Color	Rind Pattern	Fruit Shape
1	TAM 2	31.39	7.33	5.5	23.1	21.8	6.3	1.6	Yellow	Narrow Striped	Round
2	Sunshade	30.94	5.94	6.8	38.7	18.9	10.0	1.5	Coral Red	Gray	Elong
3	Chubby Gray	29.51	5.69	6.9	31.8	20.9	9.7	1.7	Coral Red	Gray	Blocky
4	Big Crimson	29.45	5.94	6.4	24.2	22.4	8.7	1.9	Coral Red	Medium Striped	Round
5	Crimson Diamond	28.79	5.06	6.8	24.9	23.1	9.2	1.7	Coral Red	Wide Striped	Round
6	Verona	28.43	5.49	6.2	24.1	22.5	10.1	1.6	Coral Red	Dark Green	Round
7	Sugarlee	28.02	6.28	5.3	23.9	21.0	10.6	1.5	Coral Red	Narrow Striped	Round
8	Muchas shandia (Chamane)	27.58	7.68	4.1	23.1	18.7	8.1	1.6	Pink	Medium Striped	Blocky
9	TAM 14	27.57	6.83	4.5	20.2	20.7	7.5	1.7	Pink	Medium Striped	Round
10	Calhoun Gray	27.49	5.45	6.8	37.3	19.7	10.5	1.6	Coral Red	Gray	Elong
11	TAM Crimson Sweet	27.28	5.87	6.2	24.6	22.3	10.6	1.6	Coral Red	Medium Striped	Oval
12	Strain II	26.55	6.34	5.4	22.9	21.6	9.6	1.5	Yellow	Light Green	Round
13	Charleston Gray	26.33	5.47	6.1	36.4	18.6	9.7	1.4	Coral Red	Gray	Elong
14	TAM 22	26.02	5.23	5.8	23.8	21.3	10.4	1.8	Orange	Gray	Oval
15	AU-Sweet Scarlet	25.97	5.03	5.6	22.4	23.1	10.7	1.5	Scarlet Red	Narrow Striped	Round
16	ZWRM50	25.90	5.82	5.6	22.0	21.8	9.8	2.0	Coral Red	Narrow Striped	Round
17	UGA147	25.35	5.77	5.1	23.5	21.3	7.9	1.8	Pink	Light Green	Oval
18	AU-Golden Producer	25.32	4.98	6.0	24.4	22.5	10.5	1.5	Orange	Medium Striped	Round
19	Grif 1732	25.08	5.01	6.3	28.9	21.2	10.3	1.7	Coral Red	Light Green	Blocky
20	Crimson Sweet	24.96	5.55	5.5	23.3	21.5	11.0	1.6	Coral Red	Medium Striped	Oval
21	Lakri Kumda	24.84	5.35	5.5	22.6	22.1	7.5	1.6	Yellow	Light Green	Round
22	Klondike Black seeded	24.74	6.65	4.8	27.8	18.4	9.0	1.5	Scarlet Red	Green	Blocky
23	Graybelle	24.64	5.77	4.8	23.2	20.3	10.5	1.5	Scarlet Red	Gray	Oval
24	Long Crimson	24.50	4.81	6.5	30.3	20.8	9.4	1.7	Coral Red	Wide Striped	Blocky
25	TAM 6	24.26	6.49	4.8	22.2	20.6	10.8	1.4	Coral Red	Gray	Round
26	TAM 4	23.70	6.85	3.9	24.0	17.9	10.9	1.7	Scarlet Red	Green	Blocky
27	TAM 9	23.60	5.23	5.5	28.6	19.4	7.2	2.2	White	Wide Striped	Blocky
28	Perola	23.35	4.93	6.0	25.0	22.5	10.1	1.5	Scarlet Red	Light Green	Oval
29	V-CI-9	23.15	6.29	4.3	19.4	19.8	9.5	1.4	Scarlet Red	Dark Green	Round
30	Handuel	22.63	6.07	3.8	20.0	19.2	10.5	1.4	Coral Red	Narrow Striped	Round
31	Wm-21	22.60	6.48	3.5	19.2	18.9	6.4	1.8	Pink	Narrow Striped	Round
32	Tastigold	22.24	4.97	4.9	21.4	20.9	10.5	1.5	Orange	Gray	Round
33	Sugar Baby	22.12	5.77	3.9	20.0	19.6	9.5	1.4	Scarlet Red	Dark Green	Round
34	ZWRM 111	20.52	5.89	3.4	18.4	18.8	11.2	1.5	Pink	Intermit	Round
Grand Mean		25.70	5.83	5.4	24.9	20.7	9.5	1.6			
CV		47.10	43.30	37.4	23.1	14.8	20.2	23.1			
Least Significant Difference (LSD)		7.94	2.48	1.4	1.9	1.9	1.1	0.2			
Median		24.30	5.35	5.2	23.6	20.8	9.9	1.6			

Table 2.3 Mean of traits for the 34 genotypes evaluated. The corresponding least square mean from two replications and three locations, mean, coefficient of variance (CV), Least Significant Difference (LSD), and median. Traits measured were TY = total yield (Mg ha^{-1}), TF = total fruit count (one thousand fruit ha^{-1}), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm). Includes fruit appearance characteristics such as flesh color, rind pattern, and fruit shape.

#	Genotype	TY	TF	FW	FL	FD	TSS	RT	Flesh Color	Rind Pattern	Fruit Shape
1	Crimson Diamond	36.19	6.31	9.1	27.6	24.9	9.4	1.8	Coral Red	Wide Striped	Round
2	TAM 2	35.72	8.39	6.1	24.1	22.0	6.2	1.6	Yellow	Narrow Striped	Round
3	Sunshade	35.63	7.17	7.9	41.4	18.9	9.9	1.6	Coral Red	Gray	Elong
4	Verona	35.37	6.69	8.5	26.2	24.1	10.0	1.7	Coral Red	Dark Green	Round
5	Muchas shandia (Chamane)	35.24	8.09	4.4	25.0	17.8	8.2	1.7	Pink	Medium Striped	Blocky
6	TAM 9	35.05	7.38	6.2	30.4	19.5	6.7	2.1	White	Wide Striped	Blocky
7	Crimson Sweet	34.80	7.15	6.3	24.0	21.8	10.7	1.6	Coral Red	Medium Striped	Oval
8	TAM 14	34.49	7.72	5.1	21.0	21.2	7.7	1.7	Pink	Medium Striped	Round
9	Chubby Gray	34.26	6.64	8.2	32.7	21.7	9.6	1.7	Coral Red	Gray	Blocky
10	ZWRM50	34.05	7.10	7.4	24.5	22.8	10.3	1.9	Coral Red	Narrow Striped	Round
11	TAM 22	33.86	6.86	6.4	24.1	22.1	9.9	1.7	Orange	Gray	Oval
12	TAM 6	33.54	7.35	5.5	23.2	21.1	10.8	1.4	Coral Red	Gray	Round
13	Calhoun Gray	33.52	6.63	7.8	39.4	19.7	10.0	1.6	Coral Red	Gray	Elong
14	TAM 4	33.42	7.84	4.3	25.1	17.7	10.3	1.7	Scarlet Red	Green	Blocky
15	Klondike Black seeded	33.05	8.02	4.4	27.5	16.9	7.9	1.6	Scarlet Red	Green	Blocky
16	Strain II	33.04	6.89	5.8	22.8	21.8	8.9	1.6	Yellow	Light Green	Round
17	Tastigold	32.76	6.82	5.9	22.5	21.6	10.2	1.6	Orange	Gray	Round
18	Charleston Gray	32.70	6.27	7.3	38.6	18.9	9.5	1.6	Coral Red	Gray	Elong
19	V-CI-9	32.61	7.14	4.8	20.7	20.2	9.3	1.4	Scarlet Red	Dark Green	Round
20	Sugar Baby	31.80	7.03	4.8	21.3	20.3	9.2	1.4	Scarlet Red	Dark Green	Round
Grand Mean		33.78	7.18	6.3	27.1	20.8	9.2	1.7			
CV		46.69	46.02	41.1	25.6	16.4	18.0	24.8			
Least Significant Difference (LSD)		44.26	3.12	1.8	9.3	2.0	1.1	0.4			
Median		32.32	6.60	5.7	25.2	20.7	9.5	1.6			

Table 2.4 Mean of traits for the 20 genotypes evaluated. The corresponding least square mean from two years, two replications and two locations. It also includes the grand mean, coefficient of variance (CV), Least Significant Difference (LSD), and median. Traits measured were TY = total yield (Mg ha^{-1}), TF = total fruit count (one thousand fruit ha^{-1}), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm). Includes fruit appearance characteristics such as flesh color, rind pattern, and fruit shape.

2.3d Correlation and Path Analysis

From the correlation and path analysis for the 2018 and three location evaluation, the highest positive direct effects on TY were from FW and TF, 0.82 and 0.72 respectively (Table 2.5). The high direct effects of FW and TF were decreased by negative indirect effects of each other, -0.39 and -0.44 respectively. The direct and indirect effects changed following an additional year and fewer genotypes evaluated. There was a decreased direct effect from FL, which went from 0.24 to 0.01 and FD from 0.37 to 0.20. While an increase in the positive direct effect of FW, went from 0.72 to 0.93 and TF from 0.82 to 0.92 (Table 2.6). The decrease in genotypes evaluated, most likely shifted the direct and indirect effects on TY and the other traits recorded. The correlation and path analysis obtain from our study pertains to the set of genotypes that we

evaluated and may differ from other studies that use a different set of genotypes. In general, there were some similarities to previous studies (Davis et al., 2011; Liu et al., 2015; Sidhu and Brar, 1981).

Sidhu and Brar (1981) evaluated F_1 hybrids, they found a negative direct effect from number fruits per plant, total soluble solids, and average weight of fruits on yield. They found a high positive direct effect from flesh weight per fruit and number of nodes of first female flower. Sidhu and Brar (1981) suggested that progeny selection should be based off of those traits that showed a positive direct effect on yield to develop improved watermelon progeny. To develop progeny with improved TY, we could indirectly select progeny with higher FW and TF early on in progeny development. We would have to find a balance between selecting for higher FW and TF, because the indirect effect between each other lowers their direct effects on TY. During selection, we should also consider SC, due to its importance as a fruit quality parameter and has a set requirement by the USDA.

	TF	FW	FL	FD	SC	RT	T. Corr
TF	**0.82**	-0.39	-0.07	-0.17	0.01	0.00	0.21
FW	-0.44	**0.72**	0.17	0.20	0.00	0.01	0.65
FL	-0.22	0.50	**0.24**	-0.07	0.00	0.00	0.46
FD	-0.38	0.40	-0.04	**0.37**	0.00	0.00	0.34
SC	-0.30	0.09	0.02	0.02	**-0.04**	-0.01	-0.21
RT	-0.08	0.19	0.03	0.05	0.01	**0.03**	0.22

Table 2.5 Path Analysis for the 34 genotypes. Traits underlined and in bold are the direct effects on TY, while the other values are the indirect effects of the traits on each other. The final column is the total correlation (T. Corr) of the traits on TY. Traits measured were; TY = total yield (Mg ha^{-1}), TF = total fruit count (one thousand fruit ha^{-1}), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm)..

	TF	FW	FL	FD	SC	RT	T. Corr
TF	**0.92**	-0.65	0.00	-0.09	0.00	0.00	0.17
FW	-0.65	**0.93**	0.01	0.12	0.00	0.07	0.48
FL	-0.33	0.53	**0.01**	-0.06	0.00	0.04	0.19
FD	-0.43	0.56	0.00	**0.20**	0.00	0.03	0.35
SC	-0.52	0.25	0.00	0.04	**0.01**	-0.05	-0.27
RT	0.00	0.29	0.00	0.03	0.00	**0.21**	0.53

Table 2.6 Path Analysis for the 20 genotypes. Traits underlined and in bold are the direct effects on TY, while the other values are the indirect effects of the traits on each other. The final column is the total correlation (T. Corr) of the traits on TY. Traits measured were; TY = total yield (Mg ha^{-1}), TF = total fruit count (one thousand fruit ha^{-1}), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm)..

In 2018 there was mild negative correlation between SC and TY, $r^2 = -0.21$ (Table 2.7 and 2.8), this was also the case for the following year, $r^2 = -0.27$. A negative correlation between total soluble solids and yield had been previously reported by Sidhu and Brar (1981). They concluded that increasing yield would lead to a decrease in total soluble solids. This can be observed in our highest TY genotype, TAM 2, which had a high TY, but low SC at 6.3 and 6.2 °brix. The high TY of TAM 2 was derived mostly from having a high TF, 7.33 thousand fruit ha^{-1} and not necessarily due to high FW. This high number of fruits may be the reason for the lowered total soluble solids in the fruits. There is a limited amount of carbohydrates and nutrients that the plant can produce, so when there is an increased number of fruit sets then those limited photo-assimilates must be spread across an increased number of sinks. In watermelon, a majority of the photo-assimilates are transferred to the fruit during the fruit setting stage (Lee et al., 2005). The major period of sugar accumulation occurs during fruit ripening, where sucrose is the main carbohydrate accumulated, thus sucrose could potentially be distributed evenly across the number of fruits available, causing a lower sugar content across the fruits.

	TY	TF	FW	FL	FD	SC	RT
TY		0.21	*** 0.65	0.46	* 0.34	-0.21	0.22
TF	0.244		** -0.54	** -0.27	** -0.46	* -0.37	-0.10
FW	0.000	0.001		*** 0.70	** 0.55	0.13	0.26
FL	0.007	0.127	0.000		-0.18	0.10	0.11
FD	0.047	0.006	0.001	0.298		0.06	0.13
SC	0.244	0.034	0.472	0.573	0.746		* -0.39
RT	0.211	0.590	0.141	0.531	0.448	0.021	

Table 2.7 Pearson's correlation for the 34 genotypes. Bottom left are the p-values and top right side are correlation coefficients, where blank squares are not significant at $P = 0.05$. * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$. Traits measured were; TY = total yield (Mg ha^{-1}), TF = total fruit count (one thousand fruit ha^{-1}), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm)..

	TY	TF	FW	FL	FD	SC	RT
TY		0.17	* 0.48	0.19	0.35	-0.27	* 0.53
TF	0.481		** -0.70	-0.36	* -0.47	* -0.56	0.00
FW	0.031	0.001		** 0.57	** 0.60	0.27	0.31
FL	0.412	0.122	0.008		-0.30	0.09	0.18
FD	0.134	0.035	0.005	0.206		0.20	0.13
SC	0.250	0.010	0.243	0.696	0.392		-0.24
RT	0.017	0.989	0.179	0.446	0.598	0.305	

Table 2.8. Pearson's correlation for the 20 genotypes. Bottom left are the p-values and top right side are correlation coefficients, where blank squares are not significant at $P = 0.05$. * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$. Traits measured were; TY = total yield (Mg ha^{-1}), TF = total fruit count (one thousand fruit ha^{-1}), FW = fruit weight (kg), FL = fruit length (cm), FD = fruit diameter (cm), SC = total soluble solids (°brix), and RT = rind thickness (cm)..

We have focused primarily on total yield and did not measure the marketable yield. However, genotypes that were round, oval, or blocky did not have as many cull fruit as oblong fruits, such as 'Sunshade' which had a higher number of fruits with bottleneck. In general, the varieties that had an overall high number of fruits, also had a high number of cull fruits. A study by Gusmini and Wehner (2005), which noted a significant ($P < 0.01$) correlation between marketable and total yield, observed a correlation of r = 0.92. Although there was a high similarity between total yield and marketable yield, a look at the marketable yield should be a priority, due

to certain genotypes having a higher level of cull fruit. Gusmini and Wehner (2005) observed a range of 69 to 99 percentage of marketable fruit, while a range in the percentage of cull fruit, 1.7% to 24.9%, was observed by Dia et al. (2016a). This indicates that there exists a variation for the number of cull fruit that a genotype may produce. Lastly, the most important reason for the focus of marketable yield is that growers are payed for the marketable fruit that they harvest and not from cull fruit produced.

The correlation between FW and TY was moderate at r = 0.65, with TF and TY at r = 0.21, both correlations were significant at $P < 0.05$ (Table 2.7). FW and TF have a correlation with TY, but a negative correlation to each other, r = -0.52. A potential explanation to this negative correlation is the sink-source relationship that exists in plants, which has been found to be complex in cucurbit fruits (Schaffer et al., 1996). Ultimately, a balance between the total fruit count and fruit weight would allow for a high yielding genotype. Lastly FW, an important trait of TY, was correlated to both FL and FD, with a higher correlation coefficient with FL, r = 0.46, than FD, r = 0.34 (Table 2.7). There was a negative correlation between FL and FD, r = -0.18. As the FL increases then the diameter decreases, this relationship also contributes to the distinct fruit shapes that are mentioned; round, oval, blocky, and oblong.

2.4 Conclusion and Future Work

There were a number of watermelon genotypes evaluated during the growing season of 2018 identified as useful potential parents for progeny and population development. The top total yielding open-pollinated performers, were ‘Sunshade’, ‘Chubby Gray’, and ‘Big Crimson’. These individuals had acceptable total soluble solid, ranging from 8.1 to 9.7 °Brix, but not as sweet as other genotypes evaluated, such as ‘Crimson Sweet’, ZWRM 111, or TAM 4. In the second year

we were able to verify and identify additional genotypes, ‘Sunshade’ and TAM 2, that performed well. From these results there were a number of F_1 crosses created in the fall of 2018, based mostly on TY and SC.

From the path analysis and correlation, we were able to find FW and TF influenced TY, with some influence from FL and FD. Early selection, $F_2 – F_5$, of progeny with high TF and FW should indirectly select for progeny with good yield. At the same time, it is important to select progeny that have a moderate to high level of SC to ensure consumer acceptance. The selection of medium sized fruits, 8 to 11 kg, would be adequate for consumer preference. TF did show negative correlation to several fruit shape parameters, such as FL and FD, as well as FW. The identification and selection of progeny with moderate fruit weight and high total fruit count would be optimal.

Although these traits showed influence on TY, there should be additional vegetative parameters evaluated that could predict or assess the potential yield of genotypes, such as vegetative growth or leaf type and size. Interestingly, ‘Sunshade’ one of the top total yielding genotypes had broad leaves, instead of lobed leaves. There is the potential that additional photo-assimilates can be obtained from broad leaves, which could allow for an increase in the TF and higher SC. Although with broad leaves, there may be an increase disease and insect incidence. During the 2019 season, ‘Sunshade’ transplants showed a higher incidence of cucumber beetle damage compared to the other varieties.

There are also fruit quality parameters that could potentially be evaluated, such as lycopene content, beta carotene, and citrulline, which add nutritional value to the genotype. There has been variation identified in theses fruit quality traits in previous studies (Davis et al., 2011; Liu et al., 2015; Perkins-Veazie et al., 2006). For “seedless” triplod watermelon development, seed size would be a trait of interest. Triploid watermelons are not truly seedless, the fruit flesh contain seed

coat of the aborted seeds, so the smaller are less distinguished. These additional quality traits are more difficult to obtain and should be evaluated at later generations, such as the nutritional fruit quality.

CHAPTER 3

3. SUMMARY OF IMPROVING THE UNDERSTANDING OF HOW ANTHRACNOSE RACE 2 AFFECTS WATERMELON

3.1 Introduction

Anthracnose (*Colletotrichum orbiculare* syn. *Lagenaria*) has affected watermelon production since the early 20th century (Gardner, 1918; Meier, 1920; Orton, 1917). This fungal disease has the ability to infect the leaves, vines, and fruits of cucurbits. The symptoms associated with anthracnose are black to brown irregular spots on the leaves, oval tan lesions on the stem and brown sunken lesions on the fruits. This disease is one of the few that is mentioned by the USDA when grading watermelons (Agriculture, 2006). Due to this, there is a decrease in marketable fruits in fields infested with anthracnose. The percentage of marketable fruits decrease, as does the total yield. Keinath (2018) showed how detrimental not controlling anthracnose race 2 can be, using water and various fungicides to control anthracnose race 2 infected watermelon plots, a 10 t ha^{-1} decrease was observed between the water control and various fungicide treatments.

Cucurbits anthracnose *C. orbiculare* can be found natively throughout the eastern and southern U.S., where warm temperatures (20-32°C), rainy conditions and high humidity are optimal for the spread of spores and germination (Monroe et al., 1997; Norton et al., 1995). The rain allows for the spread of the disease throughout fields via splashing from one plant to another. Due to easy dispersal, entire fields can be devastated with a couple of rainy days (Gay, 2017). Growers in major watermelon producing states such as Florida, Georgia, Texas, South and North Carolina have the unenviable task of combating and staying one step ahead of anthracnose.

Watermelon growers have the ability to control and manage anthracnose via cultural practices such as specific fungicides, resistant cultivars, and cultural management practices. Recently, Keinath (2018) conducted a study using 13 various fungicides that growers have been shifting towards to identify their effectiveness on anthracnose. The quinone outside inhibitors (QoI) fungicide group, which includes trifloxystrobin, azoxystrobin and pyraclostrobin, performed the best. Despite azoxystrobin's good performance against *C. orbiculare*, three species of Colletotrichum, *C. acutatum*, *C. siamense* and *C. gloeosporiodes*, have become resistant to the fungicide and eventually *C. orbiculare* may as well. Chlorothalonil and mancozeb are multi-site fungicides that have shown to be useful in combating anthracnose race 2 (Damicone and Pierson, 2013; Holmes et al., 2001). They were two of the 13 fungicides tested and they performed just as well as the QoI group. They have the added benefit of not being overcome as readily as the QoI group (FRAC, 2018). Keinath (2018) proposed the applications of mancozeb, trifloxystrobin, or chlorothalonil, within four weeks of transplanting to combat anthracnose. Despite the availability of fungicides that are able to combat anthracnose race 2, growing cultivars with resistance would decrease the amount of fungicide applied, which could ultimately decrease production costs for growers and prevent the pathogen from become resistant to fungicide.

Colletotrichum orbiculare (Berk. & Mont). Arx. is a hemibiotrophic fungus that has the ability to survive off of living cells and on dead plant tissue, allowing it to overwinter on crop debris until the following year. (Gan et al., 2013). It belongs to the *Colletotrichum* genus, which includes several species of pathogen that affect the production and quality of various important crops (Bailey, 1992). The genus is detrimental to the fruit sector damaging high-value crops such as strawberry, mango, citrus, avocado, and banana, it also affects cereal crops such as maize and sorghum.(Cannon et al., 2012). On a mannitol salt agar (MSA) dish *C. orbiculare* is olivaceous

black in appearance with large pale orange conidia masses and dark brown setae (Baxter et al., 1983). The conidia have a cylindrical to subcylindrical shape with an obtuse end and the setae are slender and erect (Arx, 1957; Sutton, 1980).

The general disease cycle of *Colletotrichum* species involves primarily the asexual reproductive cycle. The sexual cycle allows for the production of ascospores from two haploid hyphae. This ascospore has the ability to overwinter on soil or plant material, allowing the asexual cycle to initiate. Once favorable conditions arise, the ascospores germinate and produce mycelium and conidiophores. During heavy rains the conidia spread to plants, where they germinate and produce appressoria. The appressoria aid in the infection and penetration of epidermal cells. The mechanism of appressoria penetration has been well studied (Kubo and Furusawa, 1991). It involves melanized appressoria that develop a peg-like structure to penetrate the cell wall, allowing hyphae to colonize the cell and those around it. As a hemibiotrophic fungi, *Colletotrichum orbiculare* has two nutritional stages, starting with the biotrophic stage, where bulbous-shaped hyphae are produced intracellularly to obtain nutrient, secrete effector proteins to suppress plant immune system, and produce sexual acervuli (De Silva et al., 2017; Kubo et al., 2016). They subsequently develop narrower hyphae that grow rapidly and breakdown host cell wall consuming as nutrients, the necrotrophic stage (De Silva et al., 2017). This leads to necrotic brown to black lesions associated with anthracnose.

Initially there were seven identified *C. orbiculare* virulent races, but that was reduced down to three races (Jenkins et al., 1964; Wasilwa et al., 1993). Goode and Winstead (1957) tested different isolates on five watermelon varieties, ‘Congo’, ‘Charleston Gray’, ‘Fairfax’, ‘Garrison’, and ‘New Hampshire Midget’. Resistance to race 1 and 3 was found on ‘Congo’, ‘Charleston Gray’ and ‘Fairfax’, while ‘Garrison’ and ‘New Hampshire Midget’ were highly susceptible

(Goode and Winstead, 1957). (Wasilwa et al., 1993) tested several anthracnose isolates from across the United States, including several from Texas, on cucumber, watermelon, squash and cantaloupe. Four-day seedlings were inoculated at cotyledons stage with 80,000 spore mL^{-1}, which were rated from 3 to 9 days after inoculations (DAI). They identified three major vegetative compatibility groups (VCGs), which they dictated to be three races of anthracnose. The watermelon cultivars tested, were 'Black Diamond', 'Charleston Gray', 'Sugar Baby', and 'Crimson Sweet'. They found 'Black Diamond' and 'Sugar Baby' to be susceptible to all of the VCGs, while 'Charleston Gray' and 'Crimson Sweet' were only susceptible to VCG 2, indicating that they were resistant to race 1 and 3. Resistance to race 1 and 3 was derived from Africa 8, a selection made by D.V. Layton (Dutta, 1958).

Winstead et al. (1959) evaluated several watermelon varieties and found that those resistant to race 1 were also resistant to race 3. Resistance to anthracnose race 1 and 3 was due to a single dominant gene, recognized as *Ar* (Hall et al., 1960; Winstead et al., 1959). The race of the other isolate that Goode and Winstead (1957) identified was race 2, which all genotypes of watermelon are said to be highly susceptible to. Anthracnose race 2 is still an issue today, even though there exist plant introductions (PI) and cultivars with resistance to the pathogen, these resistant cultivars are unable to yield as well as current commercial cultivars and are not widely adapted (Boyhan et al., 1994b; Keinath, 2018; Sowell Jr et al., 1980; Suvanprakorn and Norton, 1980).

In 1980, two publications reported several PIs with resistance to anthracnose race 2, these were PI 270550, 326515, 271775, 271779, 203551, 299379, and 189225 (Sowell Jr et al., 1980; Suvanprakorn and Norton, 1980). Three of the seven lines were *Citrullus lanatus*, while the rest belong to a close relative, *Citrullus amarus*. These germlines were found to be resistant to

anthracnose race 2 under field conditions. From those germlines, PI 271779 and 299379 were found to be susceptible under greenhouse conditions. The inoculation concentration for the study was 20,000 spore mL^{-1} and seedlings were rated 7 DAI. In 1994, Boyhan et al. (1994b) verified the resistance in previously identified germplasm and identified an additional genotype that showed resistance to anthracnose race 2, PI 512385. In this study, the inoculation concentration was 50,000 spore mL^{-1} and seedlings were rated at 14 DAI. There have been several studies that indicate that variation exists depending on the environment that the seedlings are grown and inoculated under, as well as what plant stage the seedlings are inoculated at (Falconi et al., 2015; Love and Rhodes, 1988; Patel, 2019; Sowell Jr et al., 1980).

In Alabama, four cultivars, 'AU-Producer', 'AU-Jubilant', 'AU-Golden Producer', and 'AU Sweet Scarlet', were developed by J.D. Norton, watermelon breeder at Auburn University (Norton et al., 1993a; Norton et al., 1993b; Norton et al., 1985). These cultivars had resistance to anthracnose race 2, gummy stem blight, and fusarium wilt. The anthracnose race 2 resistance was derived from the *Citrullus amarus* PI 189225. These individuals had desirable quality traits such as high soluble solids, thin rind, flesh color, and striped rind pattern (Norton et al., 1993b). Their yield and days to maturity were on par with the popular seeded variety 'Crimson Sweet' (Norton et al., 1995). In spite of the successful incorporation of anthracnose race 2 resistance, currently no commercial cultivars with resistance to race 2 exist (Keinath, 2018).

Suvanprakorn and Norton (1980), were the first to study the mode of inheritance of anthracnose race 2 resistance. Their study specifically looked at the resistance within PI 189225, 271778, 326515, and AWB-1-AR2 (PI 326514 x PI 271778). They were crossed to susceptible cultivars, 'Charleston Gray', 'Jubilee', 'Crimson Sweet', and AWB-10, an advanced-line. The seedlings, no growth stage mentioned, were inoculated with 50,000 spore mL^{-1} and evaluated on

21 DAI. The parents, F_1, F_2, and backcross progeny were evaluated for anthracnose race 2 resistance on a 0 to 5 disease index, where 0 indicated no lesion and 5 indicates dead seedling, via a spray inoculation protocol developed by Sowell and Pointer (1962). The resistant parents and F_1 showed low disease severity (0), while the susceptible parents showed a high disease severity (5). Although some F_1 showed lesions they were classified as resistant. The F_2 generation segregated in a 3 resistant: 1 susceptible ratio. The backcrosses of F_1's to resistant parents resulted in all resistant progeny, and the backcross to susceptible parents resulted in 1 resistant: 1 susceptible progeny. These results pointed towards the resistance being controlled by a single dominant gene.

There was another inheritance study conducted by Love and Rhodes (1984), which also concluded that resistance was inherited by a single dominant gene, but they believed that there also existed minor modifier genes. The resistant PIs used for the study were, PI 189225 and 299379, while the susceptible germline was, 'New Hampshire Midget' (NHM). They also used a *Citrullus colocynthis*, R309, which is resistant to anthracnose race 2. Seedlings with 2 to 4 true leaves were inoculated with 50,000 spore mL^{-1} and rated 8 DAI. The goodness-of-fitness analysis of the F_2 populations, 'New Hampshire Midget' x PI 189225 and 'New Hampshire Midget' x PI 299379, did not reject the hypothesis of single gene dominance. The 'New Hampshire Midget' x R309 F_2 population on the other hand did not fit into a 1 or 2 gene dominant segregation ratio. They theorized that the resistance in R309 was due to multiple genes. In the F_1 and backcross populations, it was noted that the individuals did not show as high level of resistance as the parents. While in the F_2 populations they noticed that there was not a distinct bimodal distribution for resistance, as well the susceptible individuals were not as susceptible as the susceptible parent, 'New Hampshire Midget'. The two resistant germlines were crossed and the F_2 individuals were more susceptible than the resistant parents, but they did not segregate into specific susceptible

groups. This pointed to additive or recessive resistance factors in each genotype that were not in the other. A common dominant gene was theorized to be involved in resistance since the F_2 individuals did not segregate for susceptible individuals. Identifying a genetic marker associated with this dominant gene would allow for marker assisted selection for anthracnose race 2.

Recently Keinath (2015), reported the identification of anthracnose in South Carolina. In 2013 and 2014, leaf tissue with foliar lesions was collected from cantaloupe and watermelon plants in South Carolina. The pathogen was identified on the watermelon variety of 'Mardi Gras', isolates were tested on four differentials, two cucumber and two watermelon varieties, to identify the race of the isolates. The cucumber varieties were, 'H19 Little Leaf' and 'Marketer', while the watermelon varieties were 'Charleston Gray' and 'Black Diamond'. The only resistant variety to anthracnose race 2 is the cucumber variety of 'H19 Little Leaf'. Three-week seedlings were inoculated at 500,000 spore mL^{-1} and rated 14 DAI on a 0 – 100% scale with 5% intervals and 1% for minimal lesion. One of the isolates was identified as anthracnose race 2, based off of high disease severity on 'Black Diamond' and 'Charleston Gray'.

Recently Patel (2019) screened 1408 germlines from the USDA-ARS GRIN database for anthracnose race 2 resistance under greenhouse conditions. Three-week old seedlings were inoculated with a concentration of 100,000 spore mL^{-1}. The seedlings were rated 7 DAI on a 0 to 100% scale with intervals of 10%. The disease index was based off of the whole plant, which was split into different seedlings parts and rated accordingly; true leaves (50%), meristem (25%), hypocotyl (20%), and cotyledons (5%). There were 44 germlines in the first inoculation that showed more resistance than PI 189225, a highly resistant genotype. The top five most resistant genotypes identified were; PI 500303 (*C. amarus*), PI 482293 (*C. amarus*), PI 482333 (*C. amarus*), PI 244018 (*C. amarus*) and PI 494817 (*C. amarus)*. In a retest, these genotypes still showed a high

level of resistance, as did PI 189225. All of the commercial cultivars tested were susceptible to race 2, which included ‘Sweet Dawn’, ‘Top Gun’ and ‘Valentino’.

There heritability of anthracnose resistance has been calculated for various horticultural crops such as tomato, cucumber and watermelon. Tomato is affected by *Colletotrichum coccodes*, the broad-sense heritability (H^2) was calculated and determined to be $H^2 = 0.90$. Cucumbers are affected by the same species of anthracnose as watermelon, *Colletotrichum orbiculare*. The broad and narrow-sense (h^2) heritability of the anthracnose race 2 resistance in cucumbers was determined to be $H^2 = 0.71$ and $h^2 = 0.26$, indicating high broad-sense heritability of the resistance according to broad-sense. Recently Patel (2019), calculated the heritability of anthracnose race 1 and 2 resistance of watermelon via a bi-parental population. The broad and narrow-sense heritability estimates were calculated for anthracnose race 1, with $H^2 = 0.89$, and $h^2 = 0.64$. The same was done for anthracnose race 2, where broad-sense was $H^2 = 0.80$ and narrow-sense $h^2 = 0.55$. The bi-parental population used to assess the heritability was a cross between PI 189225 and ‘New Hampshire Midget’. High broad-sense heritability across the cucumber and watermelon, shows that single plant selection for anthracnose resistance could potentially be conducted and pass along the resistance.

The objectives of this study, which were to gain a better understanding of anthracnose race 2 on watermelon, were the following. The first and primary objective was to optimize a procedure for inoculating watermelon seedlings under greenhouse conditions. Several concentrations of inoculum have been used throughout the different anthracnose race 2 studies, from 50,000 to 500,000 spore mL^{-1}. There has also been a difference on the DAI in which the seedlings were rated, from 7 to 21 DAI. We first evaluated five different inoculum concentrations, 25,000, 50,000, 100,000, 250,000, and 500,000 spore mL^{-1} and rated the seedlings from 5 to 14 DAI, to identify a

potential inoculum concentration and DAI to rate the seedlings. The second objective was to screen with an isolate from South Carolina, *WmColl4,* a set of 13 genotypes that had been previously evaluated for anthracnose race 2 resistance and verify level of resistance. Lastly the final objective was to evaluation four bi-parental F_2 population, to identified mode of inheritance and the broad-sense heritability of anthracnose race 2 resistance.

3.2 Material and Methods

3.2a Plant Material

Seedlings for all anthracnose race 2 screenings were grown for two to three weeks in 24-cell trays (Growers Solutions, Cookeville, TN). The seeds were sown into Pro-line C/B growing mix (Jolly Gardener, Poland Spring, ME) and grown in the greenhouse at 25 to 30°C. Seedlings for procedure optimization were sown on April 22nd and April 24th, they were inoculated on May 13th and 15th. The germplasm screening seedlings were sown on June 10th and inoculated on July 1st. For the genetic study, Population 1 was sown on March 25th and inoculated on April 15th, Population 2, 3, and 4 were sown on July 10th, July 12th, and July 17th, inoculated on July 26th, July 29th, and August 2nd, respectively.

Procedure optimization: Six genotypes were evaluated under different inoculum concentrations and rated from five to fourteen DAI. There were two cucumber genotypes, 'H19 Little Leaf' and 'Marketer', and four watermelon genotypes. 'Black Diamond', PI 543210 (Muchas Shandia), PI 189225, and 'Charleston Gray 133'. The only genotypes with anthracnose race 2 resistance were, 'H19 Little Leaf' and PI 189225 (Sowell Jr et al., 1980; Wasilwa et al., 1993). Three of the six genotypes have been used as differential in previous studies (Keinath, 2015; Wasilwa et al., 1993). Seeds for 'H19 Little Leaf', 'Marketer', and 'Black Diamond' were obtained

from Eden Brothers (Arden, NC), while ‘Charleston Gray 133’ was obtained from Willhite Seed Inc (Poolville, TX). Muchas Shandia and PI 189225 were obtained from the USDA-GRIN database and multiplied in Uvalde, TX.

Germplasm screening: A set of 13 genotypes were screened for anthracnose race 2 resistance. The watermelon genotypes evaluated, were ‘AU-Golden Producer’, ‘AU-Producer’, ‘AU-Sweet Scarlet’, ‘Black Diamond’, ‘Charleston Gray 133’, ‘Crimson Sweet’, ‘Muchas Shandia’, ‘New Hampshire Midget’, ‘Verona’, PI 189225, PI 203551, PI 270550, and PI 271778. The cucumber variety of ‘H19 Little Leaf’, was used as a resistant check. The following had been reported to have resistance to anthracnose race 2, ‘AU-Producer’, ‘AU-Golden Producer’, ‘AU-Sweet Scarlet’, ‘PI 189225’, ‘PI 203551’, ‘PI 270550’, and ‘PI 271778’. Seeds for ‘H19 Little Leaf’ and ‘Black Diamond’ were obtained from Eden Brothers (Arden, NC), while ‘Charleston Gray 133’ was obtained from Willhite Seed Inc (Poolville, TX). The rest of the genotypes were obtained from the USDA-GRIN database and multiplied in Uvalde, TX.

Genetic study: Four F_2 populations were screened for anthracnose race 2 resistance to understand mode of inheritance and potentially use for genetic study. Population 1 was, PI 189225 x ‘New Hampshire Midget’, which had 190 F_2 individuals. There was also Population 2, Population 3, and Population 4 screened; ‘Perola’ x PI 189225, ‘Sugarlee’ x PI 271778 and ‘Verona’ x PI 189225, respectively. Population 2 had 89 F_2 individuals, Population 3 had 191 F_2 individual, while Population 4 had 113 F_2 individuals. The resistant parents used in the F_2 populations were PI 189225 and PI 271778, while the rest of the parents had been found to be susceptible. Seeds from Population 1 (parents, F_1, and F_2) were obtained from Dr. T. Wehner (North Carolina State University), while Population 2, 3, and 4 (parents and F_2) were developed in Uvalde, TX.

3.2b Experimental Design

Procedure optimization: Disease ratings for the plants were the dependent variables, while the independent variables were different inoculation concentrations and varieties. Five levels of inoculation concentrations (25,000, 50,000, 100,000, 250,000, and 500,000 spore mL^{-1}) and six levels for genotype. The evaluation was arranged in a completely randomized design (CRD), with two inoculation dates (Sets), each had two replications, which were inoculated two days apart. There were four plants per replication, with a total of sixteen plants evaluated. A single plant was considered an experimental unit, and a set of four plants was considered the plot.

Germplasm screening: Fourteen genotypes were arranged in a randomized complete block design (RCBD), where there were three replications and each had approximately eight plants, with a total of twenty-four plants evaluated. A single plant was considered an experimental unit, and a set of eight plants was considered the plot.

Genetic study: The parents and the F_2 were grown and screen at the same time. Population 1 was the only population with F_1 progeny. Population 1 had 190 F_2 and 16 F_1 individuals evaluated. In population 2, there were 89 F_2 individuals, population 3, there were 191 F_2 individuals, and lastly in population 4, there were 113 F_2 individuals. Due to genetic heterozygosity and heterogeneity of the F_2 plants, the populations were not replicated, and each plant was an experimental unit.

3.2c Inoculation Method

The isolate that we used was *WmColl4*, which was isolated by Dr. A. Keinath (Clemson University). This was isolated off of a leaf from ‘Mardi Gras’ in South Carolina. The isolate was grown on ½ potato dextrose agar for 7 to 14 days, harvested with distilled water, and poured into

a beaker through a cheese cloth. The initial inoculum concentrated was determined with a hemocytometer and then diluted down with distilled water containing Tween 80 (1 drop per 100 mL) to the final inoculum concentrations. The final inoculum concentrations used in the procedure optimization were 25,000, 50,000, 100,000, 250,000, and 500,000 spore mL^{-1}. From the evaluation of different concentrations, 100,000 spore mL^{-1} was identified as the inoculum concentration to adequately distinguish the level of resistance and used for the germplasm screening and genetic study. The seedlings were inoculated in the greenhouse with a CO_2 sprayer at 30 PSI, using a flat sprayer tip. Inoculum was sprayed to cover the leaf but did not to drip off. The inoculated seedlings were immediately moved into a humidity chamber and kept there for 48 hours at approximately 100% relative humidity with a temperature of 22 to 24°C. They were removed and placed under natural light in the greenhouse, day temperature of 30°C and night temperature of 25°C, until disease rating. The inoculations were conducted during the spring and summer of 2019 in Uvalde, TX (29.2097° N, 99.7862° W).

3.2d Disease Rating

Seedlings in the procedure optimization were rated from 5 to 14 DAI. While, seedlings for germplasm screening were rated from 5 to 9 DAI, based-off of results from procedure optimization. The bi-parental F_2 populations for the genetic study, were rated at 7 DAI, based-off of procedure developed by Patel (2019). The seedlings were rated as individuals based off of different parameters; percentage of leaves lesions (PLL) and percentage of cotyledon lesion (PCL) with 0% indicating no lesion and 100% indicating dead true leaf or cotyledon. The severity (0 – 6) of hypocotyl lesion (Figure 3.1; SHL) with 0 indicating no lesion and 6 indicating hypocotyl dried, severity (0 – 5) of petiole lesion (Figure 3.2; SPL) with 0 indicating no lesion on the petiole

and 5 indicating petiole highly diseased. There was also a whole plant rating that was given to the seedlings based off of a disease index (INDX) created by Patel (2019). The parameters measured as percentage were rated on increments of 5% with 1% given for any apparent lesion. The number of true leaves (LVE) was counted. The true leaves from each seedling were rated for PLL, the average of all the true leaves on the plant was taken and used as the PLL for that individual plant. For procedure optimization and germplasm screening a genotype's rating was based off of plot mean. The area under the disease progress curve (AUDPC) was calculated, (((0 + µ1)/2) * (D0-D1)) + ((µ1 + µ2)/2 * (D1-D2))), where µ1 = mean of disease severity for first day, D0 = day zero, and D1 = first day of disease rating.

Figure 3.1 Rating scale severity of hypocotyl lesion (0 – 6)
0 = No lesion observed; 1 = Water soaked lesions appears faintly; 2 = Water soaked lesion is more apparent, bit larger; 3 = The lesion is orangish and concaves inward; 4 = Highly concaved inward and lesion starts to spread around stem; 5 = The whole stem is infected and the plant starts to bend over; 6. can see the vascular system of the plant and stem begins to dry

Figure 3.2 Rating scale severity of petiole lesion (0 – 5)
0 = No lesion observed; 1 = Water soaked lesions appear; 2 = Water soaked lesion is more apparent; 3 = The lesion is darker and concaves inward; 4 = Highly concaved inward and stem starts to bend; 5 = Stretch of dried petiole

3.2e Data Analysis

Procedure optimization: An analysis of variance (ANOVA) for the data was done using JMP (Pro 14.0.0). A mixed – model with replication, set, their interactions as random factors and fixed factors of genotypes and inoculum concentrations. This was conducted for all of the parameters mentioned under *Disease Rating*. The Student's T test was conducted to compare means of factors and their interactions. A mean comparison with Student's T test was also conducted on genotypes for 8 to 14 DAI, as well as concentration for 8 to 13 DAI. Lastly, Pearson's correlation was calculated on the different rating parameters evaluated, to determine whether there was any similarity between the parameters.

Germplasm screening**:** The ANOVA was done on JMP Pro 14.0, as a mixed-model. The replications were considered as random factor, while genotypes were fixed. Dependent variables

were disease parameters, as well as LVE and AUDPC. The Student's T test was used to compare means of the dependent factors. Pearson's correlation was done to see whether there was any similarities between the correlation coefficient from the procedure optimization.

Genetic study: Each population evaluated had the PLL of the F_2, F_1, and parents plotted on a histogram (Appendix C). The summary statistics for each population was calculated, this includes mean, median, range, standard deviation, and number of individuals. The goodness-of-fit for a 3:1 ratio of resistant to susceptible was calculated for the populations. For a majority of the populations the cut-off for resistant and susceptible individuals was determined by taking the average of the resistant and susceptible parents, which had been previously done by Love and Rhodes (1988). In Population 1, where the F_1 was evaluated as well, the cut-off for resistance and of the F_2 population was determined using the F_1 mean. The broad-sense heritability was calculated for all of the populations using the F_2 and parents, via a method proposed by (Mahmud and Kramer, 1951), where an indirect estimate of environmental variation is conducted.

3.3 Results and Discussion

3.3a Procedure Optimization

Anthracnose is a fungal pathogen that has been studied since the early 20th century, specifically race 1 and 3 (Gardner, 1918; Orton, 1917). While anthracnose race 2 work did not start until the late 1970's, where USDA germplasm was evaluated for resistance to race 2 (Sowell Jr et al., 1980). A large amount of work on anthracnose race 2 resistance was conducted in the late 70's, 80's and early 90's, after which little work was done until the past five years (Keinath, 2015; Patel, 2019). During the periods of anthracnose research there was not a consistent method of inoculating seedlings. A difference between studies was the inoculum concentration, which has

ranged from 20,000 to 500,000 spore mL-1, with a majority of the studies using inoculums of 50,000 spore mL-1 (Boyhan et al., 1994a; Love and Rhodes, 1988; Norton et al., 1991; Suvanprakorn and Norton, 1980). There has also been a difference on the DAI in which the seedlings were rated on, with the most common being 14 DAI. It is important to note that these seedling inoculations were conducted at true leave leaf stage under greenhouse environment, which has been documented to be different to open field vining inoculation screenings (Love and Rhodes, 1988; Sowell Jr et al., 1980). This is most likely due to the growth stage that the plants are at when they are inoculated, in the greenhouse they are usually at 2 to 4 true leaf, while in the field the plants have started to vine and are more mature.

	PLL	PCL	SHL	SPL	INDX
PLL		0.92 *	0.41	0.92 **	0.97 **
PCL	0.010		0.14	0.82 *	0.74
SHL	0.415	0.799		0.59	0.73
SPL	0.001	0.046	0.216		0.98 *
INDX	0.008	0.091	0.101	0.001	

Table 3.1 Pearson's correlation of procedure optimization. Specifically observations from 100,000 spore mL-1 on 8 DAI.
Top right is the correlation coefficient with significance Bottom left is the p-value.
* significant at $P \leq 0.05$; ** significant at $P \leq 0.01$;*** significant at $P \leq 0.001$
Blank squares are not significant at $P \leq 0.05$.

In our study, five different concentrations were evaluated, 25,000, 50,000, 100,000, 250,000, and 500,000 spore mL-1. These concentrations were close or exactly the same as previous inoculum concentrations. There were several disease parameters, PLL, PCL, SHL, SPL, and INDX, that were evaluated across these inoculation concentrations. These parameters were evaluated due to earlier inoculations indicating that these seedling components are affected by the pathogen. They were separated into individual components to identify and see how they behaved independently, instead of a whole plant such as previous disease indexes. The correlation between

the different parameters was conducted, which showed PLL and INDX having the most correlation to the other parameters assessed (Table 3.1). A whole plant rating (INDX) was given to the seedlings, based off of an index created by Patel (2019). The primary focus for disease severity was PLL, since this showed the most difference between genotypes evaluated and the foliage is the plant structure most affected by anthracnose. A look at the number of leaves evaluated (LVE) was taken into account to show the approximate growth stage of the seedling.

DAI	Source	DF	PLL	PCL	SHL	SPL	INDX
7	Genotype	5	11,460.9 **	2,743.9 *	22.7 ***	32.1 **	7,973.0 ***
	Concentration	4	1,769.3 *	764.2	2.7	3.6 *	408.6 **
	Genotype*Concentration	20	261.4 **	228.7 *	0.4	0.6	65.0 **
	Set	1	1,676.5	33.3	0.2	6.7	298.2
	Set*Genotype	5	760.7 *	456.4	0.1	1.3	37.0
	Set*Concentration	4	124.8	389.4	0.8	0.3	19.9
	Set*Genotype*Concentration	20	82.4 *	101.4 *	0.4	0.3	20.4
	Rep[Set]	2	294.0	162.1	0.6	0.8	79.8
	Error	58	90.5	198.9	0.11	0.22	15.2
8	Genotype	5	16,284.2 **	4,361.7	43.9 ***	44.7 ***	10,137.2 ***
	Concentration	4	2,152.3 *	1,586.4	2.9 *	3.9 *	601.9 *
	Genotype*Concentration	20	203.8	795.0	0.6 **	0.6	93.7
	Set	1	324.7	2,417.6	0.6	5.6	199.1
	Set*Genotype	5	555.5	1,197.4	0.2	1.1	34.9
	Set*Concentration	4	177.6	2,001.5	0.3	0.5	46.6
	Set*Genotype*Concentration	20	114.9	384.2	0.2	0.4	44.8
	Rep[Set]	2	423.2	183.4	0.5	0.5	51.0
	Error	58	122.9	372.4	0.24	0.33	27.2
9	Genotype	5	19,522.1 ***	3,847.7	64.9 ***	57.9 ***	11,494.4 ***
	Concentration	4	2,748.7 **	2,112.8	3.7 *	4.8 *	838.4 **
	Genotype*Concentration	20	248.9	465.2	0.5 *	0.6	83.6
	Set	1	63.4	4,391.3	0.1	2.2	30.0
	Set*Genotype	5	434.0	1,503.7	0.3	1.1	31.1
	Set*Concentration	4	92.2	2,142.2	0.3	0.6	22.4
	Set*Genotype*Concentration	20	141.5	676.9	0.2	0.4	47.2
	Rep[Set]	2	155.5	471.0	0.1	0.3	62.8
	Error	58	128.5	460.1	0.15	0.35	41.5

Table 3.2 Analysis of variance for procedure optimization at 7,8, and 9 DAI. The mean square for the different disease parameters rated and degree of freedom (DF). * significant at $P \leq 0.05$;
** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$
† PLL = Percentage of leaf lesions (%)
‡ PLC = Percentage of cotyledon lesions (%)
§ SHL = Severity of hypocotyl lesion (0-6)
¶ SPL = Severity of petiole lesion (0-5)
# INDX = NCSU disease index (%)

Source	DF	LVE	AUDPC PLL
Genotype	5	4.2 *	1,521,610.7 ***
Concentration	4	0.2	188,403.6 ***
Genotype*Concentration	20	0.0	15,111.2
Set	1	4.1	26,606.8
Set*Genotype	5	0.4	26,698.4
Set*Concentration	4	0.2	2,981.3
Set*Genotype*Concentration	20	0.1	8,402.9
Rep[Set]	2	0.3	9,665.5
Error	58	0.1	6,745.0

Table 3.3 Analysis of variance for LVE and AUDPC PLL. The source, degree of freedom (DF), and mean square.
† LVE = number of leaves evaluated on seedling
‡ AUDPC PLL = area under the disease progress curve for percentage of leaf lesions (%)

The ANOVA for each individual rating parameter across DAI (Appendix A), indicated statistical difference for *Genotype* and *Concentration* across a majority of the DAI. The ANOVA for PLL at 5, 6, and 7 DAI showed that there was a statistically significant ($P < 0.01$) difference for *Genotype by Concentration*, none of the other DAI showed this interaction (Table 3.2 and Appendix A). The ANOVA for PLL also showed statistically significant difference ($P < 0.05$) for *Genotypes* from 8 to 14 DAI, and *Concentration* from 8 to 13 DAI (Table 3.2 and Appendix A). The ANOVA for AUDPC PLL had a statistical significance ($P < 0.001$) difference for both *Genotypes* and *Concentration* (Table 3.3). As for LVE, the ANOVA showed statistical significance ($P < 0.05$) difference for only *Genotypes* (Table 3.3). The ANOVA for PCL showed no statistical significance for *Concentration* at any DAI. This indicates that cotyledons are not affected by an increase in inoculum concentration, they may be more sensitive during early seedling stage, which was the stage that Wasilwa et al. (1993) inoculated their seedlings.

There was no difference for PLL between 100,000, 250,000, and 500,000 spore mL^{-1}, indicating no further increase after 100,000 spore mL^{-1}. This is observed in Table 3.3, the Student's T test grouping for PLL of *Genotype by Concentration* interaction, shows that genotypes have similar PLL ratings across the concentrations at 7 DAI. Muchas Shandia is an example, it shows no mean separation between 25,000 and 50,000 spore mL^{-1}, as well as between 100,000, 250,000,

Genotype	Concentration	PLL 7 DAI		INDX 7 DAI	
Muchas Shandia	25,000	35	CDEF	39	C
Black Diamond	25,000	12	EJKLMN	35	CDEF
Charleston Gray 133	25,000	11	GKLMN	26	GH
PI 189225	25,000	5	JMN	17	I
Marketer	25,000	2	LN	4	J
H19 Little Leaf	25,000	0	KLMN	0	J
Muchas Shandia	50,000	62	B	46	B
Black Diamond	50,000	17	DEIJKLMN	34	CDEF
Charleston Gray 133	50,000	11	GKLMN	28	FGH
PI 189225	50,000	9	GHIJKLMN	23	HI
Marketer	50,000	4	LN	3	J
H19 Little Leaf	50,000	0	KLMN	0	J
Muchas Shandia	100,000	75	AB	61	A
Black Diamond	100,000	35	CDEF	37	CD
Charleston Gray 133	100,000	33	CFGH	36	CDE
PI 189225	100,000	9	IJKLMN	27	GH
Marketer	100,000	8	IJKLMN	4	J
H19 Little Leaf	100,000	0	KLMN	0	J
Muchas Shandia	250,000	82	A	62	A
Charleston Gray 133	250,000	32	CDEFH	41	BC
Black Diamond	250,000	29	CDFGHI	37	CD
PI 189225	250,000	22	CDEFGHIJKM	31	DEFG
Marketer	250,000	20	CDEFGHIKL	6	J
H19 Little Leaf	250,000	0	KLMN	0	J
Muchas Shandia	500,000	86	A	56	A
Black Diamond	500,000	38	C	40	BC
Charleston Gray 133	500,000	28	CDEFHIJ	37	CD
PI 189225	500,000	12	DEFGHIJKLMN	29	EFGH
Marketer	500,000	12	DEFGHIJKLMN	6	J
H19 Little Leaf	500,000	0	KLMN	0	J

Table 3.4 Mean for PLL and INDX at 7 DAI. Across the difference concentration (spore mL^{-1}) and genotypes. The Student's T test groupings are beside the mean.

and 500,000 spore mL^{-1} at 7 DAI (Table 3.4). A similar case was observed with INDX, where genotypes are grouped together across the higher inoculation concentrations (Table 3.4). The grouped mean of PLL for the concentrations across 8 to 12 DAI shows no difference between the

three highest inoculum concentrations, 100,000, 250,000, and 500,000 spore mL^{-1} (Table 3.5). The AUDPC PLL (Table 3.5) shows a stabilization at 100,000 spore mL^{-1}, as the previous results. From these results we can conclude that it is sufficient to use 100,000 spore mL^{-1} to inoculate 2 to 4 true leaf stage watermelon seedlings with *WmColl4* isolate obtained from South Carolina.

Studies to identify appropriate inoculation methodology have been conducted on other crops affected by anthracnose. Pande et al. (1994) developed a method to screen sorghum seedlings for *Colletotrichum graminicola* resistance. They evaluated temperature of incubation, leaf wetness duration, and inoculum concentration. The different inoculum concentrations were, 4×10^3, 4×10^4, 4×10^5, 4×10^6, 4×10^7 spore mL^{-1}, and found that disease severity began to level off at 4×10^5 spore mL^{-1} and no statistical difference was seen between it and the higher concentrations. In an alfalfa study (Welty and Rawlings, 1985) looking at inoculum concentrations and temperature, they evaluated four inoculum concentrations, 1×10^3, 0.6×10^4, 1.1×10^5, 1.1×10^6. From their results no statistical difference between the three highest inoculum concentrations were found, while the values showed that the two highest inoculum concentrations caused similar severity level. Studies on other crops and diseases, show a leveling-off of the disease severity, so this is not a unique occurrence to anthracnose (Stack, 1989; Trapero-Casas and Kaiser, 1992)

According to the PLL and INDX values there are three distinct groups; Muchas Shandia (highly susceptible), 'Black Diamond' and 'Charleston Gray 133' (moderately susceptible) and PI 189225 (resistant; Table 3.4). The mean separation for PLL and INDX, *Genotype by Concentration* (Table 3.4), at 100,000 spore mL^{-1} on 7 DAI, showed three categorize. The separation could also be viewed in the grouped mean of PLL for genotypes across concentration (Table 3.6), at 9 and 10, as well as the AUDPC PLL, which showed the progression of the disease. On the later days, 11 DAI and beyond, this separation is not apparent, since Muchas Shandia and

Concentration	PLL						
	8 DAI	9 DAI	10 DAI	11 DAI	12 DAI	13 DAI	AUDPC
25,000	18 C	26 C	38 B	47 C	54 C	58 D	297 C
50,000	26 BC	35 B	43 B	54 B	62 B	65 C	363 B
100,000	36 AB	47 A	59 A	65 A	69 A	71 B	465 A
250,000	40 A	51 A	59 A	66 A	71 A	74 AB	493 A
500,000	39 A	48 A	59 A	66 A	73 A	76 A	496 A

Table 3.5 Grouped mean of PLL for concentration across 8 to 13 DAI. As well the grouping of concentrations according to Student's T test. The grouped mean of AUDPC PLL for concentrations, with grouping according to Student's T test.

Genotype	PLL								LVE
	8 DAI	9 DAI	10 DAI	11 DAI	12 DAI	13 DAI	14 DAI	AUDPC	
Muchas Shandia	82 A	91 A	95 A	96 A	98 A	99 A	99 A	828 A	3.43 A
Black Diamond	39 B	56 B	74 B	84 AB	91 AB	94 AB	97 AB	561 B	2.65 B
Charleston Gray 133	35 BC	50 B	66 B	77 B	83 B	86 B	90 B	509 B	2.45 B
Marketer	17 CD	24 C	33 C	43 C	54 C	61 C	69 C	289 C	2.36 B
PI 189225	17 CD	28 C	40 C	54 C	64 C	68 C	72 C	332 C	2.30 B
H19 Little Leaf	0 D	0 D	1 D	2 D	4 D	5 D	10 D	18 D	2.15 B

Table 3.6 Grouped mean of PLL for genotypes from 8 to 14 DAI. Grouping of genotypes according to Student's T test. The grouped mean of AUDPC PLL for genotypes, with grouping according to Student's T test. The number of leaves evaluated for PLL average

‘Black Diamond’ group together. As for LVE’s Student T test grouping, all of the genotypes fell into the same group, except for ‘H19 Little Leaf’, which had a higher number of leaves (Table 3.6). The AUDPC PLL showed that the disease progression for Muchas Shandia is faster than that of ‘Charleston Gray 133’ and ‘Black Diamond’, indicating the pathogen is able to infect and progress at a quicker rate (Table 3.6). The classification of watermelon into different resistance categories is not new, it has been previously done by Martyn (1983) with fusarium wilt (*Fusarium oxysporum* f. sp. *niveum*). The watermelon varieties were classified into four categories, highly resistant, moderately resistant, slightly resistant, and highly susceptible.

To identify an adequate DAI to rate seedlings for anthracnose resistance, a look at the difference between the three groups was calculated. The largest difference between Muchas Shandia, highly susceptible, and PI 189225, resistant, is at 8 DAI with a difference of 74%, 7 DAI was right behind at a difference of 67% (Figure 3.3 and Appendix B). For the later DAI, such as 12, 13, and 14, the difference between the highly susceptible and resistant were 33%, 29%, and 25%, respectively. This decrease in difference can make it difficult to distinguish between susceptible and resistant genotypes at later DAIs. On 8 DAI, the difference between Muchas Shandia and ‘Charleston Gray 133’ was at 41%, while at 7 DAI it had a difference of 35%. The difference between Muchas Shandia and ‘Black Diamond’ was 0% from 12, 13, and 14 DAI, showing no distinction between the two categorizes. Based off of the larger difference of PLL rating between the highly susceptible to resistant and highly susceptible to moderately susceptible, 8 DAI would be an adequate day to rate the watermelon seedlings for PLL to obtain the largest difference between groups. Since 7 DAI had similar attributes as 8 DAI and has recently been used as the DAI to rate seedlings for anthracnose race 2 resistance (Patel, 2019), one could take a rating from 7 to 9 DAI.

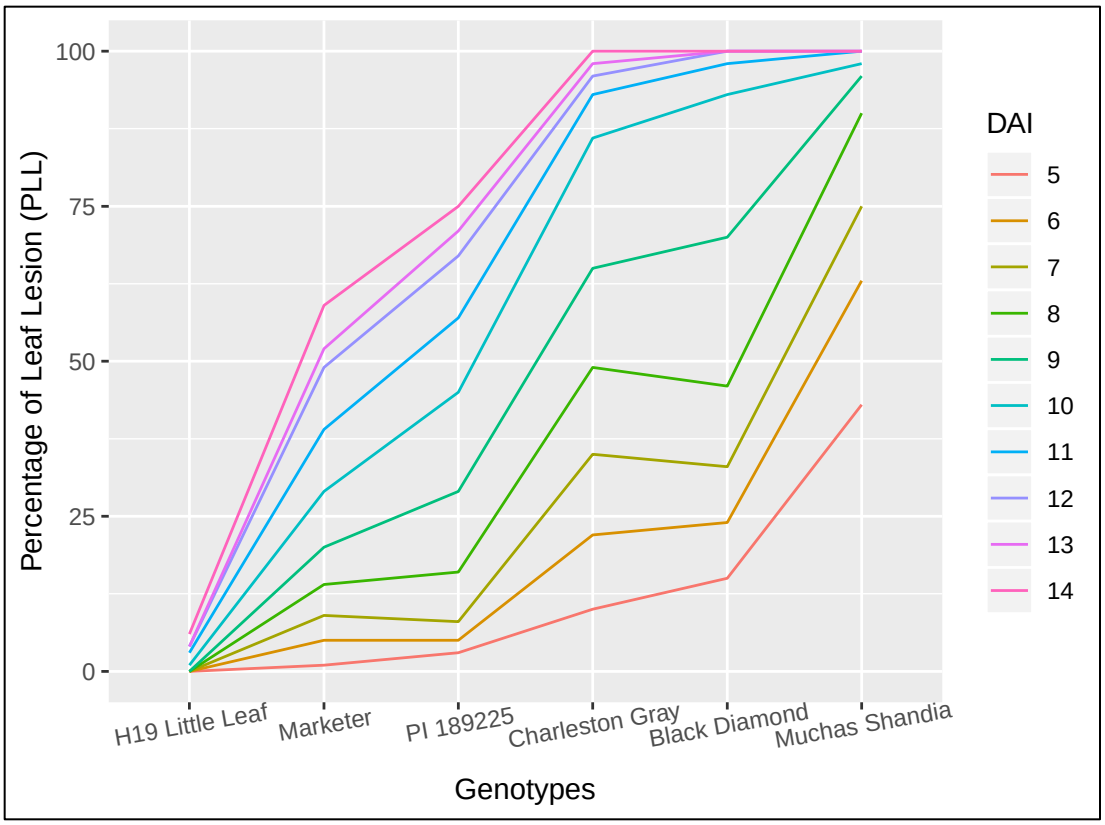

Figure 3.3 Line graph for PLL across genotypes. Specifically at 100,000 spore concentration at the different DAI across the different genotypes evaluated. Showing the difference between the genotypes

3.3b Germplasm Screening

Sowell Jr et al. (1980) were the first to conduct an anthracnose race 2 germplasm screenings, they used an isolate, '883', that was identified in Georgia. Several plant introductions (PI), 189225, 271775, 299379, 271779, 271778, and 203551, showed resistance to anthracnose race 2, under field conditions. Since the first germplasm screening study, other screening studies have been conducted using different isolates. Norton et al. (1991) used an isolate from the University of Arkansas, 'CP3', while Patel (2019) used an isolate from Dr. A. Keinath of South Carolina, both studies were conducted under greenhouse environment. In our greenhouse germplasm screening, an isolate from Dr. A. Keinath was used as well, *WmColl4*.

DAI	Source	DF	PLL	PCL	SHL	SPL	INDX
5	Genotype	13	669.4 ***	2231.1 ***	0.1	0.8 ***	588.7 ***
	Replication	2	321.6	181.5	0.2	0.4	106.7
	Error	26	81.83	207.86	0.03	0.11	25.58
6	Genotype	13	1895.2 ***	1808.4 ***	0.3 **	2.3 ***	776.5 ***
	Replication	2	381.3	49.0	0.0 **	1.4	68.6
	Error	26	66.15	95.91	0.11	0.18	39.68
7	Genotype	13	2662.3 ***	676.2 ***	0.8 ***	3.9 ***	702.6 ***
	Replication	2	189.7	59.0	0.1	1.3	52.5
	Error	26	72.23	58.11	0.19	0.23	16.09
8	Genotype	13	2718.5 ***	127.8 ***	2.6 ***	5.4 ***	800.7 ***
	Replication	2	576.5	50.6	0.7	3.1	73.0
	Error	26	83.97	21.80	0.36	0.18	15.79
9	Genotype	13	2680.7 ***	37.0	2.9 ***	5.3 ***	838.6 ***
	Replication	2	628.5	15.6	0.7	3.2	116.0
	Error	26	105.67	26.76	0.30	0.27	17.38

Table 3.7 Analysis of variance for anthracnose germplasm screening. The mean square of 5, 6, 7, 8, and 9 DAI for the different disease parameters rated and degree of freedom (DF). * significant at $P \leq 0.05$
** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$
† PLL = Percentage of leaf lesions (%)
‡ PLC = Percentage of cotyledon lesions (%)
§ SHL = Severity of hypocotyl lesion (0-6)
¶ SPL = Severity of petiole lesion (0-5)
# INDX = NCSU disease index (%)

Genotypes from our germplasm screening showed similar disease severity as previous greenhouse screenings (Norton et al., 1991; Norton et al., 1995; Patel, 2019; Sowell Jr et al., 1980). The ANOVA showed a statistically significant difference for *Genotype* across all of the traits evaluated (Table 3.7 and 3.8). Mean comparison of *Genotype* for PLL was assessed via Student's T test, where each DAI was evaluated separately (Table 3.9). Across the five DAI 'New Hampshire Midget' and Muchas Shandia were the most susceptible, at 8 DAI there was a 93 and 91% leaf lesion coverage, PLL. The Student's T test grouped the most susceptible genotypes together for all of the DAI (Table 3.9). At 8 and 9 DAI, PI 270550 was grouped with the most susceptible genotypes, indicating that the plant introduction is susceptible under greenhouse conditions. The

following genotypes showed the highest level of resistance, PI 189225, 'AU-Producer', PI 271778, and 'AU-Golden Producer'. The lowest disease severity came from PI 189225 and 271778, which had a PLL of 7 and 13%, respectively (Table 3.9). Although numerically they showed high resistance, there was no statistically significant mean separation, for PLL, to the previously identified susceptible genotypes, 'Black Diamond', and 'Crimson Sweet'. The genotype of 'Charleston Gray 133', showed similar resistance to that of PI 189225 and the AU-series. This genotype has shown high level of resistance as well in the germplasm screening study conducted by Patel (2019). 'Charleston Gray 133' was selected at Purdue University from 'Charleston Gray' for improved disease resistance to fusarium wilt. The genotype may have been improved for anthracnose resistance as well.

Source	DF	† LVE	‡ AUDPC PLL	§ AUDPC SHL
Genotype	13	1.07 ***	61,915.9 ***	18.4 ***
Replication	2	0.10	14,805.9	7.9
Error	26	0.03	2,341.9	2.4

Table 3.8 Analysis of variance for LVE, AUDPC PLL, and AUDPC SHL, screening. The source, degree of freedom (DF), and mean square of two traits.
† LVE = number of leaves evaluated on seedling
‡ AUDPC PLL = area under the disease progress curve for percentage of leaf lesions (%)
§ AUDPC SHL = area under the disease progress curve for percentage of severity of hypocotyl lesion

The similarity in our evaluation between the previously identified resistant and susceptible could potential be due to the growth stage of the seedlings. The seedlings in the germplasm screen were at an older growth stage, where they had more true leaves and begun to vine, couple of studies indicate a lower level of disease severity for older seedlings (Chongo and Bernier, 2000; Hong and Hwang, 1998; Pande et al., 1994). The seedlings for the germplasm screening were grown for three weeks, exactly as the procedure optimization study, but were at a different growth stage. The major

difference was the time of year in which they were grown. There is the potential that longer day period as well as the hotter temperatures may have influenced the growth of the plant for the germplasm screening study. It would be recommended to inoculate seedlings around the 2 to 3 true leaf stage before vining initiates, instead of going off of a time frame, such as three weeks.

Mean separation according to Student's T test for SHL showed the difference between genotype for a parameter that affects disease rating of the different parameters, if too severe than the plant starts to experience wilting and dies off, which can alter ratings (Table 3.9). From across the DAI, 8 days showed the greatest separation between genotypes, with the highest rating for the watermelon genotypes coming from PI 270550 at 3.0, while the lowest came from 'Verona' at 0.4. The most resistant watermelon genotype at 8 DAI, 'Verona', was grouped to several other watermelon varieties. The genotypes with the highest severity were PI 270550, PI 189225, and 'New Hampshire Midget', their means did not separate according to Student's T test.

The LVE for the watermelon genotypes from the germplasm screening was slightly higher than that observed for the watermelon procedure optimization, with a grand mean of 3.19 and 2.71, respectively. The lowest number of leaves was from 'AU-Producer' with an average of 2.38, while the highest LVE for watermelon genotypes came from Muchas Shandia at 3.88 (Table 3.9). The AUDPC PLL showed a similar trend as that of the PLL across the different DAI, with the highest AUDPC PLL from Muchas Shandia, 430 percentage days, while the lowest from AU-Producer at 37 percentage days (Table 3.9). The AUDPC SHL of the watermelon genotypes showed the slowest progression from 'Verona' and PI 271778, both at 1.8 percentage days. The highest AUDPC SHL value came from PI 270550, at 8.4 percentage days, followed by 'Black Diamond' at 7.0.

Genotype	PLL				SHL				LVE
	7 DAI	8 DAI	9 DAI	AUDPC	7 DAI	8 DAI	9 DAI	AUDPC	
New Hampshire Midget	89 A	93 A	99 A	430 A	1.1 BCD	2.9 AB	3.2 A	6.9 AB	3.50 CD
Muchas Shandia	81 A	91 A	97 A	405 AB	1.0 BCD	1.4 CDE	2.5 ABC	5.3 BC	3.88 B
PI 270550	66 B	78 A	89 A	338 B	1.9 A	3.0 A	3.2 A	8.4 A	3.21 DE
PI 203551	42 C	56 B	65 B	221 C	0.7 CDE	0.8 EFG	1.7 ABC	2.9 CD	3.22 DE
Verona	35 C	45 BC	59 BC	181 C	0.4 DE	0.4 FG	1.0 CD	1.8 DE	3.63 BC
Black Diamond	16 D	30 DE	44 CDE	96 D	1.3 ABC	2.0 BCD	2.9 AB	7.0 AB	2.96 EF
AU-Sweet Scarlet	15 D	33 CD	49 BCD	85 D	1.0 BCD	2.0 BCD	3.0 AB	5.2 BC	3.10 EF
Crimson Sweet	13 DE	22 DEF	29 EF	87 D	0.5 DE	0.4 EFG	0.9 CD	1.7 DE	3.06 EF
PI 271778	13 DE	16 EF	29 EF	53 DE	0.5 DE	0.6 EFG	1.4 BCD	1.8 DE	3.50 CD
AU-Golden Producer	11 DE	20 DEF	31 EF	61 DE	0.5 DE	0.9 EFG	1.5 BCD	3.0 CD	2.88 F
Charleston Gray 133	7 DE	21 DEF	38 DEF	59 DE	0.5 DE	1.0 DEFG	1.9 ABC	3.5 CD	3.23 DE
PI 189225	7 DE	16 EF	25 F	41 DE	1.5 AB	2.1 ABC	2.9 AB	6.5 AB	3.00 EF
AU-Producer	4 DE	12 FG	25 F	37 DE	0.7 CDE	1.2 CDEF	1.7 ABC	3.2 CD	2.38 G
H19 Little Leaf	0 E	0 G	0 G	0 E	0 E	0 G	0 D	0 E	4.96 A

Table 3.9 Mean for PLL and SHL at 7, 8, and 9 DAI with the AUDPC, screening. The grouping according to Student's T test is present beside the mean. The number of leaves (LVE) evaluated for PLL included.

In conclusion, the re-evaluation of genotypes for anthracnose race 2 resistance resulted in similar outcomes as previous greenhouse screenings. This points towards no mutations or shifts in anthracnose race 2 that may affect the resistance that exists in the PI lines. Interestingly, several of the previous germplasm screenings had retests to confirm their results. In these retests, the overall disease severity was usually a lower overall disease infection. These fluctuations show that variation can exist between inoculations and the need to try and reduce this variation by developing a uniform and consistent inoculation methodology for greenhouse screening.

3.3c Genetic study

Previous mode of inheritance studies identified resistance to anthracnose race 2 to be due to a single dominant gene (Love and Rhodes, 1988; Suvanprakorn and Norton, 1980). Love and Rhodes (1988) went a bit further and theorized that there were also minor modifier genes that affect anthracnose race 2 resistance. This was theorized due to the F_1's distribution shifting towards the susceptible parent and the F_2 susceptible individuals not showing the same susceptibility as the susceptible parent, 'New Hampshire Midget'. To evaluate the mode of inheritance there were four F_2 bi-parental populations screened. Population 1 and 3 had the highest number of F_2 individuals, with 190 and 191, respectively. The largest difference between the susceptible and resistant parents' PLL rating was from Population 1 with a difference of 54% (Table 3.11). The lowest came from Population 3, where the difference was 5.6%, which was nearly the exact same level of resistances. Population 1 was the only population that included the F_1 individuals, due to low number of seeds in the other population's F_1.

PLL													
Summary Statistics	† Population 1				‡ Population 2			§ Population 3			¶ Population 4		
	F1	F2	PI 189225	NHM	F2	Perola	PI 189225	F2	Sugarlee	PI 271778	F2	Verona	PI 189225
Mean	21.8	15.2	4.1	58.1	14.5	21.9	2.1	25.1	28.9	22.3	31.3	55.6	2.8
Median	22.5	12.5	3	55	12.5	20	1.5	13.3	16.7	17.3	18.3	55	1.5
Maximum	45	60	10.5	90	82.5	38.3	5.3	100	86.7	81.7	100	90	11.7
Minimum	3	1	1	27.5	0.3	10	0.3	1	1	1	0.7	20	0.7
Std Dev	15.5	10.8	3.3	23.1	14.2	7.9	1.7	26.2	31.3	21.8	32.2	20.4	2.9
N	16	190	16	9	89	16	16	191	16	13	113	11	16

Table 3.10 Summary statistics for genetic study populations
† Population 1: PI 189225 x 'New Hampshire Midget'
‡ Population 2: 'Perola' x PI 189225
§ Population 3: 'Sugarlee' x PI 271778
¶ Population 4: 'Verona' x PI 189225

Identification	Populations	† N	‡ Expect Ratio	§ Expected Resistant	§ Expected Susceptible	¶ Observed Resistant	¶ Observed Susceptible	# PLL Cut-Off R/S
Population 1	PI 189225 x NHM	190	3:1	143	47	139	51	21.8
Population 1	PI 189225 x NHM	190	3:1	143	47	172	18	31.1
Population 2	Perola x PI 189225	89	3:1	67	22	43	46	12
Population 3	Sugarlee x PI 271778	191	3:1	143	47	124	67	25.6
Population 4	Verona x PI 189225	113	3:1	85	28	66	47	29.2

Table 3.11 Expect and Observed resistant and susceptible individuals.
† Number of individuals in the population
‡ Expected ratio according to previous literature of single dominant gene
§ Number of expected resistant and susceptible
¶ Number of individuals observed resistant and susceptible
# The value used to determine the cut-off of resistant individuals

Identification	Populations	† Broad-sense Hertiability	‡ Chi-Square	§ P-value
Population 1	PI 189225 x NHM	0.35	0.34386	0.5576
Population 1	PI 189225 x NHM	-	24.428	< 0.0001
Population 2	Perola x PI 189225	0.93	33.801	< 0.0001
Population 3	Sugarlee x PI 271778	0.0074	10.347	0.0013
Population 4	Verona x PI 189225	0.94	16.593	< 0.0001

Table 3.12 Goodness-of-Fit table and broad-sense heritability.
† Broad-sense heritability, indirect estimate of single plant
‡ The chi-square for the population testing specified ratio
§ P-value of determining whether to reject the hypothesis of goodness-of fit

According to the goodness-of-fit, all of the F_2 populations rejected the hypothesis of a 3:1 ratio (3.11), when the cut-off of resistant and susceptible was the mean of the susceptible and resistant parents (3.12). While in Population 1, where F_1 individuals are evaluated as well and the mean is used as the cut-off between resistant and susceptible, the population does not reject the hypothesis of a 3:1 ratio. The chi-square obtained from the population was $\chi_2 = 0.34$ with a p-value of 0.56. This could indicate that the inheritance of anthracnose race 2 could potentially be due to a single dominant gene in PI 189225.

In Population 1, the mean of the F_1 individuals, 21.8% PLL, shifts closer to the susceptible parent, 'New Hampshire Midget', mean of 58.1% (Table 3.10). The histogram from Population 1 show a similar trend as to what Love and Rhodes (1988), had observed, which was that the susceptible F_2 individuals were not as susceptible as the susceptible parent, 'New Hampshire Midget' (Appendix C). This trend indicates that there could be other factors that influence anthracnose race 2 resistance, and that it is not as simple as a single dominant gene. The broad-sense heritability varied across the populations (Table 3.12), with Population 1 and 3, both showing low heritability, Population 3 had nonsignificant value of $H_2 = 0.0059$ and Population 1 had $H_2 = 0.35$. While Population 2 and 4 showed higher heritability, at $H_2 = 0.93$ and 0.94. The exact same bi-parental population, PI 189225 x 'New Hampshire Midget' (Population 1) was screened, but different rating method was used, where a broad-sense heritability resulted in $H_2 = 0.32$. Potentially this could be due to the different rating methods used, but from the INDX rating on the two populations with PI 189225, Populations 2 and 4, the broad-sense heritability was $H_2 = 0.92$ and $H_2 = 0.95$. This indicates that a difference in the broad-sense heritability existed.

3.4 Conclusion

An anthracnose race 2 inoculation procedure was developed to screen germplasm and populations. The ability to decrease variation across seedling inoculations and within the inoculations will allow for more precise results. We were able to identify an inoculation concentration, 100,000 spore mL^{-1} that is sufficient to differentiate seedlings for anthracnose race 2 resistance. A range of days after inoculation, 7 to 9, was determined to give the most difference between resistance levels, highly susceptible, moderately susceptible, and resistant. Potential future work on anthracnose race 2, could be to evaluate seedlings at different true leaf stages and determine an optimal stage to screen seedlings.

The greenhouse germplasm screening verified the resistance level of genotypes that had been previously evaluated. The AU-series developed in Alabama was found to show resistance to anthracnose race 2, as did two PIs, 189225 and PI 271778. An additional genotype that showed high susceptibility to anthracnose race 2 was identified, Muchas Shandia (PI 543210), this genotype could potentially be used as a susceptible parent in future population development to further understand genetic resistance to anthracnose race 2. The bi-parental populations' evaluated did not show inheritance due to a single dominant gene according to the goodness-of fit test for a 3:1 ratio. The population of PI 189225 x 'New Hampshire Midget' did show similarities to previous inheritance study that indicated a single dominant genes and minor genes that modify resistance. Ultimately, it would be best to conduct anthracnose race 2 inoculations and screening out in the field, since a difference in results have been obtained. It would allow for a more realistic appraisal of the issue that growers face during the season and determine whether germplasm is resistant to anthracnose.

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APPENDIX A

Trait	Source	DF	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI	11 DAI	12 DAI	13 DAI	14 DAI
† PLL	Genotype	5	2929.3 **	6620.8 **	11460.9 **	16284.2 **	19522.1 ***	22467.0 ***	23400.6 ***	23848.7 ***	23706.3 ***	22041.9 ***
	Concentration	4	671.6 *	1388.5 *	1769.3 *	2152.3 *	2748.7 **	2532.8 **	1902.5 ***	1404.3 **	1231.1 **	1004.4
	Genotype*Concentration	20	192.5 ***	289.3 **	261.4 **	203.8	248.9	274.8	219.3	216.8	242.5	179.4
	Set	1	951.2	1171.2	1676.5	324.7	63.4	63.4	45.4	64.8	40.9	8.1
	Set*Genotype	5	184.2	522.2	760.7 *	555.5	434.0	365.4	314.8	249.3	151.7	99.2
	Set*Concentration	4	50.9	87.4	124.8	177.6	92.2	46.4	3.9 **	33.0 *	36.9 *	201.8
	Set*Genotype*Concentration	20	38.5	91.1	82.4 *	114.9	141.5	145.0	145.2	170.4	142.1	170.8
	Rep[Set]	2	157.8	331.1	294.0	423.2	155.5	59.4	15.8 ***	37.7	87.3	217.5
	Error	58	39.279	73.0	90.5	122.9	128.5	121.6	152.1	117.1	116.5	125.0
‡ PCL	Genotype	5	170.0	816.9 **	2743.9 *	4361.7	3847.7	2310.4	1704.7	1067.2	869.6	700.7 **
	Concentration	4	59.5	189.6	764.2	1586.4	2112.8	1710.1	457.7	192.9	87.2	52.6
	Genotype*Concentration	20	55.8 *	152.4 *	228.7 *	795.0	465.2	240.2	138.5	92.5	49.9	42.5
	Set	1	3.5	18.1	33.3	2417.6	4391.3	4511.1	1743.6	621.6	248.6	112.2
	Set*Genotype	5	49.0	52.3	456.4	1197.4	1503.7	910.7	607.2	465.3	193.9	45.4 *
	Set*Concentration	4	61.3	185.2	389.4	2001.5	2142.2	1442.0	605.5	241.1	125.6	92.8
	Set*Genotype*Concentration	20	20.8 *	53.7 *	101.4 *	384.2	676.9	602.6	397.9	290.5	208.7	186.9 *
	Rep[Set]	2	8.1 **	178.5	162.1	183.4	471.0	303.5	145.3	148.1	161.9	98.9
	Error	58	43.1	122.4	198.9	372.4	460.1	377.0	190.2	110.5	77.6	58.1
§ SHL	Genotype	5	0.5	6.7 ***	22.7 ***	43.9 ***	64.9 ***	89.9 ***	106.8 ***	123.1 ***	135.8 ***	145.1 ***
	Concentration	4	0.2 *	1.1	2.7	2.9 *	3.7 *	3.4 *	2.1 *	1.3 **	1.1 *	1.3 *
	Genotype*Concentration	20	0.1	0.3	0.4	0.6 **	0.5 *	0.4	0.4	0.2	0.2	0.2
	Set	1	0.0	0.6	0.2	0.6	0.1	0.0	0.0	0.0 *	0.2	0.3
	Set*Genotype	5	0.1	0.1	0.1	0.2	0.3	0.2	0.3	0.2	0.2	0.1
	Set*Concentration	4	0.0	0.2	0.8	0.3	0.3	0.3	0.1	0.0	0.1	0.2
	Set*Genotype*Concentration	20	0.1	0.2	0.4	0.2	0.2	0.3 **	0.3 *	0.2	0.2	0.2
	Rep[Set]	2	0.1	0.1	0.6	0.5	0.1	0.1	0.0 *	0.2	0.1	0.0
	Error	58	0.04	0.09	0.11	0.24	0.15	0.13	0.11	0.10	0.10	0.07
¶ SPL	Genotype	5	8.7 **	20.2 **	32.1 **	44.7 ***	57.9 ***	68.7 **	74.5 ***	75.8 ***	74.4 ***	78.7 ***
	Concentration	4	1.4 *	3.2 **	3.6 *	3.9 *	4.8 *	5.0 *	4.9 **	5.2 ***	5.4 ***	7.9
	Genotype*Concentration	20	0.3 *	0.6 *	0.6	0.6	0.6	0.6	0.7	0.7	0.8 *	2.0
	Set	1	2.5	4.2	6.7	5.6	2.2	0.7	0.4	0.1	0.0	1.2
	Set*Genotype	5	0.4	0.8	1.3	1.1	1.1	1.0	1.0	1.1	0.7	2.4
	Set*Concentration	4	0.2	0.2	0.3	0.5	0.6	0.3	0.1	0.0 **	0.0 **	1.8
	Set*Genotype*Concentration	20	0.1	0.2	0.3	0.4	0.4	0.6	0.4	0.4	0.3	1.5
	Rep[Set]	2	0.6	1.1	0.8	0.5	0.3	0.2	0.2	0.2	0.8	2.4
	Error	58	0.11	0.15	0.22	0.33	0.35	0.35	0.37	0.28	0.43	1.52
# INDX	Genotype	5	6133.4 ***	6749.2 ***	7973.0 ***	10137.2 ***	11494.4 ***	14355.4 ***	17201.5 ***	19755.9 ***	22573.4 ***	26482.9 ***
	Concentration	4	313.3 **	477.9 *	408.6 **	601.9 *	838.4 **	919.6 **	1072.2 ***	1147.9 ***	1227.3 ***	1000.2 **
	Genotype*Concentration	20	52.7 **	60.4 **	65.0 **	93.7	83.6	80.9	106.7	133.0	142.5	109.1
	Set	1	224.6	193.8	298.2	199.1	30.0	0.3	6.1	1.4	51.7	22.6
	Set*Genotype	5	58.5	20.8	37.0	34.9	31.1	111.7	39.6	60.5	54.6	96.9
	Set*Concentration	4	6.1	40.3	19.9	46.6	22.4	19.2 *	12.4 *	15.6 **	17.4 *	37.9 *
	Set*Genotype*Concentration	20	15.9	20.4	20.4	44.8	47.2	82.8	75.0	117.1 *	100.6	145.6 *
	Rep[Set]	2	138.3	28.2	79.8	51.0	62.8	2.0 ***	1.5 ***	1.7 ***	0.3 ***	14.3
	Error	58	16.1	19.2	15.2	27.2	41.5	31.3	32.2	40.0	43.3	40.5

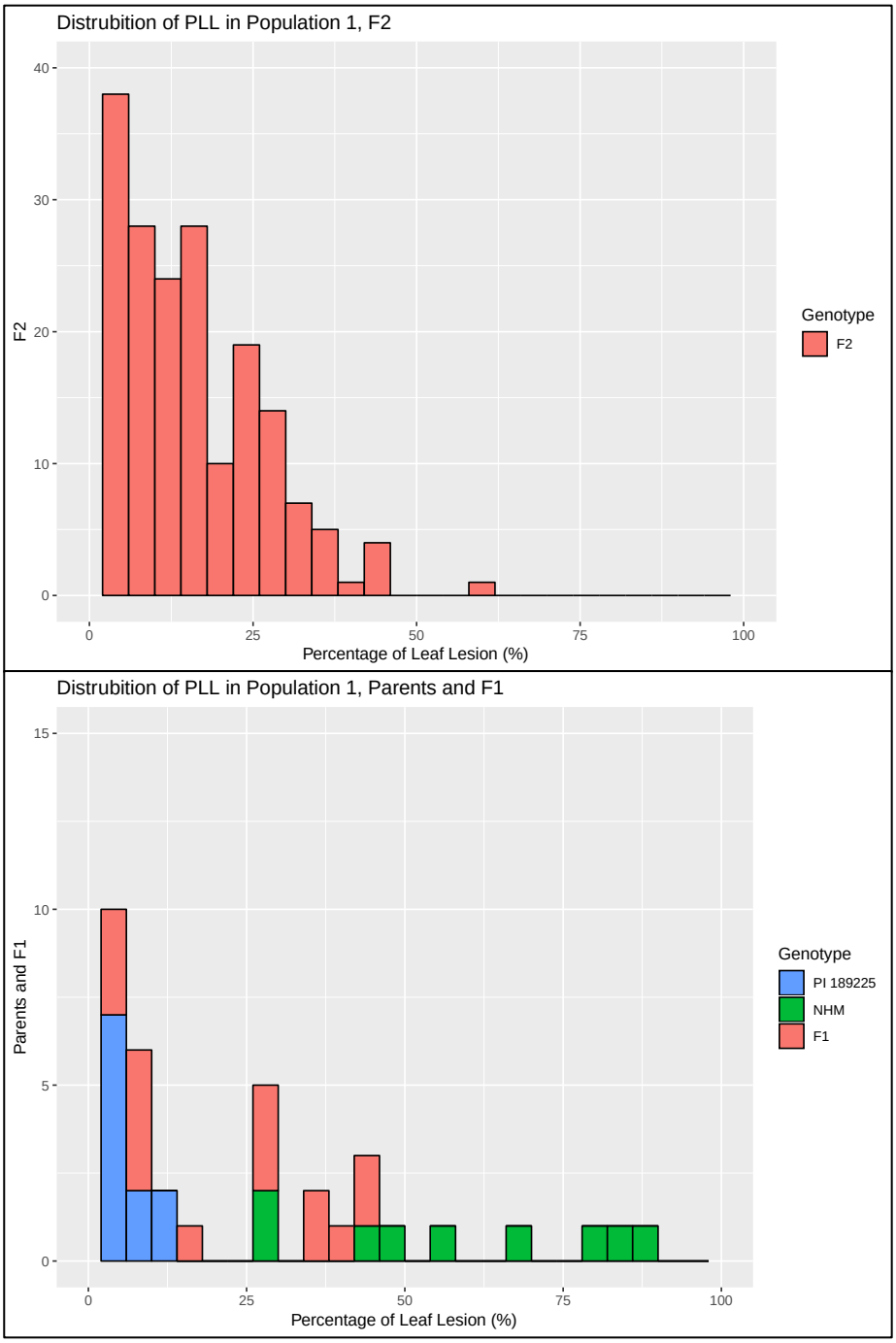
Appendix A. Analysis of variance table with the †mean square on DAI for the different disease parameters rated and ‡degree of freedom (DF). **A)** PLL = Percentage of leaf lesions (%) **B)** PLC = Percentage of cotyledon lesions (%) **C)** SHL = Severity of hypocotyl lesion (0-6) **D)** SPL = Severity of petiole lesion (0-5) **E)** INDX = NCSU disease index (%)
* significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$
Blank squares are not significant at $P \leq 0.05$.

APPENDIX B

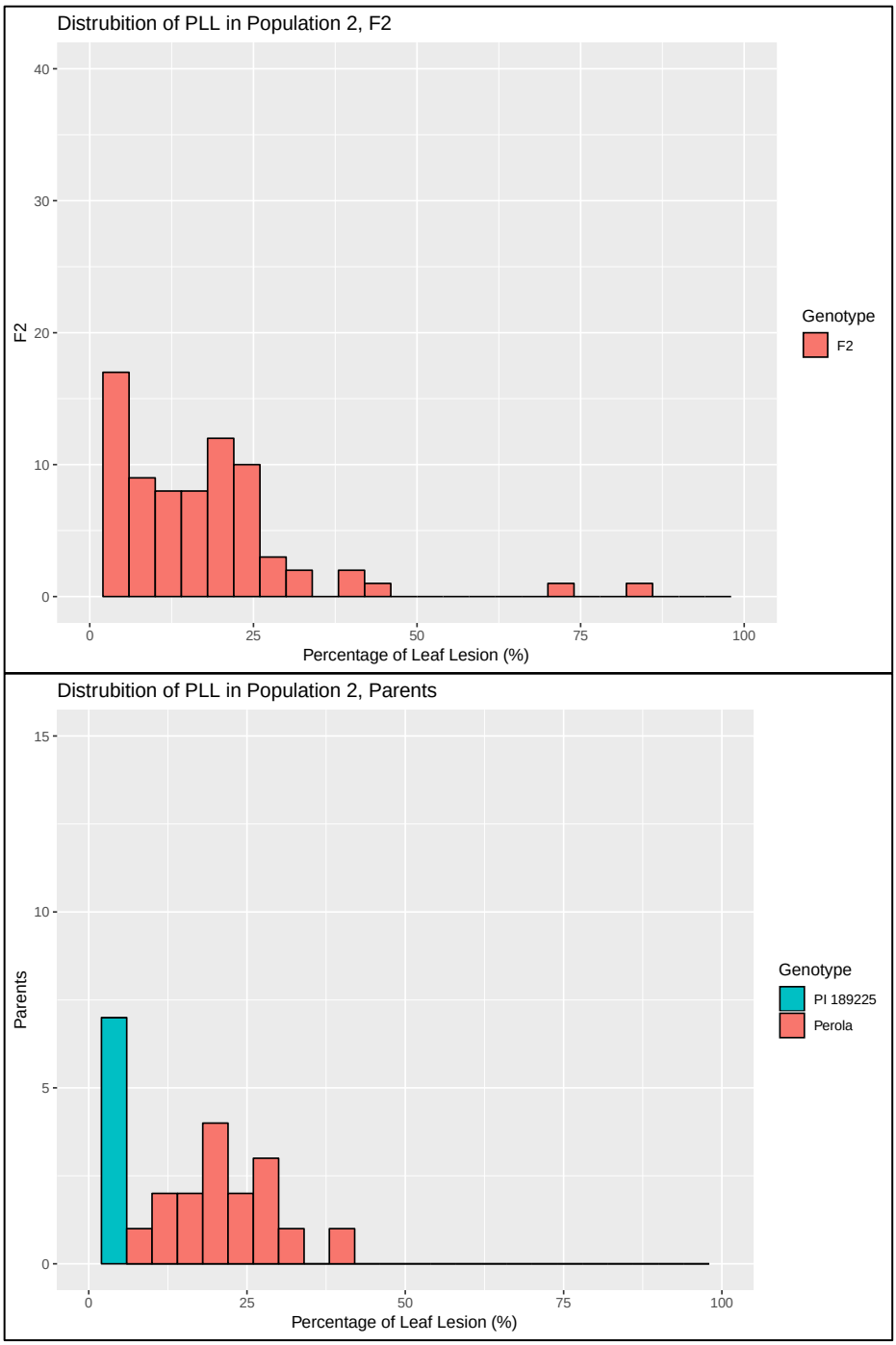
Concentration	Genotype	Percentage of Leaf Lesion (PLL)									
		5 DAI	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI	11 DAI	12 DAI	13 DAI	14 DAI
25,000	Black Diamond	4	7	12	23	33	57	73	84	88	92
25,000	Charleston Gray	3	4	11	17	29	46	56	61	66	73
25,000	H19 Little Leaf	0	0	0	0	0	0	3	6	8	10
25,000	Marketer	0	2	5	8	13	20	28	42	48	56
25,000	Muchas Shandia	9	17	35	55	66	78	83	91	95	96
25,000	PI 189225	1	2	2	6	14	25	36	41	46	50
50,000	Black Diamond	6	10	17	26	38	54	68	82	87	94
50,000	Charleston Gray	4	7	11	22	36	48	65	74	76	85
50,000	H19 Little Leaf	0	0	0	0	0	1	2	3	6	13
50,000	Marketer	2	6	9	14	19	27	39	48	56	69
50,000	Muchas Shandia	17	38	62	82	94	99	100	100	100	100
50,000	PI 189225	1	2	4	11	21	30	49	64	65	70
100,000	Black Diamond	15	24	33	46	70	93	98	100	100	100
100,000	Charleston Gray	10	22	35	49	65	86	93	96	98	100
100,000	H19 Little Leaf	0	0	0	0	0	1	3	4	4	6
100,000	Marketer	1	5	9	14	20	29	39	49	52	59
100,000	Muchas Shandia	43	63	75	90	96	98	100	100	100	100
100,000	PI 189225	3	5	8	16	29	45	57	67	71	75
250,000	Black Diamond	11	18	29	51	71	85	95	99	99	100
250,000	Charleston Gray	15	25	32	43	63	75	83	90	93	94
250,000	H19 Little Leaf	0	0	0	0	0	1	2	5	6	9
250,000	Marketer	4	12	20	27	36	42	52	59	67	73
250,000	Muchas Shandia	47	62	82	91	98	99	100	100	100	100
250,000	PI 189225	6	12	22	28	39	49	66	73	77	80
500,000	Black Diamond	16	26	38	50	66	79	87	93	97	100
500,000	Charleston Gray	15	22	28	41	58	76	86	94	96	98
500,000	H19 Little Leaf	0	0	0	0	0	1	2	3	3	13
500,000	Marketer	2	8	12	22	31	47	59	70	80	88
500,000	Muchas Shandia	48	73	86	96	99	100	100	100	100	100
500,000	PI 189225	3	7	12	24	37	53	64	77	82	83

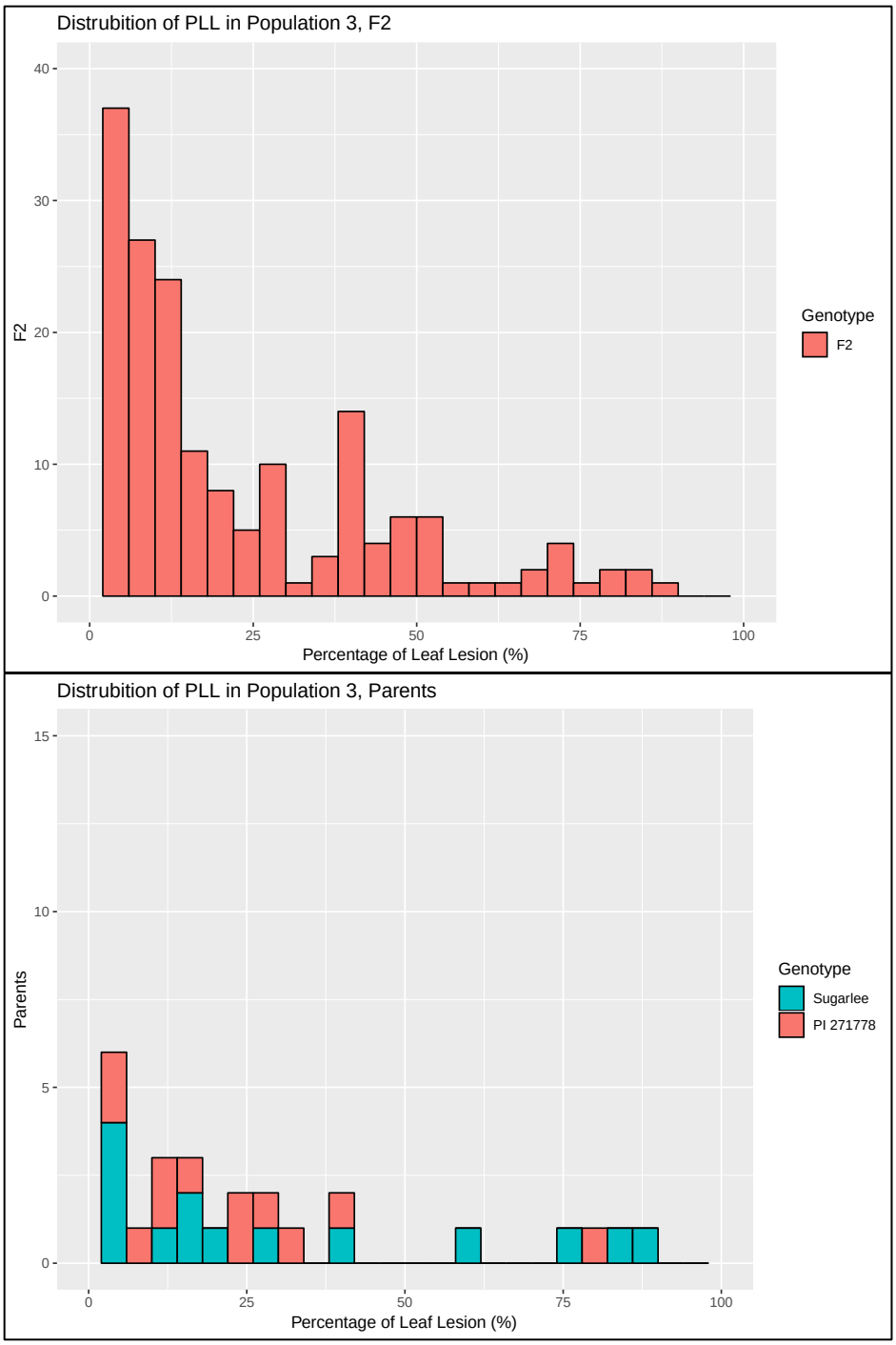
Appendix B. Mean PPL across the five different concentrations and six genotypes.

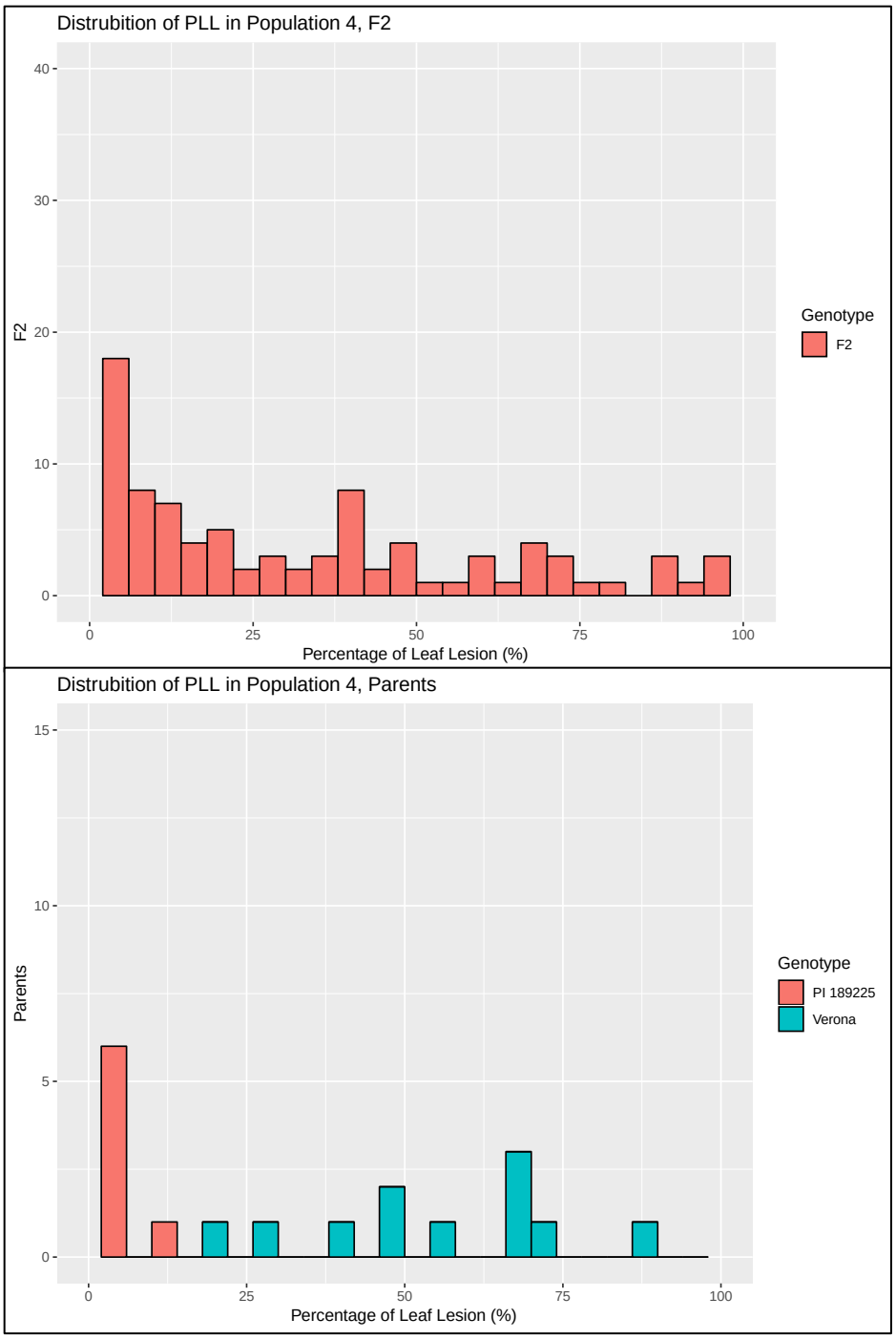
APPENDIX C



Appendix C. Histogram of genetic study populations


Distrubition of PLL in Population 2, F2
F2
40
30
20
10
0
0
25
50
75
100
Percentage of Leaf Lesion (%)
Genotype
F2
Distrubition of PLL in Population 2, Parents
Parents
15
10
5
0
0
25
50
75
100
Percentage of Leaf Lesion (%)
Genotype
PI 189225
Perola


Distrubition of PLL in Population 3, F2
F2
40
30
20
10
0
0
25
50
75
100
Percentage of Leaf Lesion (%)
Genotype
F2
Distrubition of PLL in Population 3, Parents
Parents
15
10
5
0
0
25
50
75
100
Percentage of Leaf Lesion (%)
Genotype
Sugarlee
PI 271778


Distrubition of PLL in Population 4, F2
F2
Percentage of Leaf Lesion (%)
Genotype
F2
Distrubition of PLL in Population 4, Parents
Parents
Percentage of Leaf Lesion (%)
Genotype
PI 189225
Verona