INVESTIGATING RENIFORM NEMATODE RESISTANT GENOTYPES IN COMBINATION WITH VELUM TOTAL IN COTTON *GOSSYPIUM HIRSUTUM* L.

by

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Keywords: QTLs, reniform nematode, resistant genotypes, SSR markers, upland cotton, Velum Total (nematicide)

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Abstract

Cotton, *Gossypium* hirsutum L., is an important commodity traded in the world market in terms of volume and value. Reniform nematode (*Rotylenchulus reniformis*) (RN) reduces and limits cotton yields. . At present, nematicides and crop rotations are the most effective management tool for producers. Our study investigates the use of breeding lines that have QTLs for RN resistance and the interaction effect of a nematicide under high RN pressure. The study compared these genotypes in both a non-RN field and a RN-infected field under a Velum Total treatment at two locations: Tennessee Valley Research and Education Center near Belle Mina, AL and in microplots at Auburn University. In 2017 and 2018, the lint yield values of the six lines outperformed commercial controls and the parental lines in RN infested field with Velum and without Velum. This study determined that RN stunts plants, reduces fiber quality, and reduces lint percentage, which all contributes to yield loss. Out of the six composite lines, there were five lines (B148, A202, A4142, B170, and B143) that had reduced egg numbers ($P < 0.05$) but high yields and can be considered resistant. The line A210 is considered tolerant of RN based on the high egg numbers and good yield. In addition, it is postulated that M713 Ren 4 contains introgression segments of QTLs on chromosome 21 (Ren^{barb1} and Ren^{barb2}) from GB713 that provides resistance to RN, but this information was never released. In recent studies, Ren^{barb1} and Ren^{barb2} are resolved at one locus (Ren^{barb2}), and the marker BNL3729 is strongly associated with the resistant phenotype. To validate this, progenies from these six composite lines were genotyped, and five of the six genotypes contained both Ren^{barb1} and Ren^{barb2} (excluding A210) while all six contained Ren^{barb2}. Since involving two markers demonstrated resistance while involving only one marker indicated tolerance, this data confirms that primary resistance is resolved in Ren^{barb2} since all lines had phenotypic resistance. Further work needs to be done in order to test lines of the 194 population, which were determined to be resistant, and their interaction with a nematicide.

Key words; cultivar by management, QTLs, reniform nematode resistance, SSR marker, upland cotton, Velum Total (nematicide)

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List of Abbreviations

ANOVA	Analysis of variance
bp	base pair
BT	*Bacillus thuringiensis*
cM	centimorgan
cc	cubic centimeter
°C	Celsius
Chr	chromosome
DAP	day after planting
dm^3	decimeter cup
DNA	deoxyribonucleic acid
FOV	Fusarium oxysporum f. sp. vasinfectum
GB	*Gossypium barbadense* L.
g	gram
HVI	High Volume Instrument
IPM	Ingredient pest management
log_c	logarithm 10 nematode egg count
lb/ac	bales per acre
ml	milliliter
m	meter
µl	microliter
µm	micrometer

min.	minute
max.	Maximum
MS	Mean square
NaOCl	Sodium hypochlorite
PCR	Polymerase Chain Reaction
PSS	Phenolic Separation Solution
PB	Powder Bead
QTL	Quantitative Trait Loci
RCBD	Randomized Complete Block Design
RN	Reniform Nematode
RK	Root knot nematode
RFW	Root fresh weight
SFW	Shoot fresh weight
SSR	simple sequence repeat
SFC	short fiber content
STR	fiber strength
SDHI	Succinate dehydrogenase inhibitor
s.	second
TVREC	Tennessee Valley Research and Extension Center
TAE	Tris-acetate EDTA
TBE	Tris-borate EDTA
USDA-ARS	United State Department of Agriculture, Agriculture Research Service
UV	Ultra Violet

Literature Review

Agronomic aspects of cotton

Cotton (*Gossypium* hirsutum L.) is an important commodity traded in the world market in terms of volume and value. In 2017, production was estimated at 104.2 million bales (1 bale = 480 lbs, 217.92 kg), with an 8% increase from 2016 (NASS, 2017). The primary cotton producing countries are India, China, the US, Pakistan, and Brazil and, the estimated total world trade were 35.3 million bales (USDA, 2017; NASS, 2017). Cotton production consistently fluctuates based on the current commodity prices which are dependent on the supply and demand of textile mills. (Meyer and MacDonald, 2016). In the US, it is grown in the southern states, collectively known as the cotton belt. This area ranges from CA to VA and as north as West TN, the boot hill of MO, and southern KS.

There are ~50 *Gossypium* species, with only four domesticated: *G. arboreum, G. barbadense, G. herbaceum,* and *G. hirsutum* L. (Brubaker et. al., 1999). By nature, cotton is a perennial tree; however, it is uniquely grown as an annual row crop. Cotton will germinate approximately 4 to 14 days after planting (DAP) depending on temperature and available soil moisture. Soil temperature should be at least 18°C for three consecutive days. It has a main taproot which will grow as deep as 10 inches before the cotyledon emerges, and the rate of growth is dependent on soil nutrients. (Kohel and Lewis, 1984).

Cotton has an indeterminate growth habit, i.e., the vegetative and reproductive growth occurs simultaneously. There are two types of branches on the main stem: normally one or two vegetative branches (monopodia) and several fruiting branches (sympodia). While monopodia branches contain only one meristem, sympodia branches contain multiple meristems. The intersection of the stem with a branch is referred to as a vegetative node; new nodes typically develop in three days. The fruiting branches grow in a “zig-zag” pattern with

reproductive structures forming in each point. On the first day of these structures are known as pinhead squares; it remains a square until it opens as a flower, which is approximately 21 days after planting. The first square can be visible at ~35 DAP.

Cotton flowers are complete and perfect, (contain both male and female sexual organs) and are self-pollinating. The first day of flowering, also referred to as anthesis, results in a white flower and pollination of the ovules. The following day, the petals turn pink and start to dry out. The remaining fruit is referred to as a boll. Since the plant is indeterminate, the flowers are not formed at the same time; it takes three days for a new flower to open at the node above it, and 6 days for the node beside it.

A boll requires approximately 50 days to mature after pollination; during this time, fiber develops on the seed coat as a single cell, consisting of nearly 100% cellulose. Stages of development consist of fiber initiation, primary elongation, secondary wall thickening, and maturation. The initiation phase is the start of the fiber cell and dictates how many cells develop into mature fibers. The cells elongate in primary elongation and relate directly to the final length of the fiber. This stage overlaps with secondary wall thickening, which is when cellulose begins to be deposited on the outer surface of the cell and contribute to the strength of the fiber. The last stage is maturation: the boll opens, and the fibers dry out for harvest.

In order to harvest cotton with a mechanical picker, a chemical defoliant is required to remove the leaves. This is important, as cotton is graded on its color and trash content; bits of leaf will stick to the fibers and green leaves will discolor it. Defoliant and harvest timing are affected by the temperature, environment, plant condition, product rate, and spray coverage. Harvesting can start as early as 7 days after defoliation, and requires a sense of urgency to

prevent yield loss by environmental effects. In the US, it can begin as early as July in south Texas or as late as November in Tennessee. In foreign countries where hand harvesting is common, there is no need for a defoliation spray. For example, *G. arboreum* is easy to pick but requires constant harvest with as many as 8-10 times in India, China, and Pakistan. Once cotton has been harvested, the seed cotton is processed through a saw gin to remove the fibers from the seed. The lint weight divided by total seed cotton weight is referred to as lint percentage or gin turnout. In production systems, the seed is used as feed for cattle, as ruminants are the only animals that can process the seed. This is due to a chemical called gossypol, found in all parts of the plant, which is a host plant resistance mechanism.

The lint is tested for a range of fiber quality traits such as strength, length, color, and micronaire by High Volume Instrument (HVI). All cotton bales are tested and required to maintain a base grade to prevent discounts to the selling costs. If the fiber quality is higher than the base, a premium may be given. It is significant for market demands and drives global cotton prices. Cotton color is graded on the amount of yellow and gray that is present, which is highly influenced by environmental conditions (Constable et al., 2015).

Cotton Pests and Diseases

Cotton has a range of biotic stressors such as insects, diseases, and nematodes that cause yield loss. It was a pesticide-intensive crop prior to boll weevil eradication and transgenic cotton (BT gene, *Bacillus thuringiensis*). The BT gene is found in a bacterium that produces crystals when ingested by the larva of tobacco budworms and bollworms, allowing for resistance to these pests (Hardee, et al., 2001). The major disease types are bacterial and fungal, and some can affect the plant directly while others enter by injury from insects or nematodes. There are devastating yield losses when these diseases infect a crop at maturity.

Bacterial blight caused by *Xanthomonas citri* subsp. *malvacearum* is the most common bacterial disease (Aida, et al., 2015) with symptoms including leaf spots and small circular brown lesions that prevent photosynthesis at early developmental stages (Isakeit, 2016). The most common fungal diseases are Fusarium wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) and Verticillium wilt caused by *Verticillium dahlia* (Wang, et al., 2009). The first symptom of FOV can be seen in late spring when soil temperature rise. Plants infected by Fusarium show darkened leaf veins, yellowing, and premature leaf abscission. Symptoms on older plants include stunting, chlorosis, and wilting. (Davis et al. 2006). Internal tissue of infected stems and roots are brown when the stems are cut. Yellowing starts from the lower leaves, moving up, and from the edges of individual leaves similar to Verticillium wilt.

Verticillium Wilt (*Verticillium dahlia)* effects the roots and moves into the vascular system. It restricts water and nutrient movement in the plant, resulting in wilting. Foliar symptoms include interval chlorosis, necrosis, and discoloration of the vascular system can be observed. If the inoculum level of microsclerotia in the soil is high, stunting and defoliation can be seen on the affected plants. Verticillium wilt and Fusarium wilt are easily confused due to their similar visual symptoms, but can be distinguished by signs of the fungal pathogens.

Nematodes have been identified as a serious problem on cotton since 1800s (Kirkpatrick and Rothrock, 2001). They are microscopic, bilateral, unsegmented wormlike animals that live saprophytically in water, soil, and as a parasite of plants and animals. Several types of nematodes (free living) can be beneficial for crop production because they feed on bacteria, fungi, insects, and even on each other. However, the plant parasitic nematodes encompass 10% of all nematodes and cause 14% crop losses annually all over the world

Reniform (*Rotylenchulus reniformis* (Linford and Oliveira))-(RN) and southern root knot nematode (*Meloidogyne incognita* (Kofoid & White) Chitwood]-(RK) are the most common cotton nematode pests, having large effects on yield losses. The southern root knot nematode was identified in 1889 on cotton in the U.S. (Kirkpatrick and Rothrock, 2001). RK nematodes are a sedentary endoparasitic nematode, having 5 growth stages: egg, four juvenile stages, and an adult stage (C. I. H. descriptions). The second juvenile (J2) is the infective stage which enters the roots and begins feeding ((C. I. H. descriptions) Hunt et al., 2005). Roots of infested plants are covered with small galls of different shapes depending on the amount of swelling. The nematodes limit essential water and nutrient uptake, which causes stunting, leaf reddening or discoloration, similar to nutrient deficiencies and yield losses (Kirkpatrick and Rothrock, 2001).

There is a well-documented disease complex involving root-knot nematode and Fusarium wilt. When the nematodes are controlled, cotton wilt is reduced (Shepherd, 1974). Management for nematodes includes using chemicals (nematicides) and crop rotation with non-host crops such as peanut to reduce population numbers and prevent yield loss (Weaver, et al. 2007). The best form of pest management is using genetic resistance and crop rotation together. RK and RN nematodes differ from each other by several features. RK nematodes are active and harmful in loam and sandy soil types and produce galling on the root system. RN is common in high silt-loam or clay soils; infected plant roots have small egg mass when observed under a microscope. This can make RN hard to diagnosis. Reniform females with eggs masses maybe visible with a hand lens and dissecting microscope when plants have been dug up from the soil. It is to be noted that if plants are pulled up by hand, the nematodes will not be visible.

Reniform nematode *(Rotylenchulus reniformis)*

Rotylenchulus reniformis is the most economically important species in the genus *Rotylenchulus* (Wang, 2007). It is distributed in subtropical, tropical, and warm climate zones: South America, Asia, Australia, the Middle East, Africa, Southern Europe, Caribbean, and the Pacific (Ayala and Ramirez, 1964). In 1931, RN was initially observed on a cowpea field in Hawaii and described as a pathogenic disease (Linford and Oliveira, 1940). It was later found on cotton in Georgia, and on tomato in Florida (Smith, 1940). In the US, RN has a wide distribution from Texas to North Carolina (National Cotton Council, 2005) and has become a major pathogen for cotton (Weaver, et al., 2007). Reniform nematode is a sedentary semi-endoparasitic nematode. It completes its life cycle with 4 juvenile stages, and each juvenile stage is follow by a molt. After egg hatching, the vermiform nematodes complete all the developmental molts in 1 or 2 weeks depending on the environmental conditions. The adult females are the infective stage which enters the plant, establishing a feeding site. Males mate with females and are not known to infect cotton plants. Males mature 7 days before females, but live a short time, as they never feed. A female will continue to lay eggs for an unknown period. Favorable soil conditions are 21°C with adequate soil moisture (Weaver et al., 2007).

The most common symptom of RN is stunting due to poor response to inputs such as irrigation, nutrition, etc. Bolls are smaller and lint percentage is reduced, contributing to yield losses (Birchfield and Jones, 1961). There are several management methods including nematicides, weed control, cover crops, sanitation of farm equipment, crop rotation, and biological control. Growing corn, grain sorghum, peanut, and resistant soybean are effective crops for suppressing nematodes, and should be applied for three years after host crop as it can exist without a host in deeper soils (Gazaway et al., 2007). The only truly effective measure to keep a field nematode-

free is sanitation that include implies a list of two or more things.

Management Strategies: Breeding for Resistance

The most effective form of integrated pest management (IPM) is a resistant cultivar. Yik and Brinchfield (1984) identified *G. barbadense* and *G. longicalyx* accessions as having some level of tolerance/resistance to RN, reporting that the female juveniles attack the root but never develop or produce eggs. Two breeding lines, 'LONREN-1' and 'LONREN-2', were derived from an interspecific cross between the wild species *G. longicalyx* Hutch and Lee, and *G. hirsutum* (Starr et al., 2007; Bell et al., 2014). Robinson et al. (2004) tested five *G. barbadense* accessions ('GB-49', 'GB-264', 'GB-171', 'GB-713', and 'GB-13') that had <11% RN egg production than the susceptible check 'Deltapine 16', with GB-713 displayed only 3%. Weaver et al. (2011) tested LONREN-2 in a reniform-infested field, demonstrating plant stunting with yields less than susceptible checks. Weaver et al. (2013-14) also reported LONREN-2 had good yields under low populations of RN conditions; however, under high RN intense conditions, the yields were low. This illustrates that female RN can penetrate the roots but not reproduce. The plant cells directly around the nematode die, starving the pest; however, it creates a hypersensitivity reaction with the plant, destroying its root system (Das et al., 2008). Stunting, smaller root systems, and compromised yields were observable under heavy RN pressure. Sikkens et al. (2012) tested a range of promising material in three locations and demonstrated that BARBREN-713 had high resistance to RN and good agronomic potential. In 2012, 'BARBREN-713' was released and is considered the first known germplasm to have high resistance to RN; however, it is photoperiodic (Robinson et al., 2004).

A study by Gutierrez et al., 2011, used a cross between GB713 (RN-resistance, *G. Barbadense*) X Acala Nem-X PI 590568 PVPO (RN-susceptible) to identify three QTLs:

REN^{barb1} and REN^{barb2} at 30.3 cM on chromosome 21 and REN^{barb3} at 15.3 cM on chromosome 18. The SSR markers associated with these QTLs are BNL1551_162 and GH132_199 (Ren^{barb1}), BNL4011_155 and BNL3279_106 (Ren^{barb2}), and BNL1721_178 and BNL569_131 (Ren^{barb3}). McCarty et al., (2013) crossed GB713 with 'Sure-Grow 747' (PI 656375 PVPO) and produced in total 16 BC_2F_2 populations selected based on early fruit production. M713 Ren 1 to Ren 16, comprised of different combination of the three QTLs from GB713 that provided resistance to RN (McCarty et al., 2013). 'M713 Ren1' and 'M713 Ren 2' are homozygous for the three QTLs (Ren^{barb1}, Ren^{barb2}, and Ren^{barb3}) associated with resistance. The line 'M713 Ren 5' is homozygous for the chromosome 21 QTLs but is missing the QTL found on chromosome 18 (McCarty et al., 2013). In 2017, it was concluded that the most important QTL is on chromosome 21, Ren^{barb2} (Wubben et al., 2017).

Management strategies: Nematicides and Resistant Cultivars

Nematicides are also an effective way to control RN, and there are varieties that are available to a grower. In recent years, Bayer CropScience made available a nematicide-insecticide Velum-Total (Fluopyram plus Imidacloprid). It affects both RKN and RN and early-season insects (thrips), thus enhancing seedling health and root growth in cotton. The active ingredients are Fluopyram which impacts nematode and Imidacloprid which impacts early-season insects. Fluopyram belongs to the succinate dehydrogenase inhibitor (SDHI) fungicide chemical class (FRAC Group 7). It suppresses nematodes by contact activity in the soil and disease through the xylem system (Bayer CropScience, 2016). In-furrow applications and seed treatment formulations are available and recommended at planting.

Schrimsher et al. (2014) investigated the interaction of Temik 15 G (Aldicarb), the nematicide that preceded Velum Total, on LONREN, BARBREN, and DP393. They reported

improved cotton yield and lowered reniform numbers over resistant and susceptible cotton lines. The resistant lines decreased populations by one half and all lines treated with Aldicarb had higher yields. Nematicides protect young seedlings from a multitude of stresses allowing plants to establish a better rooting structure than non-treated plants. Therefore it is anticipated that the treated lines would have higher yields however it is not known, if genotypes with increased levels of resistance, will respond the same with a nematicide treatment.

The breeding line 'M713 Ren 4', reported to have improved resistance (McCarty, personal comm.) was incorporated into the cotton breeding program at Auburn University in 2013. It was later identified to have the QTLs for tolerance/resistance to RN on chromosome 21 (McCarty et al., 2013). Several superior composite breeding lines were identified and advanced to replicated field trials. Since a more tolerant/resistant variety is now available to RN research, the interaction with a nematicide is useful information as it will determine whether a grower needs it as a management strategy when a resistant cultivar is available.

Objectives

Therefore, the objectives of this thesis are to:

(1) Evaluate the interaction of resistant/tolerant varieties and nematicide application under RN pressure.

(2) Evaluate the level of tolerance and/or resistance of six composite sister lines by phenotyping the agronomic values under RN pressure condition and validating the genotype by molecular markers

(3) Evaluate the utility of microplot as a field trial substitute in breeding for RN tolerance/resistance.

CHAPTER 1

INTRODUCTION

Cotton is one of the most important cash crops all over the world; however, production is significantly impacted by diseases and pests. Nematodes have been identified as a damaging parasite, and yield losses in cotton are rising. *Rotylenchulus reniformis* (Linford & Oliveria) (RN) is one of the most common plant-parasite nematode pests and causes almost 50% yield losses in upland cotton (*G. hirsutum L.*). It was first observed on cotton in Georgia in 1940 (Smith, 1940). Estimated losses have reached over $100 million in Alabama, Georgia, Mississippi, Louisiana, Arkansas, and Texas annually (Lawrence et al, 2018). Symptoms of RN are underdeveloped root systems, reduction of plant growth, dwarfing, and some interveinal chlorosis yellowing. It limits yield by reducing the number and size of bolls and by decreasing the lint percentage (Weaver et al., 2007).

The major management options to minimize yield losses of RN include the application of nematicides which should be used each year, as it is a short-term solution. For the long-term, crop rotations are advised; however, the rotation crop often of lower value and profit for the grower and may be expensive due to the necessity of different equipment (Yik and Birchfield, 1984). The third and most effective option is the use of resistant genotypes; however, at this time there are no commercially resistant cultivars. Therefore, combinations of nematicides and crop rotations are currently practiced.

The breeding efforts to identify sources of RN resistance ranged from minimal resistance in *G. hirsutum*, to moderate resistance in *G. longicaylx* and *G. barbadense.* These efforts have

produced the genotypes ‘LONREN’, which was found to be hypersensitive, and ‘BARBREN’, which is photoperiodic. (Das et al., 2008 and Robinson et al., 2004). In 2012, three genotypes, ‘M713 Ren 1’, ‘M713 Ren 2’, and ‘M713 Ren 5’, derived from crossing ‘GB713’ and ‘SG747’ accessions were released as resistant to RN (McCarty et al., 2012). Molecular studies have demonstrated that the resistant genes were successfully transferred into upland cotton.

(Schrimsher et al., 2014) reported that fields, microplot, and greenhouses trials indicated the phenotypic stunting of all resistant and susceptible genotypes tested was reduced by aldicarb (nematicide) and yields were increased as well. The aldicarb reduced RN population with no interaction of genotype by nematicide. Aldicarb is no longer available, and a new nematicide Velum Total (Fluopyram and Imidacloprid, Bayer CropScience) is being applied by cotton growers across the cotton belt. Concurrently, advances in cotton breeding lines have increased resistance to RN. Thus, it is unknown how RN populations may be affected by the use of increased RN resistant cultivars with the new nematicide. The hypothesis of this study is that the nematicide Velum Total, when applied to six lines with QTL markers for resistance, will not provide significant benefit. The RN resistance will be sufficient at reducing RN eggs, concomitantly reducing the population. Therefore, the objectives of this study are to evaluate the RN populations on six composite sister lines and compare these population with the yield performance. This information will also determine if these composite lines are resistant or tolerant to RN. It will also determine if Velum Total is beneficial when used in conjunction with one of these breeding lines.

MATERIALS AND METHODS

Plant germplasm

A breeding population, 194, was derived from crossing UA103 (susceptible) and M713 Ren 4 (resistant) in 2013/2014. The resistant gene of M713 lines was derived from a photoperiodic accession GB713 (PI 608139) which was crossed with Sure-Grow 747 (PI 656375 PVPO) (McCarty, et al., 2012). Population 194 was hand-selfed for 4 generations in a single seed descent fashion with no selection in order to maintain purity. At the F_4 generation, 142 lines were grown as a bulk population (also referred to as a composite population) in individual single rows. This was done to evaluate yield and increase seed for replicated trials. In the following generation, 53 bulk populations were advanced at random with no selection for any trait in a replicated field trial at Tennessee Valley Research and Extension Center (TVREC) near Belle Mina, in Northern Alabama. The soil type is classified as Decatur silt loam, with in the upper 15 cm horizon textural composition of 23% sand, 49% silt, and 28% clay (Sikkens et al., 2014).The best six lines were selected by level of RN resistance based on both yield and eggs per gram (g) of fresh root weight (g / RFW).

Field experiment

In 2017 and 2018, seasonal field tests were initiated with 10 entries including: 6 resistant breeding composite lines (194-A202, 194-A210, 194-A4142, 194-B143, 194-B148, and 194-B170), 2 commercial check cultivars (PHY444WFR, and ST4949GLT), and 2 parent germplasm lines (UA103 and M713-Ren4). All plots utilized a split-split-strip plot randomized complete block design (RCBD) and were replicated 4 times in a RN-infested and non-infested field at the TVREC. The main effect is nematode population (split) and genotype (split) nematicide (strip) application effects. The presence of nematodes is the main effect

with genotype and a nematicide, applied in-furrow at a rate of 14 oz/acre, as strip effects on both RN infested and non-infested fields.

Field Measurements

Soil samples were collected at both early and late seasons for determining RN population densities, and plant root samples were collected in mid and late seasons for determining RN egg production levels from both RN-infested and non-infested fields. Soil samples were collected as a zig-zag pattern per plot on both reniform-infested and non-infested fields, and each sample was placed in a labeled zip lock plastic bag. All sample bags were kept cool until arrival at the laboratory. At 41 days after planting (DAP), vigor ratings in the RN infested field were recorded. In addition, 2 seedling from each row (4 plants per plot) were extracted at random in both fields to determine RN egg densities. Plant heights and phenotypic characteristic of leaves were recorded in mid-season. At harvest, 25 boll samples were collected by hand for estimating yield and measuring fiber quality. These samples were ginned and a 20g fiber sample was sent to of the HVI lab at Cotton Inc. to analyze yield quality.

Nematode Extraction from Soil Samples

Nematodes were extracted from the soil samples by gravity screening followed by sucrose centrifugation. Firstly, 100 cc plastic cups was labeled like as zip lock bags and filled with well mixed soil. Each soil sample in a plastic cup added in 800-1200 ml of water and mixed vigorously for 30 second. For eliminating big soil particulates and roots, suspension was decanted slowly to on a 250 µm mesh sieve that nested on top of a 45 µm mesh sieve. Remaining nematode and soil particulates on the 45 µm mesh sieve were poured into a clean cup by means of a funnel. Nematode samples were allowed settle before the centrifugation.

Eggs Extraction from Plant Roots

Plants heights and shoot fresh weights were measured and recorded. Roots were rinsed gently to remove soil particles, weighed, and then nematode eggs were extracted. Eggs were extracted from cotton roots by placing the root system in a 0.625 % NaOCl solution and agitating the roots for 4 minutes using a rotary shaker at 120 rpm (Hussey and Barker, 1973). Eggs were rinsed with tap water, collected on a 25-µm-pore sieve, and then processed by sucrose centrifugation-flotation at 240 g for 1 minute (Jenkins, 1964). The process concluded with counting of both egg and vermiform numbers.

Microplot experiment:

Plot design

The field experiment in TVREC was duplicated in Auburn, Al, in 25 dm^3 microplot. The soil in these plots was obtained from the RN infested field in Bella Mina, AL (Figure 1). In 2017, thirty plots were treated with Velum Total and the remaining forty were the control. In 2018, thirty plots are treated with Velum Total and other thirty microplot are the control. Ten entries as the same field experiment were used and followed the same steps of field for counting nematode, eggs, plant height, phenotypic features, and fiber samples as well.

Planted and Sampling

Before planting, a soil sample was collected from each plot to confirm RN populations and record change through time. On May 26th, 2017, 15 seeds of each entry were planted with five planted in mesh for easy root removal; 4 replications of control plots and 3 replications of Velum Total plots. On May 14th, 2018, all entries planted with 3 replications in both treated and control plots as same method of previous year. In both years, 4 plants roots were dug up 41 DAP to extract and count RN eggs per gram fresh root. Plants were managed under normal field

conditions with routine watering and fertilization as needed. At the end of the season, soil samples were taken from each plot and extracted to count RN populations. All open bolls were handpicked to obtain a rough idea on cotton yield.

Statistical Analysis

Data for vermiform life stages and eggs of RN were analyzed in SAS 9.4 software (SAS Institute, Carry, NC) and using the GLIMMIX procedure. The vermiform nematodes collected from soil and eggs/g RFW extracted from plant roots required a log- normal distribution transformation to make satisfied the normality supposition. The LSMEANS transferred from lognormal distribution function were transformed back to the original data which was using PROC MEANS. The original data was presented in ANOVA table with *P*-values to determine statistical differences. Responses RN total eggs and eggs/g RFW from 2017 and 2018 experiments were analyzed together with no interaction between 2 years.

RESULTS AND DISCUSSION

The objective of this study was to evaluate the RN populations on six composite sister lines for levels of resistance and determine if Velum Total has increased benefit when used in conjunction with one of these breeding lines.

Field Experiments:

RN free field

The cotton field designated as RN-free was confirmed in the pre-plant sampling in both 2017 and 2018. The addition of Velum Total had positive effects on agronomic performances, i.e., plants had increased vigor ratings and plant height. The increase in plant height could be due to the provision of Imidacloprid, which has effects on early-season insects (Figure 2). Schrimsher et al., (2014) reported certain nematicides (aldicarb, abamectin/thiodicarb) had a significant effect on phenotypic stunting in early-season for both resistance and susceptible cultivars. As for the RN-free field, the nematicide protected cotton cultivars in the early season and increased plant height throughout the growing season.

RN infested field

Significant RN vermiform numbers at pre-plant were present and evenly distributed across the field. At 41 DAP; effects of the RN were clearly visible when comparing RN free and RN-infested trials (Figure 3 and 4). The RN-infested trial demonstrated that without a nematicide, susceptible cotton cultivars had dramatic phenotypic stunting in the early season. The population density of RN eggs/g root fresh weight (RFW), at mid-season, was significantly different for genotype and nematicide. This is expected as the nematicide kills RN at planting and the genotypes differ in their susceptibility to RN. All 194 populations had lower total egg densities and eggs/g RFW than both parents and the commercial cultivars. Line194-B148 had the lowest

total egg densities and eggs/g RFW, while the susceptible parent, UA103, had the highest (Table 1). Overall, Velum Total reduced egg numbers on both the susceptible and resistant entries. The final nematode egg densities/g RFW had a significant difference between genotypes (p=0.11). Velum Total had no effect on susceptible or resistant lines at the end-season primarily because it protects on RN population the first 10 days of growing season.

Microplot Experiments:

At pre-plant, there were no significant differences for individual effects of year, genotype, or nematicide (Table 2), this indicates that all pots are considered equal in both years. For midseason RN populations, Velum Total visually had positive impact on agronomic plant growth on both the susceptible and resistant genotypes. Evaluations of eggs/g RFW and total egg numbers demonstrated that there were no significant differences between years, genotype, or the interaction of genotype by nematicide; however Velum Total was significant (Table 3). These egg numbers of the genotypes with Velum Total are noticeably lower than non-treated genotypes. M713 Ren 4 has the lowest total egg and eggs/g RFW numbers with the nematicide while 194-A202 has the lowest total egg and eggs/g of g FRW without nematicide (Table 3). In contrast, without nematicide, ST4949 GLT the commercial susceptible standard had the greatest number of eggs/g RFW. End-season RN populations indicated that there is no significant difference between genotype, nematicide, years, and no interaction of genotype by nematicide (Table 2) because all genotypes had the same response to RN population for both initial and end season and both years as well.

Combined Field and Microplot Experiments:

In 2018, 5 individual lines were selected for end season eggs/g RFW in both the microplot and field trial location. These locations were not significantly different so egg numbers

were analyzed together. Total egg population densities and eggs/g RFW demonstrated no significant differences between nematicide but there was for genotype (Table 4). It was expected that Velum Total would not have an impact on RN population at the end of the growing season, while a genotype response was anticipated. Line 194-A210 had the lowest counts for eggs/g RFW and total egg population densities, while PHY444 WRF had the highest.

Field Experiment: *Yield, Lint Percentage, and Fiber Quality*

Yield, lint percentage and fiber quality for years were not significantly different. The RN vs non-RN fields were significantly different, as anticipated (Table 5). The means for yield, lint percentage and fiber quality traits across years in Table 6 demonstrate the effect of a RN infested field with lowered values except fiber elongation. This is in agreement with Weaver et al. 2013, who also found differences in fiber quality under RN pressure, further validating how RN impacts yield as micronaire, strength, and lint percentage are components of yield (Coyle and Smith, 1997) and demonstrates another reason why yield reductions occur. Genotype was significantly different for all traits except lint percentage and there was a significant interaction between field and genotype for yield and micronaire (Table 5). Means from both fields show that the commercial controls were the highest yielding in the non-RN but the lowest in the RN infested field (Table 7). This clearly demonstrates the need for RN resistance in commercial material. Line 194-B148 had the highest yield under the heavy RN pressure. The nematicide treatment was significant for yield and for the nematicide by field interaction (Table 7); having a significant impact on yield in only the RN infested field. Overall, yields were higher in the non-RN field, which is expected. Since this analysis was significant across fields, a separate ANOVA was performed (Table 9).

RN Field Only

The use of Velum Total was significant for yield, however; there was not a genotype interaction, primarily due to the large number of resistant genotypes. Figure 6 demonstrates this level of resistance in that the yield values are more similar in the resistant genotypes whether a Velum Total treatment is present or not. There are lines within this study that do exhibit varying levels of resistance and therefore demonstrate a trend when Velum Total is applied (Figure 7). When resistant and susceptible lines were compared, resistant lines had higher yields than the susceptible line with and without Velum Total. While the effect of Velum Total on yield is significant for both, the effect is greater on the susceptible line.

Fiber quality means of the cotton grown in the RN infested field is displayed in Table 8. The parent UA103 was chosen for its good fiber quality, but what was unknown was that M713 Ren 4 had similar if not better fiber quality. This allowed for transgressive segregants in strength, displayed in the 194- progeny lines, which is not uncommon in cotton (Meredith et al., 1984). The Velum Total treatment was positive for length uniformity and elongation but there was no interaction with genotype (Table 8). This can be expected due to the fact that Velum Total is available at the start of the season and is gone well before flowering starts (Lawrence, personal communication, 2018).

The regression of yield vs eggs/g RFW across all genotypes and both years demonstrate that population 194 decreased the negative association between yield and egg numbers/g RFW, while commercial cultivars had a much higher negative correlation (Figure 8). Identification of genotypes with resistance, tolerance, or susceptibility based on yield and egg numbers/g RFW are presented in Table 10. 194-B148 had the highest resistance response, based on the lowest egg numbers/g RFW and the highest yield. Excluding 194-A210, all 194 populations had better

response to RN pressure when compared to the resistant parent, M713 Ren 4. In contrast, A210 exhibited tolerance based on high egg numbers and moderate yield. The most susceptible line was UA103 with high egg numbers/g RFW and low yield.

CONCLUSION

This study determined RN stunts plants, reduces fiber quality, and reduces lint percentage, which all contribute to yield loss. It was concluded that screening for RN resistance can be performed in the microplots, as the results were not significantly different from the field. Although we hypothesized that Velum Total would not be necessary when using a high-resistance variety, we did find that the nematicide helps reduce stunting which was visible across all the genotypes. It was determined that the nematicide had no effect on lint percent or fiber quality, but has positive effect on yield. Out of the six composite lines, there were 5 lines (B148, B70, A4142, A202, and B143) that had lower egg numbers but high yields and can be considered resistant. There was a line (A210) with M713 Ren4 as a parent that is considered tolerant to RN based on the high egg numbers and good yield. Further work needs to be done in order to test line 194-B148, which was determined to be resistant, and its interaction with Velum Total.

CHAPTER 2

INTRODUCTION

Reniform nematode (RN) (*Rotylenchulus reniformis* Linford &Oliveira) is a cotton root damaging pest and causes yield losses in the US reaching ~$100 million (Lawrence et al., 2018). This pest can survive under harsh environmental conditions, so it is extremely difficult to control without resistant hosts or nematicides (Robinson 2007). Only a few moderately resistant genotypes have been screened in upland cotton (Robinson et al. 2004; Weaver et al. 2007) with almost 95% resistance being found in the *G. barbadense* L. photoperiodic accession, 'GB713' (Robinson et al. 2004). A quantitative trait locus (QTL) analysis has identified gene regions and markers associated with the resistance. This study involved GB713 (RN-resistance, *G. Barbadense*) crossed with 'Acala Nem-X' PI 590568 (RN-susceptible) and resulted in three QTLs: REN^{barb1} and REN^{barb2} on chromosome 21 and REN^{barb3} on chromosome 18 (Gutierrez et al., 2011). The SSR markers associated with these are BNL1551_162 and GH132_199 (Ren^{barb1}), BNL4011_155 and BNL3279_106 (Ren^{barb2}), and BNL1721_178 and BNL569_131 (Ren^{barb3}) (Gutierrez et al., 2011; Wubben et al., 2017). Romano et al., (2009) also reported similar genetic positions for RN resistance, with QTLs linked with BNL3279 and BNL4011 on chromosome 21 in *G. aridum*. McCarty et al. (2013) crossed GB713 with 'Sure-Grow 747' (PI 656375) and screened the progenies as the generation was advanced to the BC_2F_2. A total of 16 BC_2F_2 populations were selected using SSR markers and early fruit production. Three lines were released: 'M713 Ren 1' and 'M713 Ren 2', homozygous for all three QTLs (Ren^{barb1}, Ren^{barb2}, and Ren^{barb3}) and 'M713 Ren 5' homozygous for two chromosome 21 QTLs (Ren^{barb1} and Ren^{barb2}) but is missing Ren^{barb3} (McCarty et al., 2013). This same population later proved that there is only one major QTL on chromosome 21, Ren^{barb2} (Wubben et al., 2017). 'M713-Ren 4', which

was part of the original population but not released, has the chromosome 21 QTLs (McCarty, personal communication). This line was incorporated into the Auburn University cotton breeding program, and 6 composite F_6 breeding lines are now under evaluation in replicated field trials. These lines have been phenotyped, but the QTLs found in the M713 Ren 4 population were never verified.

Therefore, the objective of this study is to validate the phenotypic resistance by confirming the presence of the QTLs from M713 Ren 4 in a different genetic background.

MATERIALS AND METHODS

In 2018, 6 F_5 composite sister lines (194-A202, -A210, -A4142, -B143, -B148, and –B170), 2 commercial controls (PHY 444WRF and ST4949GLT), accession GB713, M713 Ren 1, Ren 2 and SG 747 were planted in the greenhouse (Figure 9). All genotypes were compared to M713 Ren 4 and UA 103 by DNA extraction to determine polymorphisms.

Field measurements

In 2017 and 2018, seasonal field tests were initiated with 10 entries: 6 resistant breeding composite lines (194-A202, 194-A210, 194-A4142, 194-B143, 194-B148, and 194-B170), 2 commercial check cultivars (PHY444WFR, and ST4949GLT), and 2 parent germplasm lines (UA103 and M713-Ren 4). Soil samples were collected at both early and end seasons for counting RN, and plant root samples were collected in mid and end seasons for counting RN eggs from both RN-infested and non-infested fields.

DNA extraction and Molecular Marker Analysis

DNA extraction was done by DNeasy PowerPlant Pro Kit method per the manufacturer's instructions (quick start protocol from QIAGEN Sciences, Germantown, USA). This method was designed to facilitate fast and easy purification of DNA from plant tissues, seeds and cells, and also helps to remove PCR inhibitors from plant tissue during extraction isolations. Fifty milligrams (50 mg) fresh leaf tissues, 5 plants per genotype, were collected in 2 ml PowderBead tubes at 21 DAP for DNA extraction. The six markers identified by Gutierrez et al. (2011) and Wubben et al. (2017), on chromosome 21 were used to screen the parents to identify which markers were polymorphic. BNL1551_162 (Ren^{barb1}) and BNL3279_106 (Ren^{barb2}) markers were selected to use for the progeny test. Tubes containing 410 µl bead solution, 40 µl phenolic separation solution (PSS), 50 µl sl solution, and 3 µl RNase solution were vortexed, placed in a

homogenizer for 2 minutes, then centrifuged for 2 more minutes. The supernatants were transferred to a 2 ml collection tube, 175 µl IR solution was added, then the solution was vortexed for 5 seconds and incubated for 5 min. at 2-8 °C. The samples were then centrifuged at 13,000 x g for 2 min and 600 µl pure supernatant was transferred to a 2 ml clean collection tube. Aliquots of 600 µl PB solution and 600 µl ethanol were added and the sample was vortexed for 5 s., 600 µl lysate was transferred into MB Spin Column tubes and the sample was centrifuged at 10,000 x g for 30 s. The follow-through solution was discarded and the replaced filter back into same tubes. This step was repeated 3 times. A 500 µl CB solution was transferred into MB Spin Column tubes and centrifuged at 10,000 x g for 30 s. A 500 µl aliquot of ethanol was transferred to the same MB Spin Column tube then centrifuged at 10,000 x g for 30 s. Solution was discarded and the filter replaced back into Column tube. A 60 µl EB solution was added and incubated for 2 min. in room temperature. Samples were centrifuged at 10,000 x g for 30 s., the EB solution was transferred onto a white filter membrane and centrifuged again. MB Spin Column was discarded and DNA retained. Six markers,BNL3279, BNL4011, BNL1551, BNL3649, NAU2152, and NAU3158 on chromosome 21 and 9 markers, BNL0569, BNL1079, BNL2571, BNL3479, NAU2443, JESPR0056, DPL0807, Gh055 and DPL0229 on chromosome 18 identified by Gutierrez et al. (2011) and Wubben et al. (2017), were used to screen the parents to identify markers that were polymorphic. BNL1551_162 (Ren^{barb1}) and BNL3279_106 (Ren^{barb2}) markers were selected for chromosome 21 along with all 9 markers for chromosome 18 to use for progenies test.

Nano Drop

Before following the polymerase chain reaction (PCR) protocol, the ratio of nucleic acids and proteins were measured for DNA quality and concentration in each sample by using the

Nano Drop program (Thermo Fisher Scientific, USA). Before checking DNA concentration, all samples were centrifuged for purification. Nucleic acids have an absorbance maximum (max) of 260 nm, while protein is at 280 nm. Historically, the ratio of absorbance at these wavelengths has been used as a way to measure both nucleic acid and protein purity.

PCR Protocol

The PCR is a sensitive and powerful technique for DNA amplification. The manufacturer's instruction recommended all reaction components be on ice and quickly transferred to a thermocycler preheated to the denaturation temperature (95 ^{0}C). For this reaction, a 0.45 µl aliquot from each reverse and forward primer, and a 1.8 µl DNA template was mixed in a PCR tube and a master mix was prepared. This master mix consisted of 6.13 µl distilled water, 1 µl buffer, 0.2 dNTP, and 0.07 Taq polymerase (New England Biolabs, Inc., Ipswich, MA, USA), and the total reaction volume was adjusted to 10 µl using nuclease-free water. The DNA solution was stirred and 7.4 µl was distributed to each PCR tube. It was then analyzed with the SSR markers for both chromosome 18 and 21. Typical cycling conditions for PCR consist of an activation step of 95°C for 30 sec followed by 35 cycles of 20 sec at 95°C, 1 min annealing at 52 °C, and 1 min at 72°C, followed by a final extension step of 10 min at 72°C. The amplification was resolved on 2% of agarose gel using ethidium bromide and visualized under Ultraviolet (UV) light.

Running Agarose Gel

Depending on the process, one of two buffers, TAE (Tris-acetate EDTA) or TBE (Tris-borate EDTA) were used for an agarose gel preparation. A 2% ratio of buffer to agarose powder was prepared in a flask and warmed until all powder was dissolved in the buffer. Ethidium Bromide (~3% solution) was used for gel staining when the solution cooled. This solution was

poured in a PCR block that had a comb (used for producing wells) and was left to solidify. A gel electrophoresis separates DNA by size of base pairs (bp) for purification and visualization. The DNA moves from negative (-) electrode to the positive (+) electrode. The first well was filled with 3µl to form a 100 bp ladder of known DNA fragment lengths. The other wells were filled with 5 µl DNA that mixed with 1 µl blue or orange DNA gel loading dye. Time and speed of the electrophoresis was adjusted and ran for 45 minutes to 1 hour. When the DNA had separated to the positive side, it was removed from the gel and visualized under an UV light. The lower bands demonstrate resistant markers, and the upper bands like demonstrate susceptibility.

RESULTS AND DISCUSSION

BNL_1551 for Ren^{barb1} and BNL_3279 for Ren^{barb2} produced clear, distinct bands on each of the parents. Five out of the six progeny lines (B143, B170, B148, A4142, and A202) derived from these parents demonstrated both QTLs, whereas one line (A210) only contained Ren^{barb2} (Figure 10 and 11). Wubben et al. (2017) created isolines with each of the three QTLs and in different combinations to determine their magnitude in RN resistance. They determined that RN resistance is depend on Ren^{barb2} and Ren^{barb3}; however, Ren^{barb2} has the most important role in resistance. Our study confirms their findings by demonstrating that the genotype associated with only Ren^{barb2} has no significant difference in yield compared with the genotypes associated with Ren^{barb1} and Ren^{barb2}.

Nine markers, BNL0569, BNL1079, BNL2571, BNL3479, NAU2443, JESPR0056, DPL0807, Gh055, and DPL0229 for Ren$^{\mathrm{barb3}}$ were analyzed by DNA extraction and PCR protocol; however, there were no clear and distinct bands for chromosome 18 markers (Figure 12). There was no difference between resistant and susceptible genotypes for any of the 9 markers on chromosome 18. Ren 2, GB713, and Ren 4 demonstrated resistant, while UA103, SG747, and PHY444 indicated susceptible to RN. Our data indicate that Ren$^{\mathrm{barb3}}$ is not necessary for achieving high yields under high RN pressure (Table 11). Group 1 is identified as no QTLs and therefore classified as susceptible. It is the average of the susceptible parent, UA103, and the control cultivars, PHY444WRF and ST4949GLT. Group 2 has one QTL line A210, and group 3 has 2 QTLS and consists of the resistant parent, M713 Ren4, and lines B148, B170, B143, A4142, and A202. The eggs/g RFW and yield results corroborate those of Wubben et al. (2017). Genotype 194-B148 had a low eggs/g RFW and a high yield (1480 lb/ac) when compared with both parents and commercial check cultivars. This genotype has both Ren$^{\mathrm{barb1}}$ and Ren$^{\mathrm{bar2}}$

markers, indicating it could be the best resistant line to RN based on high yielding and low egg numbers/g RFW. Genotypes from group 3 demonstrated that low eggs/g RFW and high yield, indicating that without the Ren^{barb3} marker, plants still have a resistant response to RN due to the Ren^{barb1} and Ren^{barb2} markers. Although, 194-A210 has high yield (1093 lb/ac), the eggs/g RFW are not significantly different then the susceptible parent (UA103) and commercial cultivars (PHY444WRF and ST4949GLT). Therefore, this genotype maybe considered more tolerant than resistant to RN, despite the fact that it only has Ren^{barb2} markers.

CONCLUSION

The 2017 and 2018 yield results demonstrate that the six bulk breeding lines outperformed the commercial checks in a RN infested field. Molecular analysis confirmed that five lines contain both Ren^{barb1} and Ren^{barb2} whereas one line only contains Ren^{barb2}. However, phenotypic data demonstrated no significant difference between the six lines in the RN-infested field's yield, suggesting the most important QTL region on chromosome 21 is Ren^{barb2}. Although all composite sister lines demonstrate the presence of at least the Ren^{barb2} marker, yield and eggs/g RFW indicate line A210 could be considered tolerant while lines A202, B148, B143, A4142 and B170 could have resistance to RN based on their response to yield and RN egg numbers under heavy RN pressure condition. This demonstrates that the breeding program was able to transfer the resistance genes into a different genetic background. We also conclude that since these lines contain both the markers, and show varying levels of resistance to tolerance, other genes are potentially being expressed and future studies are needed to further understand this resistance mechanism. In addition, linkage maps for all these populations need to be conducted to discover if there are markers closer to the resistance QTLs because the markers used are 30.3 cM from chromosome 21, indicating 70% chance of recovery. If there is another marker which is closer to the QTL, the chance of the recombination is lessened.

Appendix 1

Table 1: Means for RN eggs/g RFW and the total egg population per plant at 41 days after planting, averaged across 2017 and 2018 at TVREC trials

Effects		Eggs/g RFW		Total eggs	
Genotype	194-A202	317	CD	1585	BCD
	194-A210	450	ABC	2113	ABCD
	194-A4142	282	CD	1540	BCD
	194-B143	395	BCD	1778	BCD
	194-B148	183	D	904	D
	194-B170	254	CD	1090	CD
	UA103	1016	A	4870	A
	M713-Ren4	465	ABC	1939	BCD
	PHY444 WRF	494	ABC	2207	ABCD
	SG747 WRF	787	AB	3255	AB
Nematicide	No Nematicide	832	A	3.4888	A
	Nematicide	203	B	3.0688	B
P-value	Genotype	0.011		0.015	
	Nematicide	<.0001		<.0001	
	Genotype*Nematicide	0.1182		0.1182	

Table 2. ANOVA p-values for microplot trial, averaged across 2017 and 2018 for initial and end-season RN populations.

Effects	Number of DF	F Value	Initial season *R. reniformis*	End-season *R. reniformis*
Nematicide	1	1.05	0.9640	0.3529
Genotype	9	1.21	0.2941	0.7349
Genotype*Nematicide	9	1.01	0.438	0.3438

Table 3. Microplot RN means for eggs/g RFW and total eggs at 41 days after planting, across 2017 and 2018.

Effects		Treatment	Eggs/g RFW	Total eggs
Genotype	194-A202	no nematicide	101	361
	194-A202	nematicide	15	121
	194-A210	no nematicide	146	675
	194-A210	nematicide	9	32
	194-A4142	no nematicide	378	1303
	194-A4142	nematicide	16	76
	194-B143	no nematicide	202	796
	194-B143	nematicide	16	45
	194-B148	no nematicide	255	1303
	194-B148	nematicide	11	56
	194-B170	no nematicide	202	1487
	194-B170	nematicide	9	121
	AU103	no nematicide	223	1347
	AU103	nematicide t	14	76
	M713-Ren4	no nematicide	378	1143
	M713-Ren4	nematicide	6	20
	PHY444 WRF	no nematicide	321	721
	PHY444 WRF	nematicide	10	63
	ST4949 GLT	no nematicide	492	1347
	ST4949 GLT	nematicide	9	74
Nematicide		No nematicide	389 A*	158 A
		Nematicide	28.2 B	7.3 B
P-value	Year		0.280	0.274
	Genotype		0.3235	0.653
	Nematicide		<.0001	<.0001
	Genotype*Nematicide		0.5131	0.351

*Letters in the same column demonstrate significant difference at p=0.05.

Table 4. RN means for eggs/g RFW and total eggs at 120 days after planting averaged across microplot and RN infested field in 2018.

Effects		Eggs/ g RFW	Total eggs
Genotype	194-A210	33 C*	280 B
	194-A4142	75 AB	509 AB
	UA103	69 ABC	508 AB
	M713-Ren 4	49 BC	608 AB
	PHY444 WRF	138 A	1238 A
P-value	Location	0.778	0.158
	Genotype	0.015	0.037
	Nematicide	0.109	0.796
	Genotype*Nematicide	0.267	0.592

*Letters in the same column are significantly different at p=0.05.

Table 5. P-values for yield, lint percentage, and fiber quality across years and RN fields.

Effects	df	Yield	Lint percentage	Micronaire	Length	Length uniformity	Strength	Elongation
Year	1	0.358	0.295	0.243	0.243	0.241	0.241	0.372
Field	1	<.0001	<.0001	0.001	<.0001	<.0001	0.003	0.023
Genotype	9	0.019	0.831	<.0001	<.0001	<.0001	<.0001	<.0001
Field*Genotype	9	<.0001	0.156	0.002	0.593	0.967	0.396	0.248
Nematicide	1	<.0001	0.813	0.82	0.366	0.477	0.934	0.918
Genotype*Nematicide	9	0.996	0.772	0.205	0.211	0.183	0.903	0.932
Field*Genotype*Nematicide	10	0.985	0.802	0.472	0.258	0.49	0.406	0.853

Table 6. Fiber quality, lint percentage and yield means averaged across years and fields.

Field	Lint percentage	Yield	Micronaire	Length	Length Uniformity	Strength	Elongation
	%	lb/ac		in	%	g/tex	%
Non-RN	0.409 A	1490 A	4.62 A	1.24 A	84.7 A	32.8 A	6.6 B
RN-infested	0.3951 B	1110 B	4.54 B	1.21 B	83.9 B	32.4 B	6.7 B

Table 7. Yield means for RN-infested and non-RN fields, across years.

Effects		Non-RN Yield		RN Yield	
		lb/ac			
Genotype	194-B148	1427	CDEF*	1304	DEFGH
	194-A4142	1494	BCD	1269	EFGH
	194-B143	1447	CDE	1229	GH
	194-B170	1393	CDEFG	1211	GHI
	194-A202	1488	CD	1187	HI
	194-A210	1491	BCD	1187	HI
	M713-Ren 4	1251	FGH	1026	IJ
	PHY444 WRF	1694	A	917	J
	UA 103	1543	ABC	902	J
	ST4949 GLT	1680	AB	873	J
Treatment	No nematicide	1456	A	1013	C
	Nematicide	1525	A	1208	B

*Letters in a column are significantly different at p=0.05.

Table 8 . Fiber quality trait means and p values for genotypes tested in a reniform infested field located at TVREC in 2017 and 2018.

	Genotype	Micronaire	Length (in)	Uniformity (%)	Strength (g/tex)	Elongation (%)
	194-A202	4.7 AB	1.19 CD	83.5 DE	33.9 AB	NS
	194-A210	4.5 DE	1.21 BCD	83.8 BCDE	34.1 AB	NS
	194-A4142	4.6 BCD	1.19 D	84.1 BCDE	33.8 ABC	NS
	194-B143	4.4 E	1.21 BC	84.2 BC	34.5 A	NS
	194-B148	4.6 CDE	1.20 BCD	83.5 E	33.9 AB	NS
	194-B170	4.7 AB	1.19 D	83.9 BCDE	33.1 BCD	NS
	UA103	4.4 E	1.22 B	84.3 AB	32.5 DE	NS
	M713-Ren4	4.5 DE	1.20 BCD	83.5 E	33.0 BCD	NS
	PHY444 WRF	4.1 F	1.26 A	84.8 AB	32.7 CDE	NS
	SG747 WRF	4.7 ABC	1.51 E	83.7 CDE	31.6 E	NS
P-value	Genotype	<.0001	<.0001	<.0001	<.0001	0.203
	Nematicide	0.397	0.8531	0.0385	0.1423	0.09
	Genotype*Nematicide	0.4534	0.6676	0.606	0.5733	0.287

Means with the same letter are no significantly difference at the 0.05 probability level as calculated by Tukeys LSD

Table 9. ANOVA results for yield in the RN-infested field averaged across 2017 and 2018

Effect	df	*P* value
Year	1	0.241
Genotype	9	<.0001
Nematicide	1	<.0001
Genotype*Nematicide	9	0.857

Table 10. Identification of resistance, tolerance, or susceptible genotypes based on yield and eggs/g RFW.

Ranking	Genotype	Yield		Eggs/g RFW		Classification
		lb/ac				
1	194- B148	1305	A	183	D	R
2	194-B170	1212	A	259	CD	R
3	194-A4142	1270	A	290	CD	R
4	194-B143	1230	A	398	BCD	R
5	194-A202	1188A	B	325	BCD	R
6	M713 Ren4	1026B	C	460	ABC	T
7	194-A210	1236	A	466	ABC	T
8	ST4949GLT	848	D	794	AB	S
9	PHY444WRF	917	CD	487	ABC	T
10	UA103	903	CD	1029	A	S

Table 11. Effect of SSR Markers on *R. reniformis* egg production on six composite sister lines, both parents and two commercial check cultivars.

	Ren^{barb} QTL combination						
	Chr 21	Chr 21	Chr 18	Eggs	Yield		
Genotypes	Ren^{barb1}	Ren^{barb2}	Ren^{barb3}	/g RFW	lb/ac		Genotype
Group 1	-	-	-	1615 A	757	B	3
Group 2	-	+	-	1155AB	1093	A	1
Group 3	+	+	-	579B	1126	A	5

Group 1 (ST 4949 GLT, PHY444 WRF, UA103), Group 2 (A210), and Group 3 (M713 Ren4, A4142, A202, B148, B143 and B170)

Appendix 2

Figure 1. Microplot Design at Auburn University

Figure 2. Effects of the Velum Total on PHY444WRF (commercial control) for plant growth and health at no-RN trial in Belle Mina, AL.

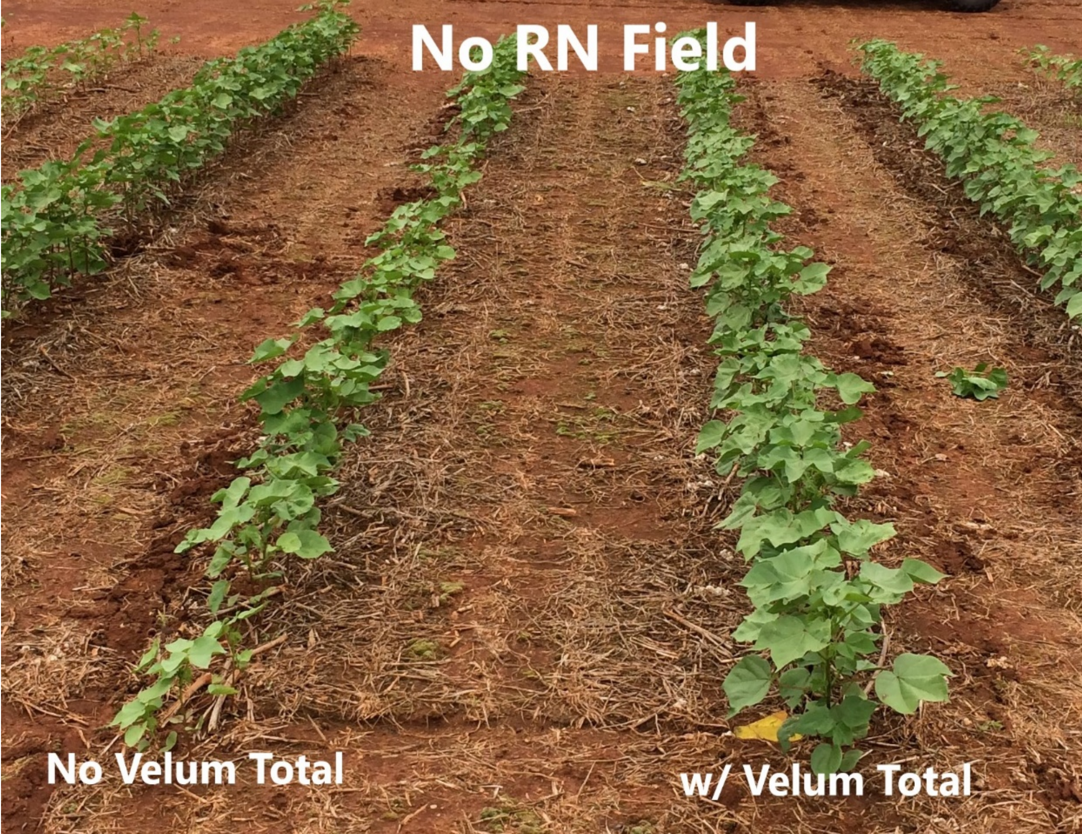


Figure 3: Velum Total effects on plant growth at 41 days after planting in RN infested field.

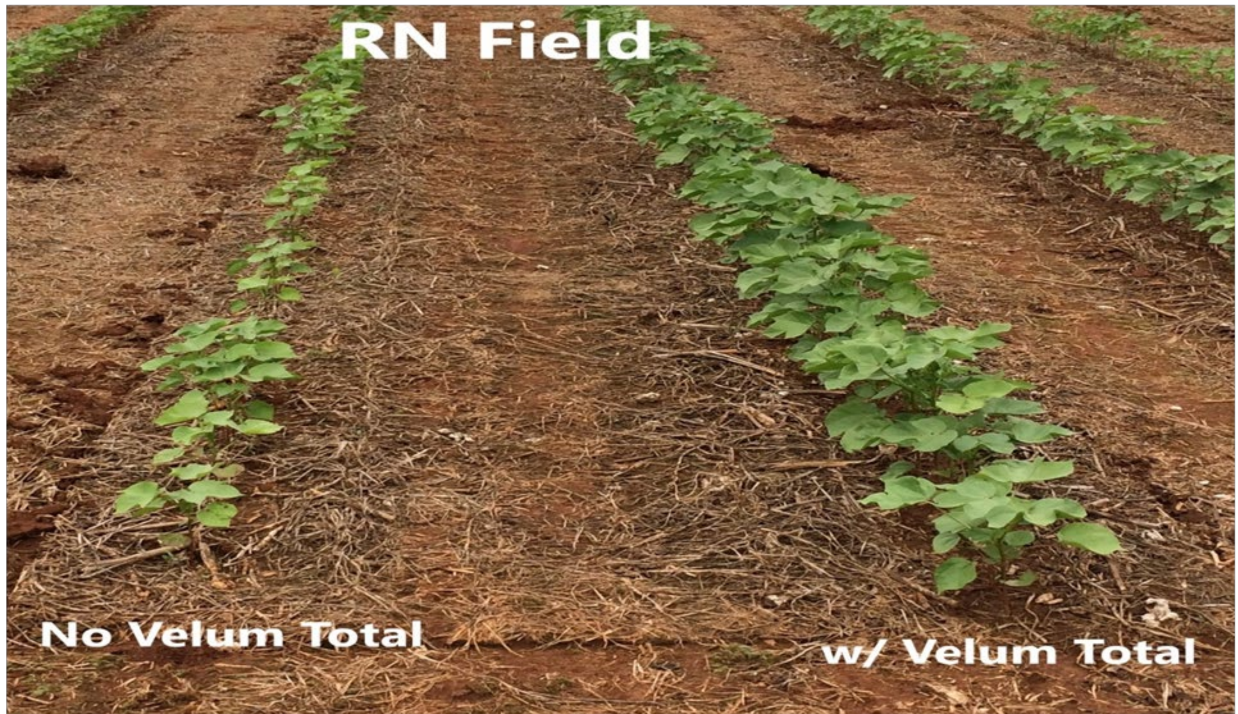


Figure 4: Effects of the RN on resistant parent M713 Ren 4 at 41 days after planting.

Figure 5. Effects of Velum Total on cotton growth at 45 days after planting in 2017

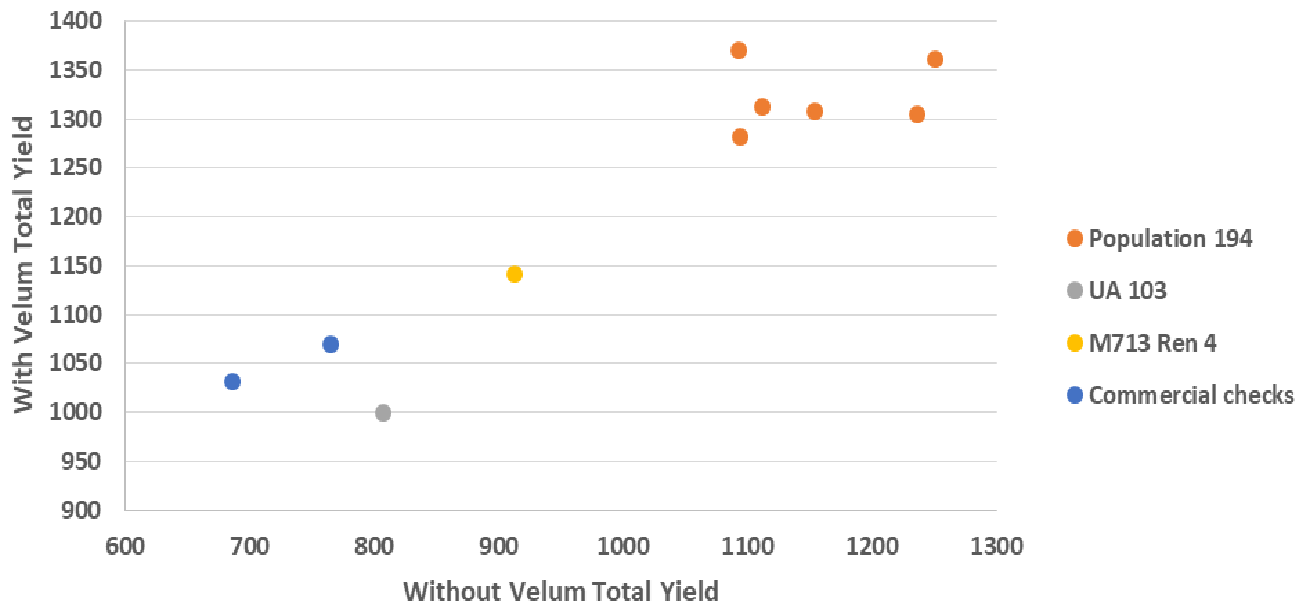


Figure 6. Lint yield values in RN infested field at Belle Mina, AL, averaged cross 2017 and 2018; with and without Velum Total treatments.
1400
1350
1300
1250
1200
1150
1100
1050
1000
950
900
With Velum Total Yield
600
700
800
900
1000
1100
1200
1300
Without Velum Total Yield
Population 194
UA 103
M713 Ren 4
Commercial checks

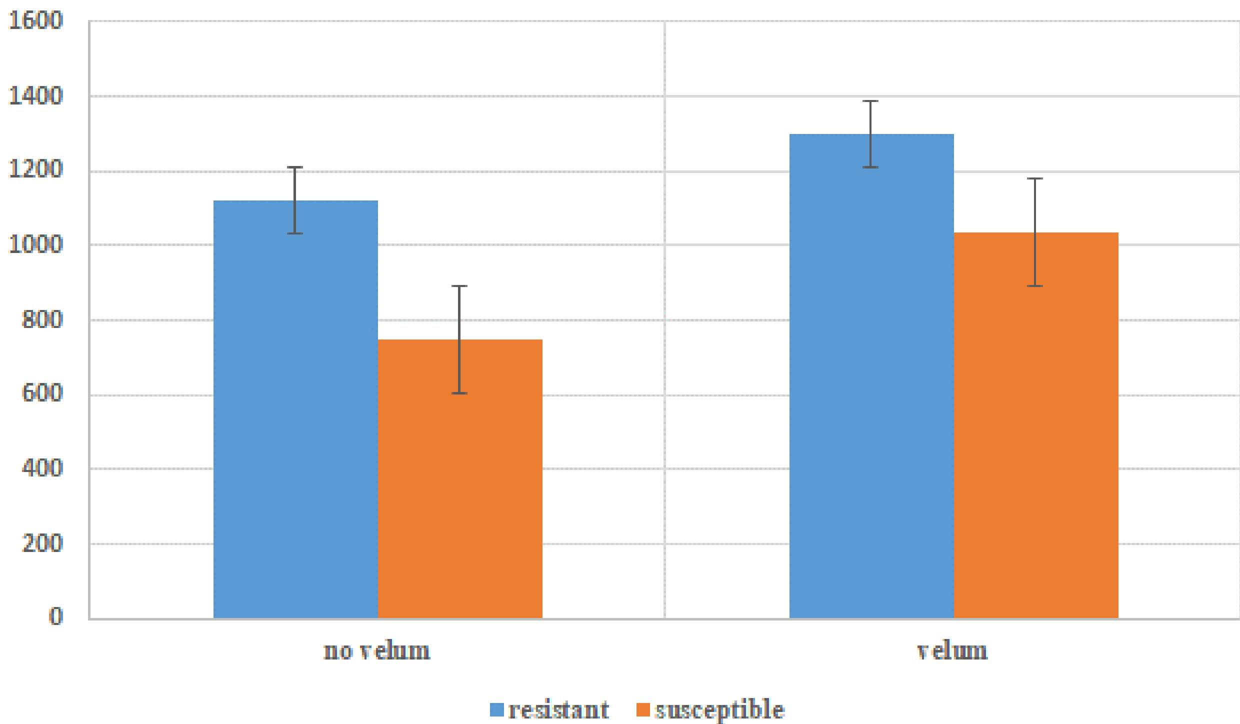


Figure 7. Yield of resistant vs susceptible cultivars averaged together with and without a Velum Total treatment.
1600
1400
1200
1000
800
600
400
200
0
no velum
velum
resistant
susceptible

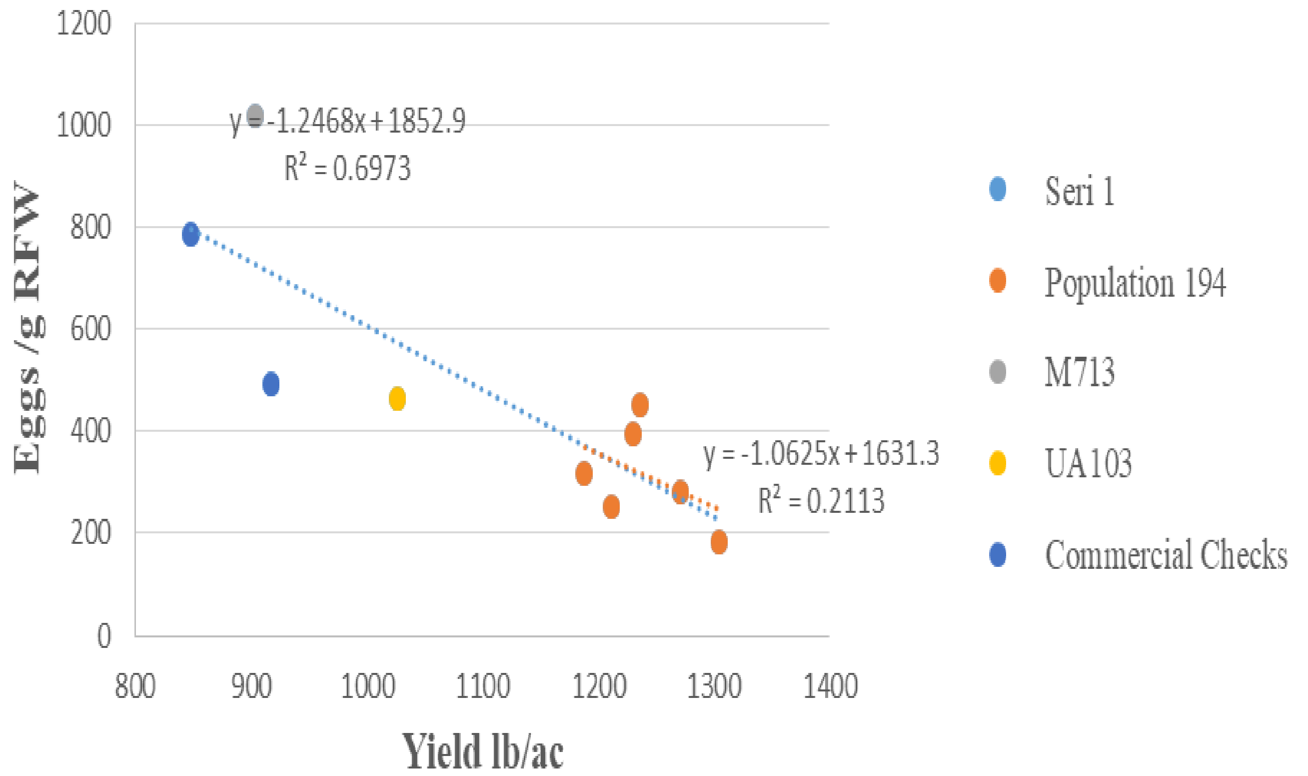


Figure 8. Regression of yield vs eggs / g RFW cross all genotypes and both years.
1200
1000
800
600
400
200
0
Eggs /g RFW
y = -1.2468x + 1852.9
R² = 0.6973
y = -1.0625x + 1631.3
R² = 0.2113
800
900
1000
1100
1200
1300
1400
Yield lb/ac
Seri 1
Population 194
M713
UA103
Commercial Checks

Figure 9. Plant material used for molecular marker validation.

Figure 10. Agarose gel for BNL1551 primer. Resistant Ren4 (R), Susceptible UA103 (S) parents and B143 (C), A4142 (D), A202 (E), A210 (F), B170 (G), and B148 (H) progenies.

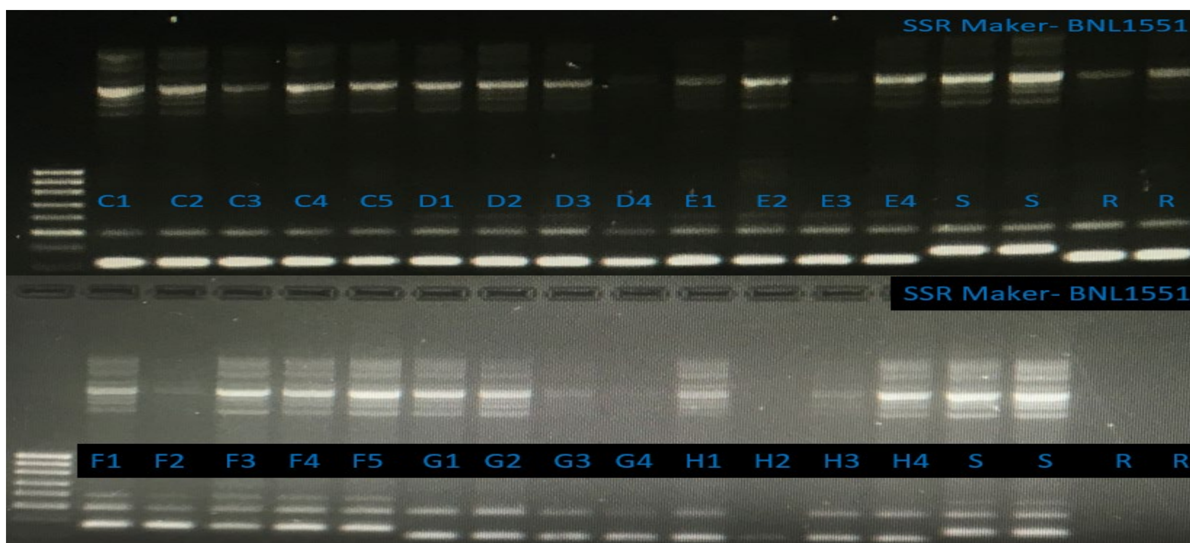


Figure 11. Agarose gel for BNL3279 primer. Resistant Ren 4 (R), Susceptible UA103 (S) parents and B143 (C), A4142 (D), A202 (E), A210 (F), B170 (G), and B148 (H) progenies

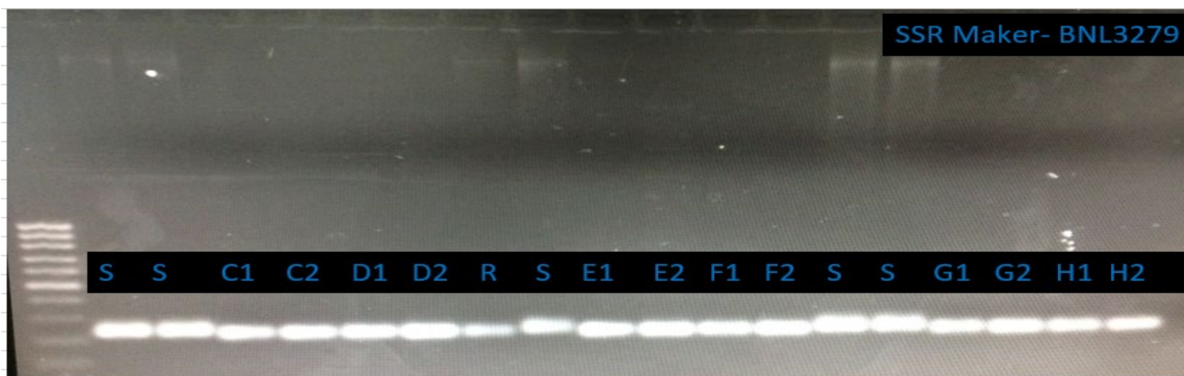
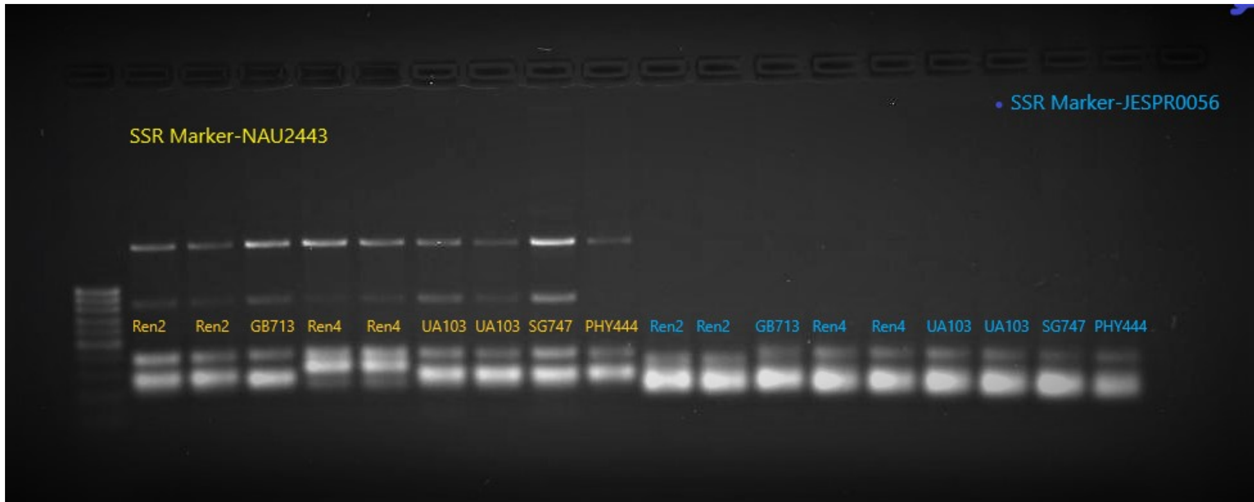


Figure 12. Cotton genotypes in agarose gel for chromosome 18 primers



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