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The Response of Gossypium spp. to Biotic and Abiotic Stresses in Louisiana and the Modeling of Yarn Performance

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THE RESPONSE OF *GOSSYPIUM* SPP. TO BIOTIC AND ABIOTIC STRESSES IN LOUISIANA AND THE MODELING OF YARN PERFORMANCE

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This dissertation is dedicated to the memory of my late grandmother, Maya Bhandari, and to my beloved family.

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ABSTRACT

Developing improved cotton cultivars depends on how cotton cultivars perform the best when under stresses. Reniform nematode is a major plant pathogen, causing 4-6% yield loss in southern United States. A variation in reproduction and pathogenicity across reniform isolates collected from Louisiana on susceptible cotton was reported. This study was conducted to determine the response of resistant/tolerant cotton genotypes to multiple reniform isolates by inoculating 10,000 juveniles into seven days old seedlings. Across genotypes, the Evan and Avoyelles isolates had significantly higher vermiform nematodes (33,793 and 27,800/250 g soil, respectively) than other isolates. Across isolates, the number of juveniles on A_2-190 and Lonren-2 (5,573 and 6,013, respectively) were significantly lower than that on other genotypes. There was a significant interaction between the genotypes and isolates suggesting that the response of genotypes to reniform isolates was different.

Salt stress is a major abiotic stress, affecting cotton production in the Macon Ridge and Red River regions in Louisiana. In a preliminary study, 150 day neutral primitive cotton accessions were screened at 0, 125, 250 mM NaCl under hydroponics. A promising subset was rescreened for salt tolerance in pot culture. MT11 had the lowest reduction in plant height and dry shoot weight (32% and 47%), significantly less than FM958 (43% and 66%) across salt concentrations. MT1219 had the lowest accumulation of Na^+ (1,026.37 mM) at 250 mM NaCl, and significantly lower than FM958 (2,135.39 mM). Based on reduction in plant parameters, MT11, MT1219, MT45, and MT245 performed better than other genotypes. This study also showed that both hydroponics and pot culture are effective in the screening of a large number of cotton genotypes against elevated salt concentrations.

In addition to stresses, cotton breeders are interested to develop a selection index, which aids in an efficient selection of multiple fibers traits. Using the data mining techniques, all developed models agreed that fiber length and strength are the most important fiber properties in determining the spinning consistency index (SCI). This study showed that SCI can be used as alternative selection index for combining the multiple fiber traits to enhance yarn spinning.

CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

Cotton (primarily *Gossypium hirsutum* L. and to a lesser extent *G. barbadense, G. arboreum,* and *G. herbaceum*) is the leading natural textile fiber as well as one of the most important oilseed crops in the world. In terms of total area harvested, cotton ranks fourth after corn, soybean and wheat in the United States. Globally, US cotton production is ranked third after China and India. In the US, it is estimated that 16.08 million bales were produced in 2014/2015, which is 25% higher than in 2013/2014. The production increase in 2014 vs 2013 is largely a result in an increase in production area from 3.05 to 3.93 million hectares (USDA, 2014). As an oilseed, cotton is also ranked in the third position, worldwide, in terms of volume behind soybean and corn. The oil produced from cotton is largely used for human consumption. The cake left after oil extraction is a high protein animal feed principally used in the beef and dairy industries (National Cottonseed Products Association, 2014). Collectively, these uses contribute to cotton's prominence as one of the most important agricultural row crops in the US.

Reniform nematode (*Rotylenchulus reniformis*) is a significant pathogen in upland cotton production and causes an estimated 1.48% yield loss in the United States. In the southern United States, i.e., Louisiana, Arkansas, Georgia, Texas, and Tennessee, more severe losses (>4%) were observed in 2013 (Lawrence et al., 2014). The loss caused by reniform nematode may be exaggerated under water-stressed conditions, while foliar symptoms may not appear in well-managed cotton fields (Robinson, 2007). Symptomatically, reniform nematode infection reduces seedling growth at early stages (2-3 leaf stages), which results in severe stunting (pathogenicity). In addition, it causes a yellowing of lower leaves, a 1-2 node delay in fruit set a browning of the

lower leaf margins and tips that result in a delay in maturity, and yield reduction (pathogenicity) (Birchfield and Jones, 1961; Jones et al., 1959).

The reniform nematode is a sedentary semi-endoparasite, which feeds on more than 350 plant species across 77 families in warm temperate, sub-tropical, and tropical regions of world (Dasgupta and Seshadri, 1971; Gaur and Perry, 1991). Unlike the root-knot nematode (*Meloidgyne incognita*), the infective stage is the immature female, which penetrates and disrupts the cortex cells as it moves into a root and establishes a feeding site on the stele (Bird, 1984). While feeding on the endodermis, it produces a multinucleated cell resulting from cell wall dissolution and hypertrophy without hyperplasia of pericycle cells, which is known as a syncytia (Cohn, 1973; Heald, 1975). Due to disruption of the cortex and dissolution of pericycle cells, reniform nematode infestation hinders the movement of water and nutrient throughout the root system. After establishing a feeding site, the reniform nematode develops further and forms the typical kidney shape. Reproduction is by amphimixis resulting in a lay of 60-200 eggs in a gelatinous matrix outside of the root. Male reniform nematodes have a less developed stylet and oesophageal glands than females and can't feed and produce syncytial cells (Bird, 1984; Gaur and Perry, 1991; Leach et al., 2009).

The geographical infestation and intensity of reniform nematodes in the Cotton Belt has been rapidly increasing over years. In Louisiana, reniform nematode is well established in most of the cotton producing parishes. Over a period from 1961-2010, reniform infestation has increased from three to twenty four parishes (McGawley et al., 2010). Since an active horizontal movement of reniform nematode is minimal (2 meters per year), it is believed that the rapid infestation is due to cotton monoculture and movement of equipment from infested fields to other fields (Moore et al., 2010a; Robinson, 2007). Once it is established, the reniform nematode

can spread horizontally and vertically throughout a cotton field by tillage and water flow (Moore et al., 2010a). To manage the reniform infestation in cotton fields, cotton growers have implemented various management practices, such as crop rotation with non-host species, use of nematicides, planting of tolerant or resistant cotton varieties, sometimes combined with site specific management (Burris et al., 2010; Davis et al., 2003; Lawrence and McLean, 2000; Lawrence et al., 1990; Rich and Kinloch, 2000; Robinson, 2007; Starr et al., 2007; Wolcott et al., 2005). Until recently, aldicarb (Temik) was a cheap, effective, and widely used nematicide to suppress the reniform nematode population in the reniform infested fields, but its usage was restricted after 2014 due to concerns about its acute toxicity. At present, there are no commercial reniform resistant/tolerant cotton varieties available for cotton growers. The use of a reniform resistant/tolerant cotton genotype would be an alternative and economically viable management option to manage reniform nematodes in the infested areas.

With the increasing prevalence of field infestation with reniform nematodes and its elevation to being a primary pest for cotton in recent years, cotton breeders initiated the evaluation of cotton germplasm accessions to identify a source of reniform resistance. Yik and Birchfield (1984) evaluated four different cultivated and wild species of the genus *Gossypium* and found that *G. longicalyx*, collected from Africa, has an immune response to reniform nematode. They also reported that *G. barbendense* 'Texas 110' demonstrated a high degree of resistance. Bell et al. (2014) developed two highly reniform resistant lines: Lonren-1 and Lonren-2 using a hexaploid bridging strategy to incorporate the diploid *G. longicalyx* source of resistance into a tetraploid upland cotton background. Robinson et al. (2004) evaluated the entire collection of Pima (*G. barbadense*) and upland primitive cotton accessions and found that GB-713 was highly tolerant to infestation by the reniform nematode. The study also found that most

upland cotton accessions were moderately to highly susceptible to the reniform nematode. Stewart and Robbins (1994) reported that the Old World cultivated diploid cotton *G. arboreum* (A_2-190) was highly tolerant to the reniform nematode. As a result of the long standing interest in reniform nematode resistance, most of the wild and cultivated diploid and tetraploid cotton species, such as *G. hirsutum, G. longicalyx, G. barbadense. G. herbaceum, G. somalense, G. aridum,* and *G. african* have been evaluated for their reaction. Currently, the Lonren-1, Lonren-2, GB-713, and TX-110 sources of resistance are the most commonly used sources in cotton breeding programs.

Historically, research on the reniform nematode has been conducted using only a single isolate collected from a specific geographical region of US, typically a locally infested field. However, variations in both morphological and genetic, as well in reproduction and pathogenicity, of the isolates have been observed (Agudelo et al., 2005; Arias et al., 2009; Dasgupta and Seshadri, 1971; McGawley et al., 2010; Tilahun et al., 2008). Based on reproduction on host species, Dasgupta and Seshadri (1971) designated race A and race B of the reniform nematode in India. More recently, four races of reniform nematode were reported in India (Singh and Azam, 2011). Agudelo et al. (2005) reported a variation in morphology and reproduction among reniform populations and found that a population collected from Texas had the highest reproduction. McGawley et al. (2010) reported that reniform nematode populations collected from Mississippi and Louisiana had a higher level of reproduction than other populations. It is now well established there is a variation in reniform nematode populations collected from different states. However, there is still a lack of information about variation in reproduction and pathogenicity among reniform isolates collected within Louisiana. Common to all these prior studies as well is that when cotton was used as a host species that only a single or

a very few genotypes were used and that variation in species source of resistance (e.g. *longicalyx, barbadense, hirsutum*) was not incorporated. This study seeks to investigate how the source of reniform nematode resistance/tolerance in cotton genotypes interacts across different reniform isolates collected from different cotton production regions in Louisiana. Results may enable the identification of cotton genotypes (sources of resistance), which display a favorable reaction across reniform isolates that could be used to develop reniform resistant cultivars in a cotton breeding program.

In addition to biotic factors, abiotic stresses, such as drought and salinity are major environmental limiting factors, which affect the growth and productivity of crop species. Soil salinity is one of agriculture's major abiotic stress factors, affecting 23% and 20% of the total irrigated land in the US and the world, respectively (Ghassemi et al., 1995; Wang et al., 2003). Salinity is a severe problem in areas of high evaporation and low rainfall, i.e. arid and semi-arid regions (e.g. Southwest and West regions in US). In these regions, rainfall is not enough to leach accumulated salts out from soil surface, which results in rapid accumulations (Bernstein, 1975; Brady and Weil, 2009). In Louisiana, salinity is a problem in the Upper Red River and Macon Ridge regions where cotton is one of the major crops grown. In these regions, water quality is one of the major issues with irrigation water from Red River, which contains nearly 2600 ppm salt (Morgan, 2010).

Although cotton is moderately tolerant to salinity with a threshold of 7.7 dSM^{-1} (4,928 ppm) (Maas and Hoffman, 1977), the effect of salt concentrations on the growth and development of cotton during different growth stages may be observed in these regions. Due to a long spell of dry weather during the growing season in recent years, cotton growers are irrigating fields through surface or sprinkler irrigation to supplement the water requirements at critical

stages of cotton growth. In these regions, 40% of cotton fields are irrigated and the percentage is likely to increase to maximize production and reduce a risk of crop failure. It is likely that irrigating cotton fields with elevated saline water from Red River will increase the accumulation of salt residues on the soil surface over time, exacerbated because of a shallow hard pan and poor drainage in these areas. The increased use of the Red River as a source of irrigation water is due to high salt concentrations in the ground water and high cost of well pumping, and salt levels in the irrigating water have been increasing over the last 20 years in this regions (Morgan, 2010). As irrigation becomes more prevalent in cotton production, salinity might become a significant issue in the near future, which will need to be managed either through the soil reclamation /management practices or through the development of salt tolerant genotypes. Though soil salinity can be temporarily reclaimed by crop management practices to some extent, use of improved salt tolerant cotton genotypes would be an alternative and economically viable management option to manage cotton production in the salt affected regions.

Salt concentrations in the soil surface impair the absorption of macro- and micro-nutrients required for plant growth and development. The increased concentration of Na^+ and Cl^- within the plant system may partially or fully inhibit the metabolic, physiological, and biochemical processes, and all these effects together reduce plant growth and development at different developmental stages (Hasegawa et al., 2000; Munns and Tester, 2008). It has been well documented that salinity reduces seed germination and emergence, primary and secondary root growth, plant height, fresh and dry shoot weight, shoot/root ratio, and stem thickness, and all these effects together cause in dwarf plants with necrosis and chlorosis of old leaves in many crop species (Chen et al., 2010; Hamdy et al., 1993; Khan et al., 1995; Latif and Khan, 1976; Reinhardt and Rost, 1995; Wang et al., 2011; Ye et al., 1997; Younis et al., 1987). Munns and

Tester (2008) reported two distinct growth responses resulting from elevated salt concentrations: a rapid decrease in growth due to sudden exposure to high salt concentrations (external osmotic pressure) followed by a slow response as the Na^+ accumulates in the leaves. A reduction in leaf expansion is a direct result of salt stress because increased external osmotic pressure causes a rapid loss in cell turgidity, which results in a rapid reduction of shoot growth (Wang and Nii, 2000). The shoot growth reduction causes a delay in emergence of leaves and lateral buds, which reduces the number of lateral branches (Munns and Tester, 2008). In term of production, cotton yield is reduced as the salt concentration increases due to higher boll shedding and lower number of fruiting branches (Chen et al., 2010; Longenecker, 1974). Additionally, excess salinity has been shown to reduce lint percentage, fiber fineness, maturity, length, strength, and micronaire, which combine to reduce fiber quality (Ashraf and Ahmad, 2000; Korkor et al., 1974; Longenecker, 1974).

Exclusion of Na^+, ion regulation and compartmentalization, osmotic adjustment, induction of antioxidants, and synthesis of solutes are well known salt tolerant mechanisms observed in many plant species (Munns and Tester, 2008; Parida and Das, 2005). Janardhan et al. (1976) reported Na^+ exclusion in salt tolerant Indian cotton varieties, which prevents Na^+ accumulation to toxic levels in the leaves. At a cellular level, compartmentalization of Na^+ into the vacuoles from the cytosol through a Na^+/H^+ anti-transporter was observed in Avp1 expressing cotton genotypes (Pasapula et al., 2011). For cotton breeders, identification of inter- and intra-specific sources of variation and the identification of the mechanisms of salt tolerance across accessions are important to the development of salt tolerant cotton cultivars. Compared to the total number of germplasm accessions in US cotton germplasm collection, even

cumulatively, the number of cotton germplasm lines included in past studies for screening and characterizing their salt tolerance is quite low.

With regard to salt tolerance, there is scant data available on the variation in the cotton germplasm pool. The lack of information hinders efforts to understand the mechanism of salt tolerance and to select appropriate salt tolerant cotton genotypes for use in the development or breeding of salt tolerant cotton varieties. There is a need for more systematic studies of salt tolerance response over a larger number of germplasm accessions to provide the foundation upon which to develop salt tolerant cotton cultivars. This study provides an opportunity to identify the degree of salt tolerance among one hundred fifty genotypes obtained from the Mississippi Converted Race Stock program. The information collected, in regard to salt tolerance will be available in the National Cotton Germplasm collection so that cotton breeders can use this information to develop and improve the salt tolerant cotton cultivars.

In addition to biotic and abiotic stresses, cotton breeders from public and private institutions are interested is to develop high yielding cotton varieties with improved fiber qualities to meet the requirements of standard yarn properties. The improved fiber quality is a key to success in the competitive global textile industries. Knowledge of the relationship between yarn and fiber properties is important for cotton breeders to select high quality genotypes/offspring in the breeding program. In the textile industry, yarn quality is a vital component which determines the quality of fabric and clothes (Zhu and Ethridge, 1996).

High volume instruments (HVI) and Advanced Fiber Information System (AFIS) are widely used instruments in selection of high quality cotton bales in the textile industry (Sasser, 1981; Shofner et al., 1990). For cotton breeders, HVI is the most popular tool in selection of progenies and cultivars with high quality fibers because a large number of fiber samples can be

processed in short periods of time at low cost (Suh and Sasser, 1996). Although various fiber properties are determined by using HVI and AFIS, it is still challenging to give priority to a parameter or group of parameters to select the best fibers for industrial uses (Majumdar, 2010). The interrelationship between the various HVI and AFIS parameters is not represented and it is their interplay, along with spinning equipment variables that lead to the production of usable yarn. In essence, some sort of selection index could be useful if it was able to reasonably and reliably predict yarn quality. Two recent attempts to develop such an index, based on the HVI data and consultation with the textile professionals, are the fiber quality indices: Qscore 1 and Qscore 2. Although these indices were developed as a single index incorporating four different fiber properties, most cotton breeders hesitate to use this score in their breeding program because this algorithm gives an arbitrary weight for each fiber property and the optimum weight of each fiber property in relation to yarn quality is still unknown (Bourland et al., 2010).

With an advancement of computational and analytical tools, a number of data mining and machine learning techniques, such as multiple linear regression, path analysis, regression tree, random forest, boosting and artificial neural network, are increasingly popular and widely used to develop predictive models for simple to complex data in many scientific disciplines (Breiman, 2001; Gurney, 1997; James et al., 2014; Kang et al., 1983; Kutner et al., 2004). There are limited studies in the application of other data mining tools and techniques in cotton breeding. Since as early as 1980, cotton breeders have investigated two data mining and machine learning techniques, such as classical linear regression and artificial neural network (ANN) to determine the functional relationship between yarn and fiber properties (Cheng and Adams, 1995; Ramesh et al., 1995). The varieties used in these older experiments and their limited data sets may no longer be relevant. Additionally, none of the published classical linear regression and ANN

models used AFIS data. From HVI it is possible to calculate a spinning consistency index (SCI), which suggests the overall quality and spinning ability of cotton fibers and can be used to evaluate the technological value of cotton fibers. Unfortunately, this index is a "black box" for the cotton breeder, as the research that led to its development provides little rationale about how and what fiber parameters were considered in its development and SCI's ability to predict yarn properties. Therefore, the objective of this research is to develop a number of statistical models using data mining and machine learning tools to identify the important fiber properties, which affects SCI and to compare this index with yarn strength to determine its applicability in the textile industries.

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CHAPTER 2: RESPONSE OF FIVE RESISTANT/TOLERANT COTTON CULTIVARS TO ISOLATES OF *ROTYLENCHULUS RENIFORMIS* COLLECTED FROM RENIFORM INFESTED FIELDS OF LOUISIANA

2.1 INTRODUCTION

Cotton (primarily *Gossypium hirsutum* L. and to a lesser extent *G. barbadense, G. arboreum,* and *G. herbaceum*) is the leading textile fiber as well as one of the most important oilseed crops in the world. In terms of total area harvested, cotton ranks fourth after corn, soybean and wheat in the United States. Approximately two-thirds of the cotton grown in the US is exported, amounting to 10.50 million bales (500 lbs lint/bale) in 2012/13. Exports have been steadily rising as a percent of total production largely due to strong demand from China (USDA, 2014). Globally, US cotton production is ranked third after China and India. In the US, it is estimated that 13.19 million bales were produced in 2013/2014, 24% lower than in 2012/2013. In comparison, world cotton production in 2013/14 (117.81 million bales) decreased 4% relative to 2012/2013. This worldwide production decrease is a direct response to a decrease in planted area from 34.13 to 33.12 million hectares in 2013/14 to 2012/13, respectively. In the US, the production decrease in 2013/14 vs 2012/13 is largely a result of a decrease in production area from 3.79 to 3.10 million hectares (USDA, 2014). As an oilseed, cotton is ranked third, worldwide, in terms of volume behind soybean and corn. The oil produced from cotton is largely used for human consumption. The cake left after oil extraction is a high protein animal feed principally used in the beef and dairy industries (National Cottonseed Products Association, 2014). Collectively, these uses contribute to cotton's prominence as one of the important agricultural row crops in the US.

Cotton is vulnerable to several plant insects and diseases that decrease production. Out of 12% loss in cotton production caused by various insects and diseases, the loss caused by

reniform nematode (*Rotylenchulus reniformis*) is estimated to be 1.48% in the US (Lawrence et al., 2014). The most severe yield losses (> 4%) to reniform nematode are observed in Louisiana, Arkansas, Georgia, Mississippi, Texas, and Tennessee (Lawrence et al., 2014). Depending upon the level of infestation, cultivars grown, and environment conditions, yield losses caused by reniform nematode have been estimated to be as high as 40% (Farias et al., 2002). The reniform nematode was first reported as a cotton parasite in Louisiana in 1941 (Smith and Taylor, 1941). Since the initial report of its occurrence in Louisiana, the reniform nematode has spread, increasing from 3 to 24 parishes during the period of 1961 to 2010 (McGawley et al., 2010). Compared to the root knot nematode (*Meloidogyne incognita*), the area infested by reniform nematode has increased rapidly over the years because of its short life cycle (16-22 days), its ability to establish feeding sites along primary, secondary and tertiary roots, as well as its ability to survive in desiccated weather and soil conditions (Gaur and Perry, 1991; Rebois, 1973). Due to its aggressive nature, the reniform nematode out competes root knot nematode populations in cotton fields and has rapidly begun the major nematode pathogen affecting cotton production (Robinson, 2007).

Reniform nematode is a sedentary, amphimictic and semi endoparasite, which feeds on more than 350 plant species across 77 families in warm temperate, sub-tropical, and tropical regions of world (Gaur and Perry, 1991). The mature female is easily identified by her kidney shape, while the male is vermiform in shape and shorter than females. The life cycle of the reniform nematode is comprised of four vermiform stages i.e. eggs, J1, J2 J3, J4 and adults. A mature female can lay from 60-200 eggs in a gelatinous matrix she exudes on the surface of plant roots (Dasgupta and Seshadri, 1971). It takes 7-10 days for eggs to hatch before entering the different vermiform stages, which are demarcated by molting. Upon infection by root

penetration, a multinucleated cell is formed from the dissolution of cell walls between adjacent cells forming a syncytia (Cohn, 1973; Heald, 1975). Upon infestation, the anterior portion of female is embedded in the root, whereas posterior portion remains outside the root surface. After establishing a feeding site in the root cortex, females develop further and form the typical kidney shape (Gaur and Perry, 1991). The life cycle of the reniform nematode normally takes about 16-22 days, but is dependent upon the host species, temperature, and soil conditions (Bird, 1984; Gaur and Perry, 1991; Leach et al., 2009). Host plant symptoms include stunting, yellowing of lower leaves, browning of the lower margins and tips, a delay in maturity, and yield reduction (pathogenicity) (Birchfield and Jones, 1961; Jones et al., 1959).

Cotton growers have various management options available to reduce yield loss due to reniform nematode infestation. These include crop rotation, the use of nematicides or the planting of resistant/tolerant varieties to manage reniform nematodes in the field (Burris et al., 2010; Davis et al., 2003; Robinson, 2007; Starr et al., 2007). Crop rotation with non-host crops, such as peanut, corn, resistant soybean or sorghum is effective in reducing the reniform population (Davis et al., 2003; Gazaway et al., 2000; Koenning et al., 2004). Nematicides are a reliable option for growers because they are easy to apply at the time of planting and effectively reduce initial nematode population densities (Lawrence and McLean, 2000; Lawrence et al., 1990; Rich and Kinloch, 2000; Wolcott et al., 2005). However, there are environmental concerns associated with nematicide use and they can be expensive. Host plant resistance is an effective, viable, and typically profitable management option to manage and control nematode infestations in cotton fields. To date, several cotton germplasm lines that show moderate to high levels of resistance or tolerance to the reniform nematode have been released (Bell et al., 2014; McCarty et al., 2013; McCarty et al., 2012; Robinson et al., 2004; Robinson and Percival, 1997; Yik and

Birchfield, 1984). No commercial cultivars that have high level of resistance to reniform nematode are available.

Due to increasing infestation of the reniform nematodes in cotton fields, researchers started screening wild and cultivated species of cotton genotypes to identify a source of resistance for reniform nematode in late 1980. Yirk and Birchfield (1984) evaluated four different species of *Gossypium* and found that the germplasm line TX-110 was highly tolerant to reniform nematodes. Robinson et al. (2004) screened 1866 primitive accessions of *G. hirsutum* and 907 of *G. barbendese* against reniform nematodes. They reported that a majority of the *G. hirsutum* accessions were moderate to highly susceptible, while six primitive accessions of *G. barbendese* were moderately tolerant to reniform nematode. Out of these six accessions, GB-713 was highly tolerant to reniform nematodes and has been widely used to develop reniform resistant breeding germplasm. Bell et al. (2014) developed two highly reniform resistant lines; Lonren-1 and Lonren-2 by introgression of a source of reniform resistance from *G. longicalyx* into upland cotton. Stewart and Robbins (1994) evaluated Asiatic cotton germplasm and found that *G. arboreum* (A_2-190) was highly tolerant to reniform nematodes. Although moderate levels of reniform resistance were observed in wild species of *G. aridum* and *G. herbaceum*, they are not extensively used for breeding because of genetic incompatibility and linkage drag.

Past research on the reniform nematode was conducted by using a single isolate collected from a specific geographical region of US, typically a locally infested field. However, variations in both morphological and genetic, as well in reproduction and pathogenicity of the isolates, have been observed (Agudelo et al., 2005; Arias et al., 2009; Dasgupta and Seshadri, 1971; McGawley et al., 2010; Tilahun et al., 2008). Dasgupta and Seshadri (1971) designated two races of reniform nematode, i.e. race A and race B, based on host assay and the rate of reproduction on

castor, cowpea and cotton in India. Out of ten isolates, nine isolates of similar morphology reproduced on all three hosts, while one isolate reproduced only on cowpea. In Japan, Nakasono (2004) classified the reniform nematode into three categories: small, medium, and large based on body size and three different biological types, i.e. male-numerous type, male-rare type, and male-absent type. Rao and Ganguly (1998) reported a variation in body length and width, stylet length, distance from head to vulva, and position of the dorsal esophageal gland orifice among reniform populations from different geographic regions in India. Agudelo et al. (2005) observed variation in nematode morphology and reproduction among isolates collected from different geographical regions. They reported that a reniform population collected from Hawaii has a larger body than other isolates, while a population collected from Limestone, Alabama has a small body size. Morphological variations i.e. size and length of stylet, position of esophagus gland orifice, and esophagus length were also observed among reniform populations. The population collected from Limestone, Alabama had a higher rate of reproduction on the hosts than isolates collected from Huxford, Alabama, Louisiana, and Hawaii.

Based on the 18S ribosomal DNA and first internally transcribed space (ITS1), genetic variation was observed among the populations collected within reniform infested fields of Alabama (Tilahun et al., 2008). Arias et al. (2009) reported that 88 microsatellite markers are polymorphic across six isolates collected from Texas, Louisiana, Mississippi, and Georgia. The isolate collected from Georgia had the highest reproduction and pathogenicity as compared to other isolates. McGawley et al. (2010) showed that reniform populations collected from Mississippi and Louisiana had higher reproduction than populations collected from Arkansas, Texas, Hawaii, and Alabama. A common feature of all of these studies, however, is that the

reproduction and pathogenicity tests were conducted upon a single host genotype (but not necessarily the same one across the studies).

It is now established that there is variability in reproduction and pathogenicity among various reniform nematode isolates collected from different US States. The variation in reproduction and pathogenicity may have an impact on host plant resistance management. It is unknown if there is variation among reniform nematode isolates collected from reniform infested fields within Louisiana. Furthermore, if variation does exist, is it detectable by the use of different host genotypes of the same genus. It would be valuable to establish a differential response of resistant/tolerant lines of cotton to different reniform isolates if such variation exists. Therefore, this study seeks to evaluate the response of tolerant cotton cultivars to reniform nematode isolates collected from reniform infested fields in Louisiana and provide information useful to plant breeders for future research to develop cotton cultivars with resistance/tolerance to the reniform nematode.

2.2 MATERIALS AND METHODS

2.2.1 Reniform isolates and cotton cultivars

Five isolates collected from reniform nematode infested fields in Louisiana were used in this study (Table 2.1). Using a dissecting microscope, 25 egg masses were collected from each isolate and transferred to previously established tomato seedlings (*Lycopersium esculentum* L. cv. 'Rutgers') planted in 20.3 cm (diameter) terra cotta pots filled with steam pasteurized sandy loam soil in a greenhouse under natural light conditions. The reniform isolates were carefully handled and maintained in the greenhouse to maintain isolate purity. Reniform inoculum was extracted on the day of inoculation by using the centrifugal sugar flotation technique (Jenkins, 1964).

Table 2.1 Reniform isolates and cotton genotypes used in this study.

Reniform isolates		Cotton genotypes		
Isolates	Parishes	Name	Sources	References
Evan	Evangeline	Lonren-1	*G. longicalyx*	Bell et al. (2014)
LA	Rapides	Lonren-2	*G. longicalyx*	Bell et al. (2014)
Avoyelles	Avoyelles	Barbren-713	*G. barbadense*	Robinson et al. (2004)
Oak Tree cut	Tensas	TX-110	*G. barbadense*	Yik and Birchfield (1984)
Old Crop rotation	Tensas	A_2-190	*G. arboreum*	Stewart and Robbins (1994)
		Delta Pearl	*G. hirsutum*	

2.2.2 General information

Seed of resistant and susceptible cotton cultivars was planted in 3.8 L plastic pots filled with steam-sterilized sandy loam soil in summer 2013; two seeds per pot. The pots were arranged in randomized complete block design (RCBD) with a factorial arrangement of treatments (reniform isolates and genotypes) and five replications per treatment in the greenhouse. The experiment was repeated in the early fall of 2013. The cotton variety "Delta Pearl" (PVP 20000061, Delta & Pine Land, Co., Scott, MS) was used as the susceptible check. Plants without reniform nematode inoculation were used as controls. After seed germination, pots were thinned to one seedling per plot. At 7 days after germination, 10,000 vermiform nematodes from each isolate were used to inoculate each pot. The inoculum was injected 2-5 cm deep into the soil at three spots 1-2 cm away from the plant stem to facilitate vermiform contact with the host root system. The pots were watered via drip irrigation as required to maintain adequate soil moisture to support the plant growth. Fertilizers and pesticides were applied as needed. The pots were harvested at 9 weeks (63 days) after inoculation. This should allow the reniform nematode to complete at least four complete reproduction cycles.

Before plant harvest, plant height was recorded. Harvested shoot and root of each genotype was oven dried at 65° C for 72 hours and weight was recorded. Soil from individual pots was carefully transferred to a flat plastic pan and any root materials removed from the soil.

After thoroughly mixing the soil, 250 g of soil was taken for extraction of vermiform nematodes using an elutriator (customized by Agriculture Engineering, University of Georgia, 1998) (Byrd et al., 1976). A soil suspension was poured through the elutriator and collected on stacked sieves arranged 100 mesh sieve on the top followed by a 400 mesh sieve. The materials collected on the 400 mesh sieve was transferred into a 50 mL centrifuge tube and centrifuged at 1500 rpm (revolutions per minute) for 5 minutes. The suspension at the top of centrifuge tube was carefully discarded without disturbing the soil pellet at the bottom. About forty (40) mL of sugar solution (450 g sucrose/L) was added and thoroughly mixed in the centrifuge tube. This was then centrifuged at 1500 rpm for 1 minute. The supernatant was quickly poured into 400 mesh sieve and washed thoroughly with tap water. The suspension was collected in graduated sample beakers and adjusted to the final volume of 100 mL. For vermiform counting, 10 mL of suspension was pipetted onto a petri dish having 5 mm cross section lines. Using the dissecting microscope, vermiform nematodes across a cross section (2 or 4 lines) were counted at 4X (or 10X) and multiplied by 800 (counted across 2 cross section line) or 400 (4 cross sectional lines) to calculate the total number of reniform nematodes in 250 gram soil. The number of nematodes in 250 gram of soil was multiplied by 10 to get the total number of vermiform nematodes (pf) in each pot. The reproduction value (Rf) was determined by dividing the final population (pf) by the initial inoculum level (pi).

2.2.3 Statistical analysis

Analysis of variance (ANOVA) were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC) for number of vermiform nematodes per 250 g soil, plant height, dry shoot and root weight. Prior to ANOVA, the number of vermiform nematodes was log transformed to meet an

assumption of normality. To determine the difference among isolates and genotypes, T-grouping was used for mean comparisons.

2.3 RESULTS

2.3.1 Reproduction of reniform isolates on cotton genotypes

The reproduction value of reniform isolates across the different genotypes is presented in Table 2.2. Based on the reproduction on Delta Pearl (susceptible check), the Evan isolate had the highest reproduction value (Rf) followed by the Avoyelles isolate, while the lowest reproduction was reported in the Oak Crop rotation isolate. The reproduction values of reniform isolates on Lonren-1, Lonren-2, and *G. arboreum* (A_2-190) were lower than on TX-110 and Barbren-713 genotypes (Table 2.2).

Table 2.2 The reproduction values (Rf) of reniform isolates across cotton genotypes.

	Reniform isolates				
Genotypes	Evan	LA	Old Crop rotation	Oak Tree cut	Avoyelles
Delta Pearl	107.60	66.80	51.04	61.76	86.08
TX-110	29.52	14.96	20.28	17.00	32.00
Barbren-713	29.32	10.56	13.76	12.72	21.52
Lonren-1	17.72	10.40	11.76	3.12	10.64
Lonren-2	9.36	7.52	8.44	3.36	7.40
A2-190	9.24	4.76	7.08	3.20	9.16

The two nine week duration experiments were combined for analyses of variance because there were not significant differences for a number of vermiform nematodes between two set of experiments. There were significant differences among reniform isolates and genotypes for a number of vermiform nematodes ($P<0.01$). There was a significant interaction between genotypes and isolates for a number of vermiform nematodes implying that there was a differential response of different cotton genotypes across reniform isolates ($P<0.01$) (Table 2.3). This might be expected due to the different sources of reniform resistance genes among the

tested genotypes. Lonren-1 and Lonren-2 are derived from *G. longicalyx*, while Barbren-713 derives its resistance from *G. barbadense* L. accession GB713.

Table 2.3 Number of vermiform nematodes as affected by reniform isolate and cotton genotype.

Source	df	Mean square	F value
Isolate	4	1.52	36.54**
Genotype	5	8.41	201.89**
Isolate x Genotype	20	0.12	2.73**

**=Significant at $P \leq 0.01$.

The Evan and Avoyelles reniform isolates had the highest mean number of vermiform nematodes (33,793 and 27,800/250 g soil, respectively), and both were significantly higher than other isolates (Figure 2.1). The Oak Tree cut isolate had a significantly lower number of vermiform nematodes than the other reniform isolates (16,860/250 g soil), while the LA and Old Crop rotation isolates were intermediates (Figure 2.1).

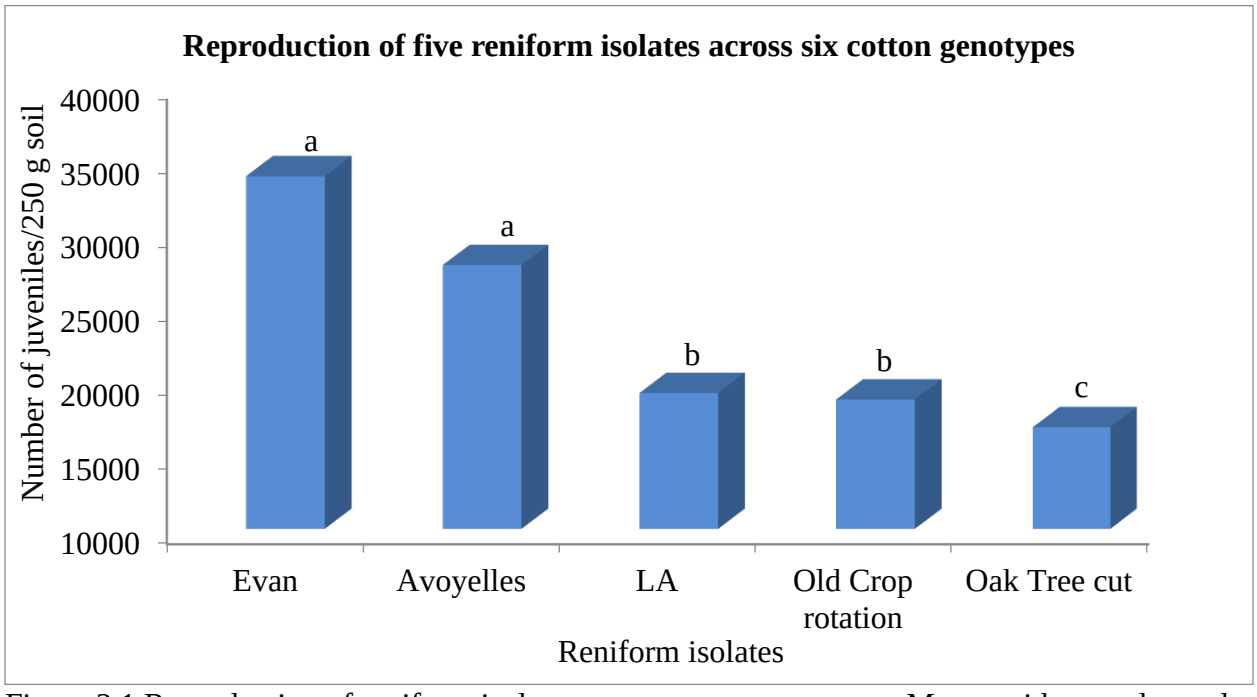

Figure 2.1 Reproduction of reniform isolates across cotton genotypes. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

Across reniform isolates, Delta Pearl had the highest number of vermiform nematodes (74,656/250 g soil) followed by TX-110 (22,752/250 g soil), and Barbren-713 (17,576/250 g

soil) and all were significantly different for number of juveniles from each other (Figure 2.2). Lonren-2 and A_2-190 (diploid cotton) had the lowest number of vermiform nematodes (6,688/250 g soil) (Figure 2.2).

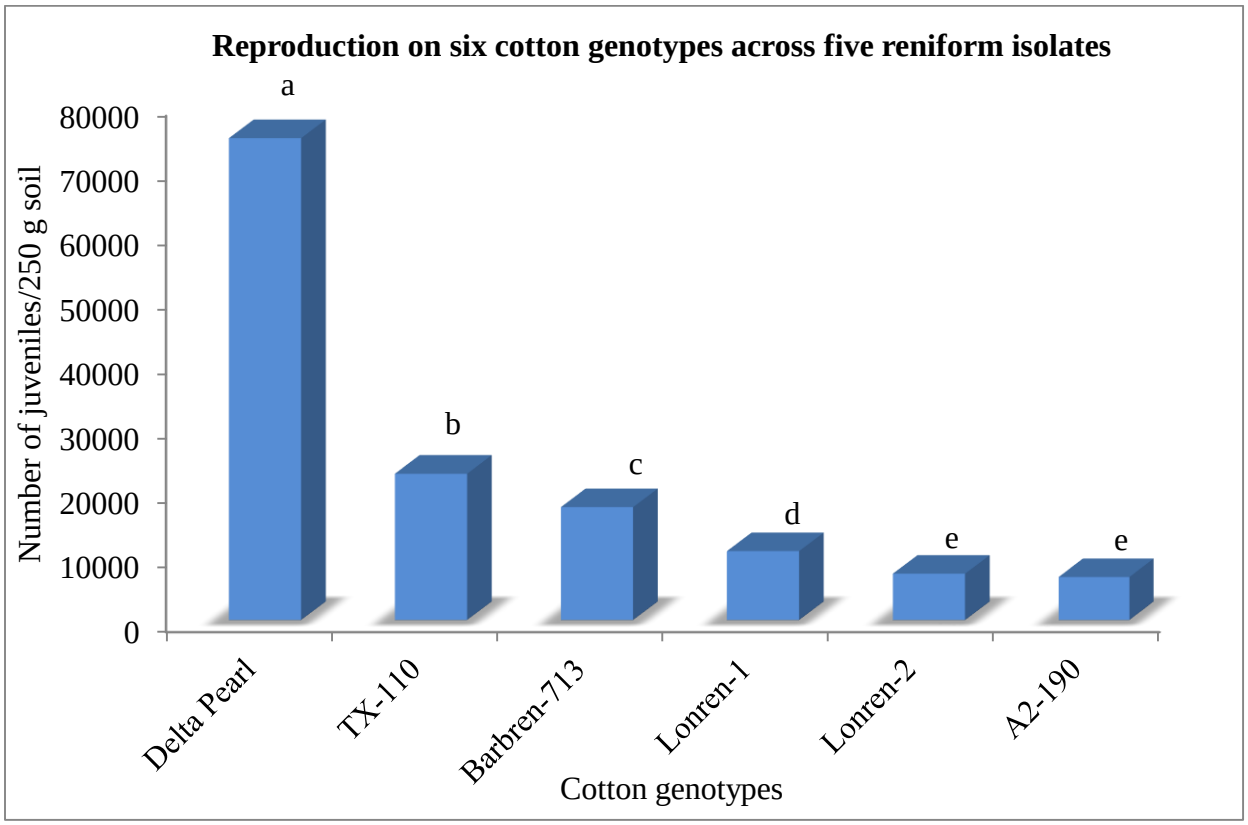

Figure 2.2 Reproduction on six cotton genotypes across five reniform isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

On Delta Pearl, the Evan isolate had the highest rate of reproduction (107,600/250 g soil) followed by the Avoyelles isolate (86,080/250 g of soil) and both were significantly higher than the Old Crop rotation isolate (51,040/250 g soil) (Figure 2.3). In contrast to reproduction on Delta Pearl, the Avoyelles isolate had the highest rate of reproduction on TX-110 (32,000/250 g soil) followed by the Evan isolate (29,520/250 g soil) and both were significantly different from the Oak Tree cut and LA isolates. On Barbren-713, the Evan isolate had the highest number of vermiform nematodes (29,320/250 g soil), but it was not significantly different than the Avoyelles isolate (21,520/250 g soil). The LA isolate reproduced the lowest number of

vermiform nematodes (10,500/250 g soil) and was not significantly different than the Old Crop rotation and Oak Tree cut isolates (Figure 2.3). The data also showed that the differences in number of juveniles of reniform isolates across the cotton genotypes were much wider than the differences in reproduction of reniform isolates within the cotton genotypes.

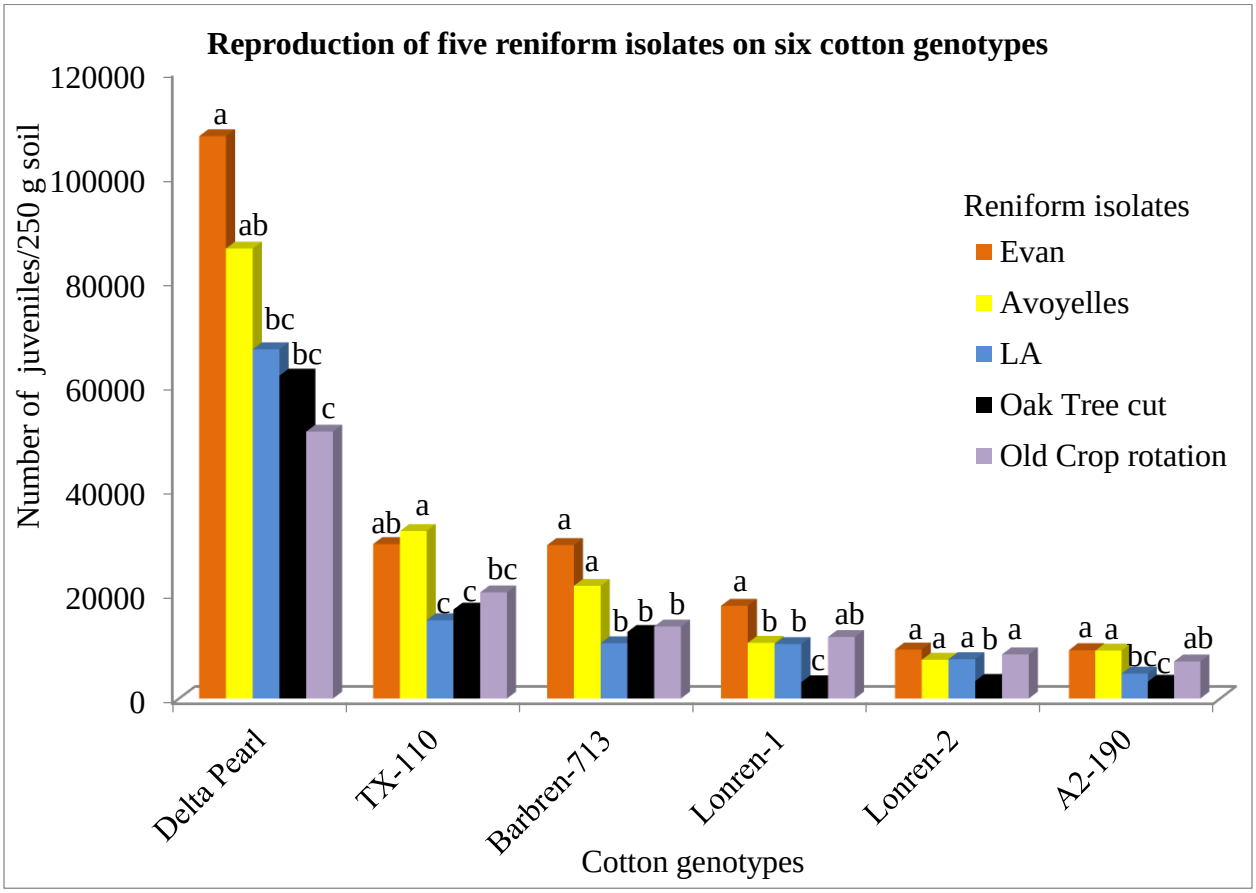

Figure 2.3 Reproduction of reniform isolates on cotton genotypes. Within genotypes, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

On Lonren-1, all reniform isolates reproduced fewer than 20,000 vermiform nematodes. On Lonren-1, the Evan isolate had the highest number of vermiform nematodes (17,720/250 g soil), but was not significantly different from the Old Crop rotation isolate (11,760/250 g soil). The Oak Tree cut isolate (3,120/250 g soil) had significantly lower reproduction than the other isolates (Figure 2.3). All reniform isolates reproduced fewer than 10,000 vermiform nematodes on Lonren-2. The Evan isolate had the highest number of vermiform nematodes on Lonren-2

(9,360/250g soil), but it was not significantly different compared to the Old Crop rotation, LA and Avoyelles isolates. Since both Lonren-1 and Lonren-2 have resistance from *G. longicalyx* source, they demonstrated a similar pattern of response to the different reniform isolates. Lonren-1 and Lonren-2 suppressed reproduction the most of all tetraploid genotypes across all isolates. Even the order of the isolates is generally preserved, although Lonren-2 limited reproduction almost twice as much as Lonren-1. On the diploid cotton genotype, *G. arboreum* (A_2-190), the Evan isolate had the highest reproduction potential (9,240/250 g soil) followed by the Avoyelles isolate (9,160/250 g soil), but they were not significantly different with each other or the Old Crop rotation isolate.

2.3.2 Effect of reniform isolates on plant height

As was true for reproduction, there were significant differences among reniform isolates and cotton genotypes for plant height ($P<0.01$). There was also a significant interaction between the genotypes and isolates for plant height suggesting that there is a differential pathogenicity of reniform isolates across the cotton genotypes ($P<0.01$) (Table 2.4). Mirroring the reproduction numbers, the Evan and Avoyelles isolates reduced plant height the most across the genotypes (Figure 2.4).

Table 2.4 Impact of cotton genotype and reniform isolate on plant height, dry shoot and root weight.

		Plant height		Dry shoot weight		Dry root weight	
Source	df	Mean square	F value	Mean square	F value	Mean square	F value
Isolate	5	2470.91	15.04**	116.80	7.50**	25.27	17.47**
Genotype	5	12265.00	74.65**	408.94	26.25**	63.08	43.60**
Isolate* Genotype	25	418.28	2.55**	21.07	1.35	1.73	1.20

**=Significant different at $P \leq 0.01$.

The controls were significantly taller than the inoculated cotton genotypes averaged over the genotypes. The Old Crop rotation treatments gave the smallest average reduction in plant

height (103.1 cm). Across genotypes, the Evan isolate resulted in the short plant (92.9 cm), followed by the Avoyelles isolate (94.8 cm), but they were not significantly different from each other (Figure 2.4).

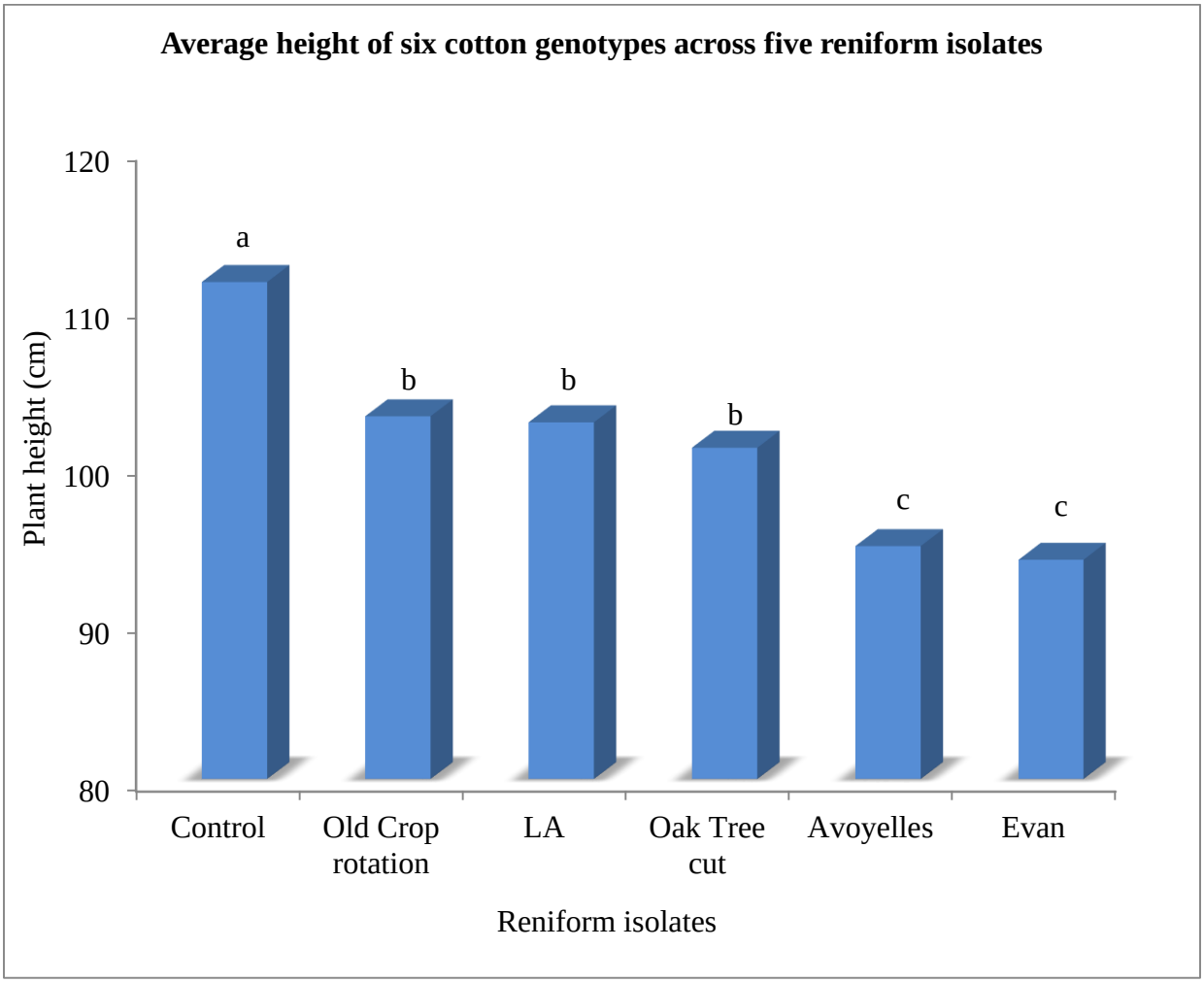

Figure 2.4 Average height of six cotton genotypes across the five reniform isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

The LA isolate reduced plant height of Delta Pearl the most, but was not significantly different from the other isolates except for the control (Figure 2.5). On Lonren-1, Lonren-2, and *G. arboreum* (A_2-190), the Evan isolate reduced the plant height the most followed by the Avoyelles isolate, and both were significantly shorter than the control. There were no significant differences among reniform isolates for plant height on Barbren-713 (Figure 2.5).

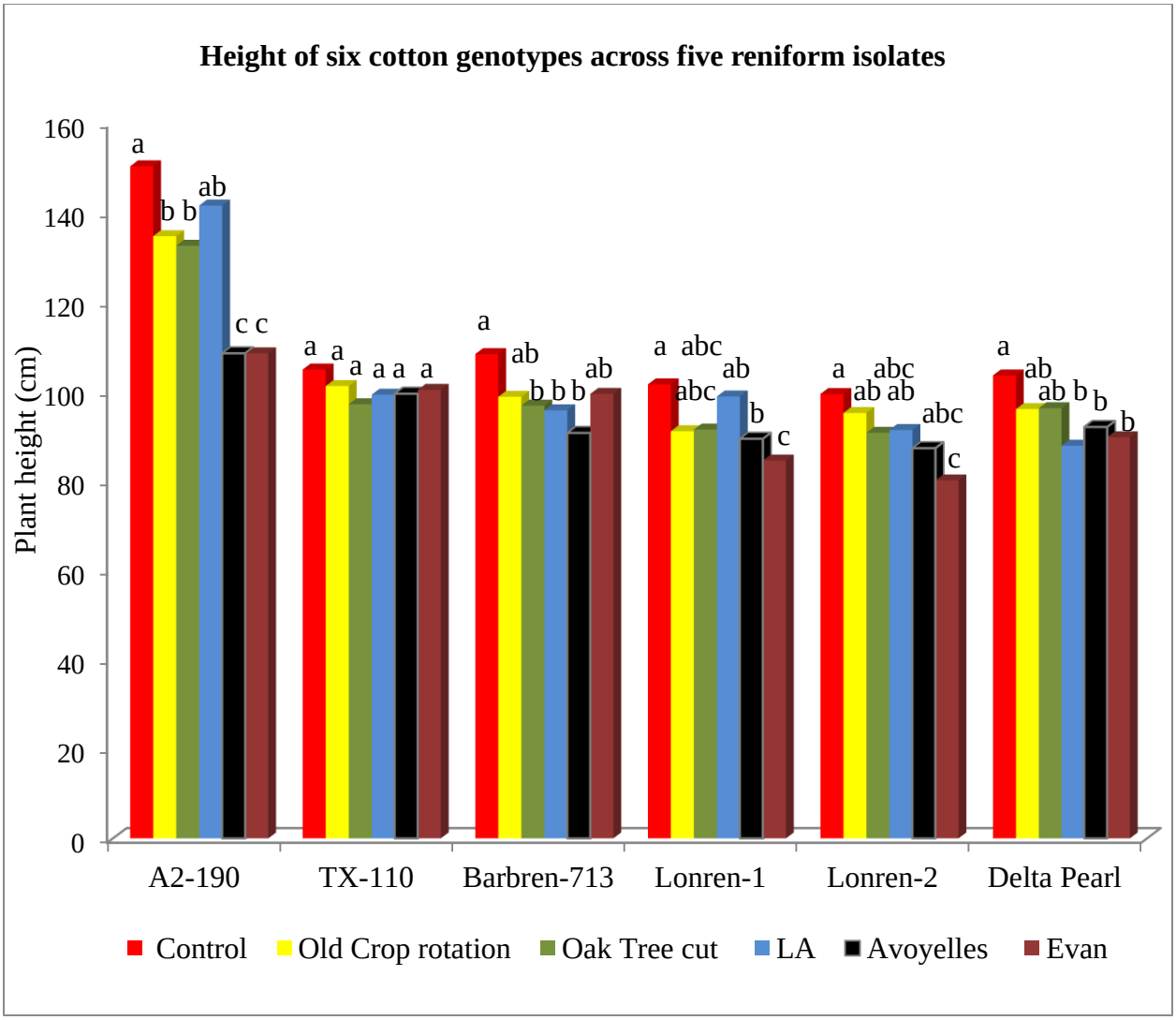

Figure 2.5 Height of six cotton genotypes across five reniform isolates. Within genotypes, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

2.3.3 Effect of reniform isolates on dry shoot weight

There were significant differences among reniform isolates and genotypes for dry shoot weight ($P<0.01$), and the differences were consistent across genotypes ($P=0.12$) (Table 2.4). Average dry shoot weight of the control (18.6 g) was significantly higher than reniform inoculated genotypes (Figure 2.6). The genotypes inoculated with the Avoyelles isolate had the lowest dry shoot weight (14.5 g), but it was not significantly different from the Evan and Old Crop rotation isolates.

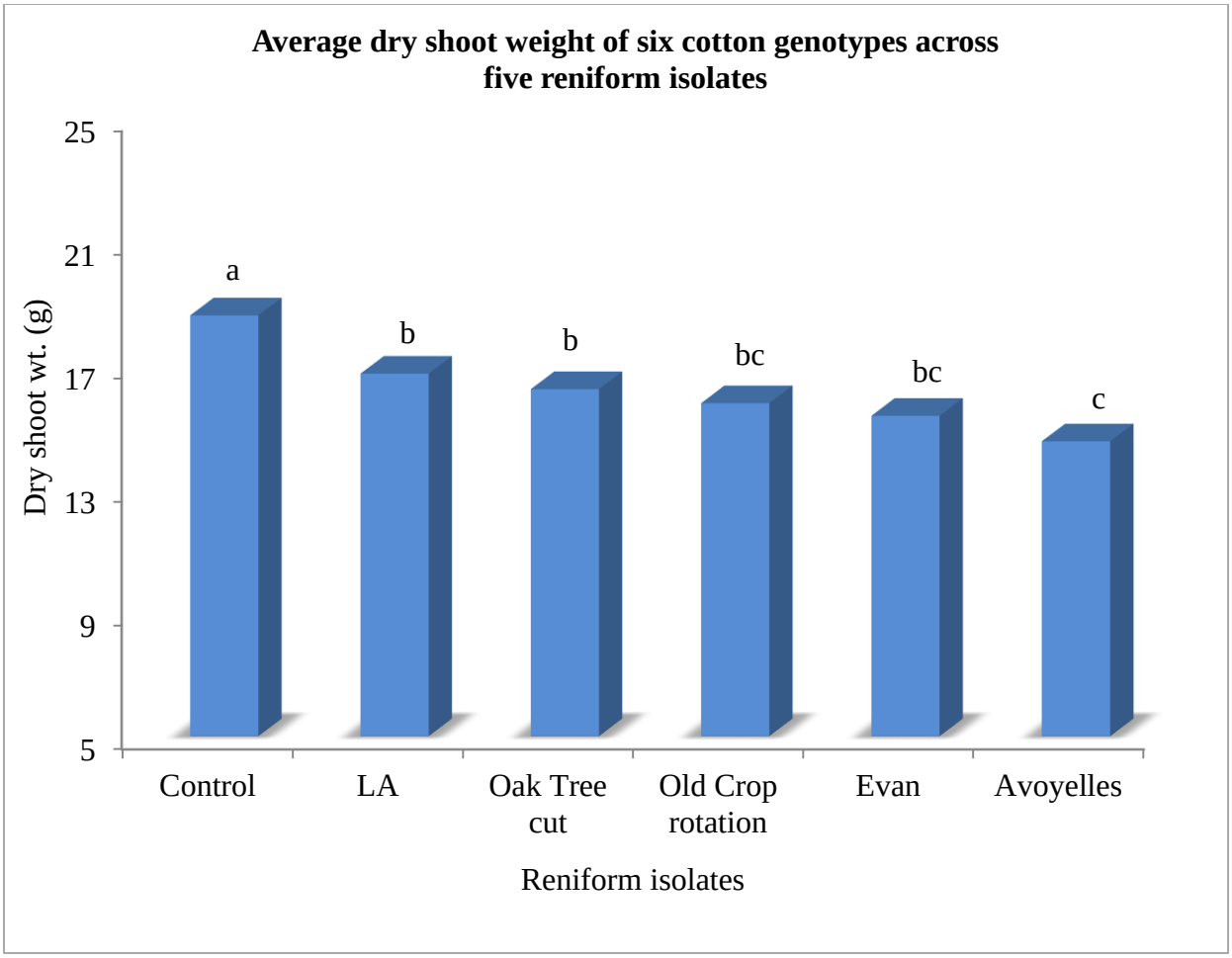

Figure 2.6 Average dry shoot weight of six cotton genotypes across the five reniform isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

2.3.4 Effect of reniform isolates on dry root weight

There were significant differences for dry root weight among reniform isolates and genotypes ($P<0.01$), but no interaction between reniform isolates and genotypes was found ($P=0.24$) (Table 2.4). Across genotypes without reniform nematode infestation (control) was observed the highest dry root weight (4.7 g) and this was significantly higher than for genotypes inoculated with reniform isolates (Figure 2.7). Across genotypes, those inoculated with the Evan isolate had the lowest dry root weight (2.9 g) and were significantly lower than the Old Crop rotation, LA, Oak Tree cut, and Avoyelles isolates (Figure 2.7).

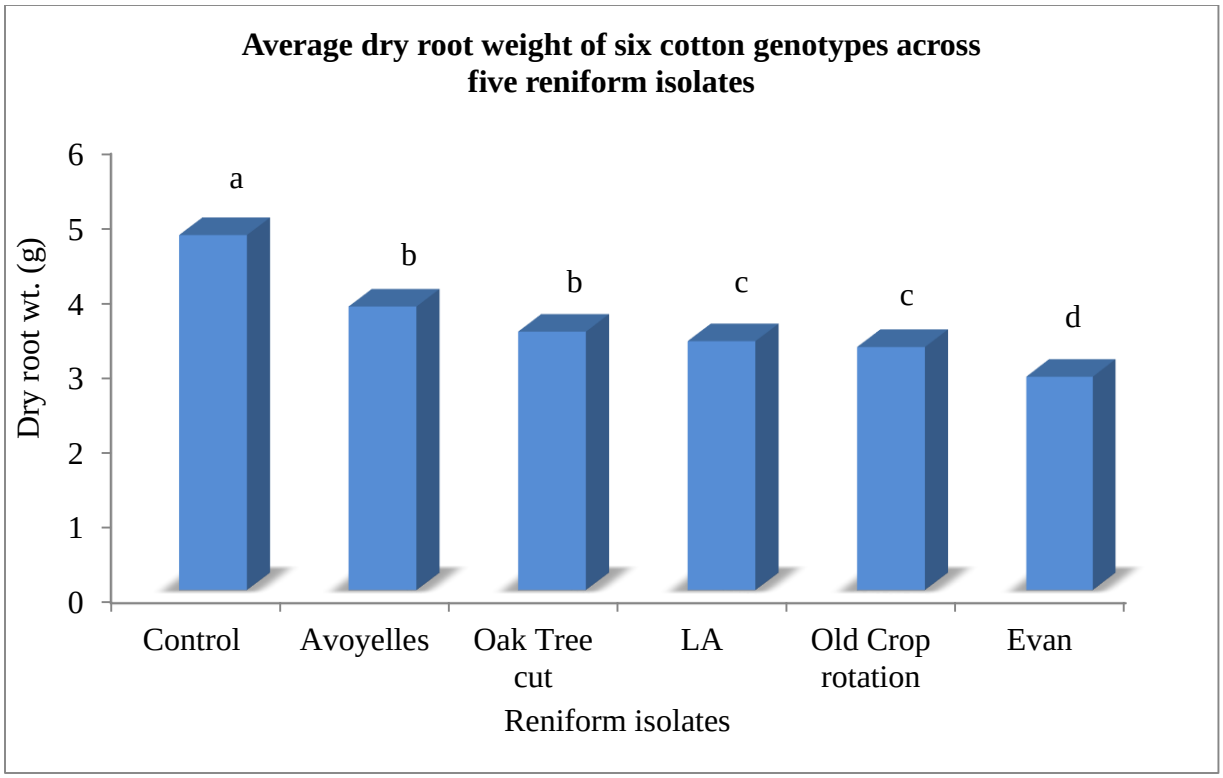

Figure 2.7 Average dry root weight of six cotton genotypes across the five reniform isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

2.4 DISCUSSION

This study revealed significant variation in reproduction and pathogenicity (reduction in plant height, dry shoot and root weight) among reniform isolates collected from cotton fields in Louisiana. The data showed that the response of cotton genotypes reported to be tolerant/resistant were varied for the number of vermiform nematodes across reniform isolates. Variation in reproduction among isolates collected from reniform infested soil might be due to their adaptation to different soil textures under the site-specific crop management system (Koenning et al., 1996; Sturhan, 2012). Differences in reproduction and pathogenicity might occur because of a genetic variation in reniform isolates (Arias et al., 2009; Tilahun et al., 2008). In addition to the polymorphism across the reniform populations collected from different US states, Arias et al. (2009) reported that twenty-two SSR markers showed the polymorphism across three reniform populations collected from reniform infested fields within Mississippi.

Phenotyping and the identification of polymorphic molecular markers within segregating progenies are essential for successful quantitative trait loci (QTL) mapping and eventual marker assisted selection. After identifying reniform resistant germplasm, cotton breeders have been developing mapping populations and identifying QTL linked to reniform resistance loci. Robinson et al. (2007) reported a single dominant gene was associated with reniform resistance in *G. longicalyx*. Dighe et al. (2009) mapped a single dominant QTL locus, designated (Ren^{lon}), on chromosome 11 in *G. longicalyx*. Romano et al. (2009) reported that a single dominate QTL locus (Ren^{ari}) on chromosome 21 is responsible for reniform resistance in *G. aridium*. Gutiérrez et al. (2011) found two major QTLs linked to reniform resistance on chromosome 21 (Ren^{bar1}, Ren^{bar2}) and one minor QTL on chromosome 18 (Ren^{bar3}) in the *G. barbadense* L. accession 713. The underlying assumption in all these studies was that there is no variation among reniform populations regardless of geographic origin and/or that the response of cotton genotypes across reniform isolates is uniform. There is still a lack of information about whether these QTLs are stable across different reniform isolates. In this study, the reproduction of reniform isolates on Lonren-2 and *G. arboreum* (A_2-190) was significantly lower than on other cotton genotypes, Lonren-2 and *G. arboreum* (A_2-190) also had significantly different responses across the multiple reniform isolates. It would be valuable to investigate if QTL map differently for reniform resistance across diverse reniform isolates.

Based on the reproduction potential, cotton fields infested with the Evan isolate are likely to build reniform populations faster than fields infested with the Old Crop rotation or Oak Tree cut isolates. It is anticipated that cotton fields infested with the Evan isolate may require a longer crop rotation with corn, sorghum, resistant soybean or peanut non hosts than fields infested with other reniform isolates to suppress the juvenile's populations. Due to differential reproduction

and host preferences, Kirkpatrick and Sasser (1984) recommended a specific crop rotation scheme for each race of root-knot nematode (*Meloidogyne incognita*) to suppress root-knot populations in cotton. Due to differential rate of reproduction, application rate of nematicides may need to vary to manage the reniform isolates in cotton fields in specific agro-ecological regions. With respect to reproduction, the source of reniform resistance is also important to manage the reniform nematodes in infested fields. Based on nematode reproduction on TX-110 and Barbren-713, improved cotton varieties derived from these two sources are likely to build up the reniform population to an economic threshold level after two growing seasons and is wise to do a crop rotation with corn, sorghum or resistance soybean after two years. Utilization of reniform resistance sources A_2-190, Lonren-2, and Lonren-1 provide better resistance than TX-110 and Barbren-713, but growing resistant cotton year after year may lead to the resistance breaking down to the reniform populations. Although Lonren-1 and Lonren-2 display a hypersensitive reaction at high reniform populations, improved cultivars from these sources can be utilized to manage Oak Tree cut and Old Crop rotation isolates because the reproduction of these reniform isolates on Lonren-1 and Lonren-2 are quite low and may not build up enough juvenile's populations that cause hypersensitivity. Crop rotation with reniform resistant/tolerant cultivars is recommended to manage reniform infested cotton fields because it maintains the reniform population below economic threshold level and reduces the vulnerability to the development of resistance breaking reniform populations in the field. The reproduction and pathogenicity of specific reniform isolates as well as a degree of resistance among cotton cultivars will dictate the type of management needed for acceptable control.

The results of this study justify further investigation into the interaction between the reproduction of reniform isolates on different sources of resistance in cotton. It also implies that

different management strategies may need to be applied to reduce damage from specific reniform nematode isolates that are specific to geographical regions. Furthermore this study suggests that both Lonren-2 and *G. arboreum* (A_2-190) exhibit a high level of resistance regardless of the reniform isolates geographic origin. Within a cotton breeding program, both Lonren-1 and Lonren-2 (both tetraploids) are good sources of resistance and relatively amenable to use though they both, especially Lonren-1, have other agronomic performance deficiencies. The diploid cotton *G. arboreum* (A_2-190) exhibited the highest level of resistance across the reniform isolates, but would be more problematic to use within a breeding program.

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CHAPTER 3: IDENTIFICATION OF DAY NEUTRAL PRIMITIVE COTTON ACCESSIONS TOLERANT TO ELEVATED LEVELS OF SALT CONCENTRATIONS

3.1 INTRODUCTION

Cotton (primarily *Gossypium hirsutum* L. and to a lesser extent *G. barbadense, G. arboreum,* and *G. herbaceum*) is the leading textile fiber as well as one of the most important oilseed crops in the world. In terms of total area harvested, cotton ranks fourth after corn, soybean and wheat in the United States. Approximately two-thirds of the cotton grown in the US is exported amounting to 10.50 million bales (500 lbs lint/bale) in 2012/13. Exports have been steadily rising as a percent of total production largely due to strong demand from China (USDA, 2014). Globally, US cotton production is ranked third after China and India. In the US, it is estimated that 13.19 million bales were produced in 2013/2014, which is 24% lower than in 2012/2013. In comparison, world cotton production in 2013/14 (117.81 million bales) decreased 4% relative to 2012/2013. This worldwide production decrease is a direct response to a decrease in planted area from 34.13 to 33.12 million hectares. In the US, the production decrease in 2013/14 vs 2012/13 is largely a result of a decrease in production area from 3.79 to 3.10 million hectares (USDA, 2014). As an oilseed, cotton is ranked in the third position, worldwide, in terms of volume behind soybean and corn. The oil produced from cotton is largely used for human consumption. The cake left after oil extraction is a high protein animal feed principally used in the beef and dairy industries (National Cottonseed Products Association, 2014). Collectively, these uses contribute to cotton's prominence as one of the important agricultural row crops in the US.

Abiotic stresses, i.e. drought, salinity, temperature, and flooding are major problems in crop production that can reduce yields by 50% (Bray et al., 2000). Of these, soil salinity affects 20% of the total irrigated land in the world (Wang et al., 2003). In the US, soil salinity is a

significant problem in the Southwest and West regions that affects 23% of the irrigated land (Ghassemi et al., 1995). Globally, Wang et al. (2003) predicted that 30% of arable land will be deteriorated by salinity in the coming 25 years and further project that salinity will affect 50% of total arable land by 2050. Over this same period, food production needs to be increased by 38% by 2025 and by 57% by 2050 to supply the current levels of food for a growing world population (Wild, 2003). In the near future, salinity might become a significant enough issue that it will need to be managed for crop production either through the soil reclamation /management practices or through the development of salt tolerant crops and forest trees by breeding techniques to meet food and fiber demands from a growing global population.

Salinity is a severe problem in areas of high evaporation and low rainfall i.e. arid and semi-arid regions. In these regions, the amount of rainfall is not enough to leach salt from the surface resulting in salt accumulations (Bernstein, 1975; Brady and Weil, 2009). Though salt naturally originated from the weathering of parent materials, the accumulation of salts on the soil surface is accelerated by the application of fertilizers, soil amendments, and irrigation with salt-rich water (Chhabra, 1996). The increased availability of sodium ions in the soil surface may depress other macronutrients available for plant absorption and increases the external osmotic potential, which hinders the influx of water into the root system (Grattan and Grieve, 1998). Increased sodium ion concentrations also damage soil structure and cause the dispersal of soil particles, and ultimately reduces the overall soil aeration (Brady and Weil, 2009).

High concentration of sodium ions within the plant system may partially or fully inhibit various metabolic, physiological, and biochemical processes and collectively effect, in a negative manner, plant growth and development at different developmental stages (Hasegawa et al., 2000; Munns and Tester, 2008). *Gossypium* spp. are generally considered to be moderately tolerant to

salinity with an injury threshold of 7.7 dSM^{-1} (Maas and Hoffman, 1977). However, even low levels of salt (concentrations less than 1 dSM^{-1} or 640 ppm) in the surface soil have been shown to affect the growth and development of cotton plants (Ahmad et al., 2002; Ashraf, 2002; Ashraf and Ahmad, 2000; Chachar et al., 2008; Qadir and Shams, 1997; Razzouk and Whittington, 1991). The effect of salinity is more severe when cotton is exposed to salinity for the longer periods (Ashraf and Ahmad, 2000). Seed germination and emergence are both drastically decreased with increasing salt concentrations in the soil (Hamdy et al., 1993; Khan et al., 1995; Latif and Khan, 1976; Younis et al., 1987). Salinity reduces both primary and secondary root growth, vegetative growth, leaf size and expansion, shoot/root ratio, and stem thickness resulting in dwarf plants with necrosis and chlorosis of leaves (Chen et al., 2010; Khan et al., 1995; Reinhardt and Rost, 1995; Wang et al., 2011; Ye et al., 1997). Shoots are more sensitive than roots in response to salinity (Babu et al., 1987). The effects of salinity on older leaves are more prominent than on younger leaves because Na^+ accumulates over time and at higher levels is toxic (Munns and Tester, 2008).

The number of cotton bolls per plant is drastically reduced with increasing salt concentrations due to higher boll shedding and a concomitant decrease in fruit positions (Chen et al., 2010; Longenecker, 1974). Additionally, salinity has been shown to reduce lint percentage, fiber fineness, maturity, length, strength, and micronaire, eventually reducing fiber quality (Ashraf and Ahmad, 2000; Korkor et al., 1974; Longenecker, 1974). At higher salt concentrations in the soil beyond the threshold level for cotton, salinity kills cotton plants completely. Overall, high salinity reduces the economic return of cotton by reducing cotton lint production and the fiber's quality.

In Louisiana, cotton is one of the major crops grown in the Upper Red River region and on the Macon Ridge. The cotton grown in these areas is susceptible to drought due to low rainfall during the growing season and shallow hardpans. In such situations, irrigating the field through surface or sprinkler irrigation methods is the main option to supplement the plant water requirements at critical stages of development. In these regions, 40% of cotton fields are irrigated and the percentage is likely to increase to maximize production and reduce risk. Due to high salt concentrations in the ground water and high cost of pumping, the Red River is the main source of water for growers in the Red River Valley of northwest Louisiana (Branch, 2004).

Water quality is a key factor in irrigation systems, and is determined by amount of salt concentration in the irrigating water. Good quality irrigation water should contain less than 400 ppm salt. In the Red River area, some irrigation water contains nearly 2600 ppm salt; which causes severe injury and limits soybean production (Morgan, 2010). In this region, salt levels in the irrigation water have been increasing over the last 20 years (Morgan, 2010). As irrigation becomes more prevalent in cotton production, salinity is likely to become a more serious problem and the area affected by salinity is expected to rise. To some extent, salinity levels can be managed by surface drainage, leaching or cultural practices. On the other hand, use of salt tolerant cotton cultivars would be an alternative option to manage salt affected production regions. Currently, there are no commercial salt tolerant cotton varieties available for growers.

A logical first step is the identification of variability in response to elevated salt concentrations among cotton plants. To date, there are a limited number of studies seeking to identify salt tolerant cotton germplasm. Abul-Naas and Omran (1974) reported that *G. barbadense* is more tolerant to salt relative to *G. hirsutum*. Bhatti and Azhar (2002) evaluated the root growth of nine cotton cultivars under saline conditions and identified two genotypes as

being the most tolerant. Higbie et al. (2010) evaluated six cultivars of pima and upland cotton and found three genotypes moderately tolerant to salinity. Basel (2011) evaluated five upland cotton genotypes and found three varieties with moderate to high tolerance against salinity. Abbas et al. (2011) screened fifty cotton genotypes against different salt concentrations and identified six genotypes as tolerant. They further reported that salt tolerance traits have a moderate to high genetic variability and are highly heritable. Castillo (2011) screened 209 wild primitive TX accessions using a hydroponic technique and found that the accession TX307 was the most salt tolerant genotypes out of 109 surviving genotypes. Compared to the total number of germplasm accessions in US cotton germplasm collection, even cumulatively, the number of cotton germplasm lines included in these past studies for screening and characterizing their salt tolerance is quite low.

With regard to salt tolerance, there is no comprehensive data available in cotton germplasm pool. The lack of information hinders researcher's efforts to understand the mechanism of salt tolerance and to select appropriate salt tolerant cotton genotypes for use in the development or breeding of salt tolerant cotton varieties. There is a need for more systematic studies of salt tolerance response over a larger number of germplasm accessions to provide the foundation upon which to develop salt tolerant cotton cultivars. This study provides an opportunity to identify the degree of salt tolerance among one hundred fifty genotypes obtained from the Mississippi Converted Race Stock program. In this program to date, 169 photoperiodic primitive accessions collected from Mexico and Central America have been converted to be day neutral through a series of backcrosses with a day neutral donor "Deltapine 16". The day neutral progenies in F_2 were selected and backcrossed four times to their original race stock and day neutral F_2 progenies were selected in each backcross (McCarty and Jenkins, 1993; McCarty and

Jenkins, 2002; McCarty et al., 2004). The day neutral primitive accessions provide a source of genetic variation for agronomic and fiber traits, insect and disease resistance (Knutson et al., 2014; McCarty et al., 1996; McCarty et al., 2006). The information collected, in regard to salt tolerance will be available in the National Cotton Germplasm collection so that cotton breeders can use this information to develop and improve the salt tolerant cotton cultivars.

3.2 MATERIALS AND METHODS

3.2.1 Plant materials

Out of one hundred sixty nine day neutral primitive cotton accessions, one hundred fifty genotypes of *G. hirsutum* (2n=4x=52) were utilized in this study. The 150 lines were obtained from Dr. Jack McCarty, USDA-ARS, and are from the Converted Race Stock program.

3.2.2 Hydroponic technique

A preliminary screening was conducted using a hydroponic system consisting of plastic tubs aerated with an air pump in the greenhouse. The study was conducted in summer, 2013. The flat plastic tubs (64.4 L) with dimensions of 15x45x100 cm were fitted with one aquarium air pump and 2 bubble stones of approximately 90 cm in length per tub. A split plot design with two replications (five plants/replication) was used. A nutrient solution was prepared by dissolving 1 g of Peter® fertilizer (20:20:20), 150 mg of calcium nitrate and 150 mg of magnesium sulfate per liter (Castillo, 2011). To limit algal growth, tubs were painted white to reduce light penetration through the tubs.

Germination towels were used for seed germination. Uniform seven day old seedlings were transferred individually into holes bored into a Styrofoam insulation panel floating on the nutrient solution. The dimension of each hole was 1 cm in diameter and holes are spaced in a 2 cm grid. A fine (1mm x 1mm) nylon mesh was attached to the bottom of the styrofoam to hold

the seedlings more firmly. An X-shaped hole was made in the nylon mesh that works like a valve to prevent root girdling. Four days after being transferred to the hydroponic system, holes were plugged with a small amount of cotton fiber to support upright seedling growth. Seven days after seedlings being transferred to X hole in hydroponics, sodium chloride (NaCl) was added in an increments of 62.50 mM every 24 hours until the final concentrations of 125 or 250 mM NaCl were reached in each tub. Tubs without added sodium chloride were used as a control. The hydroponic system was monitored daily and solution pH maintained between 6.5-7.0. The electrical conductivity (EC) was measured daily and adjusted as necessary. The seedlings were harvested at 18 days after initiation of salt treatments. At harvest, seedling height, fresh shoot and root weight were measured (from cotyledonary scar). Harvested shoot and root of each genotype was oven dried at 65°C for 72 hours and weight was recorded.

3.2.3 Advanced salt screening

Based on the performance of day neutral primitive cotton accessions (primarily percent reduction in seedling height) across salt concentrations under hydroponic technique and availability of seeds, ten genotypes were selected for further analyses. In addition to the ten selected genotypes, FiberMax958 (FM958) (Bayer CropScience, Indianapolis, IN) was used as a check variety. In this study, seedlings were treated with salt solution for one week longer than in the hydroponics system (preliminary screening) to determine salt tolerance levels among genotypes. The hydroponic system was felt to have a limitation in its ability to support the seedlings beyond 18 days after initiation of salt treatments as seedlings became bigger. Therefore, potting mixture was used in this study.

Due to limited availability of seeds, a single seed was sown in one quart plastic pots (8.9x6.4x12.7 cm) filled with the Miracle Gro potting mixture (Scotts Company LLC). Seeds of

each cultivar were also sown in additional pots to swap the pots (if it was necessary). Seedlings were watered every 24 hours for two weeks before salt treatments. It was observed that pots still held a good amount of moisture after 24 hours. After germination, seedlings were fertilized by adding 300 mL of nutrient solution prepared by dissolving 4 g of Peter® fertilizer (20:20:20) per liter once each week. At two weeks after sowing, NaCl was added in an increment of 62.50 mM every 24 hours until the final concentrations of 125 or 250 mM were reached. Seedlings without added sodium chloride was used as the control. Salt treated plants were treated with 300 mL of 125 and 250 mM salt solutions every 24 hours for 21 days, while control plants were watered with 300 mL of tap water. It is expected that accumulation of salt in the pots is unlikely because excess salt or water was well drained out from the bottoms of the pots and the salt concentrations were maintained in each pot. The seedlings were harvested at 25 days after initiation of salt treatments. After harvesting, seedling height, fresh and dry shoot weight, fresh and dry root weight were measured.

3.2.4 Physiological measurement

After measuring dry leaf weight, the tissue was ground with a mortar and pestle. Ground leaf tissue was processed through a flame photometer to determine Na^+ and K^+ content in the leaves.

3.2.5 Statistical analysis

Analysis of variance (ANOVA) was conducted using the SAS 9.3 (SAS Institute Inc., Cary, NC). T-grouping was used to compare the plant parameters (percent reduction in plant height, fresh and dry shoot weight, fresh and dry root weight, and accumulation of sodium (Na^+), potassium (K^+), and K^+/Na^+ ratios) among the cotton genotypes under 0, 125, and 250 mM salt concentrations.

3.3 RESULTS

3.3.1 ANOVA for hydroponics technique

Sixty six day neutral primitive cotton accessions survived at 250 mM salt treatments. The ANOVA showed that there were significant differences between salt treatments and genotypes for percent reduction in plant height, fresh and dry shoot weight, and fresh and dry root weight ($P<0.01$) (Tables 3.1 and 3.2). There was no interaction between genotypes and salt treatments, suggesting that there is a similar trend in response of all genotypes against elevated salt concentrations. Under hydroponic technique, the performance of day neutral primitive cotton accessions across salt concentrations for a number of plant parameters (percent reduction in plant height, fresh and dry shoot weight, and fresh and dry root weight) are presented in Table 3.3.

Table 3.1 Impact of genotype and salt concentration on percent reduction in plant height, fresh and dry shoot weight.

		Plant height (%)		Fresh shoot wt. (%)		Dry shoot wt. (%)	
Source	df	Mean square	F value	Mean square	F value	Mean square	F value
Genotype	65	242.93	2.75**	484.46	1.80*	421.63	1.68*
Salt	1	60871.00	469.26**	46494.00	1339.16**	39722.00	3371.94**
Genotype*Salt	65	107.72	1.22	248.64	0.92	327.71	1.30

**=Significance at $P \leq 0.01$.

Table 3.2 Impact of genotype and salt concentration on percent reduction in fresh and dry root weight.

		Fresh root wt. (%)		Dry root wt. (%)	
Source	df	Mean square	F value	Mean square	F value
Genotype	65	2936.97	1.64**	3956.25	2.33**
Salt	1	89520.00	83.37	45378.00	15.98
Genotypes*Salt	65	1148.21	0.64	1689.03	1.00

**=Significance at $P \leq 0.01$.

Table 3.3 Percent reduction in plant parameters across salt concentrations under hydroponic technique.

Genotype	Plant height (%)	Fresh shoot wt. (%)	Fresh root wt. (%)	Dry shoot wt. (%)	Dry root wt. (%)
MT45	55.76^{a}	67.66^{ab}	64.04^{ab}	59.37^{a-c}	62.71^{a-d}
MT113	52.38^{ab}	53.32^{a-g}	-34.91^{m-o}	62.04^{ab}	3.50^{f-n}
MT201	52.02^{a-c}	44.27^{c-j}	5.13^{b-n}	54.38^{a-g}	26.71^{a-m}

(Table 3.3 continued)

Genotype	Plant height (%)	Fresh shoot wt. (%)	Fresh root wt. (%)	Dry shoot wt. (%)	Dry root wt. (%)
MT117	51.10^{a-d}	51.36^{a-h}	14.64^{a-m}	50.81^{a-h}	26.30^{a-m}
MT641	49.67^{a-e}	62.71^{a-c}	50.21^{a-f}	56.67^{a-e}	74.55^{ab}
MT104	49.56^{a-f}	47.69^{b-j}	-9.28^{g-o}	50.16^{a-h}	-30.86^{m-p}
MT175	49.03^{a-g}	56.49^{a-d}	51.98^{a-e}	38.90^{c-n}	41.18^{a-j}
MT106	48.04^{a-g}	25.23^{j-l}	-24.68^{j-o}	52.60^{a-g}	-2.60^{h-o}
MT320	47.74^{a-g}	44.21^{c-j}	8.49^{a-m}	59.08^{a-d}	54.89^{a-h}
MT188	47.54^{a-g}	47.30^{b-j}	34.78^{a-i}	43.19^{a-m}	40.90^{a-j}
MT171	47.15^{a-g}	49.64^{a-i}	29.24^{a-j}	44.42^{a-l}	28.16^{a-l}
MT246	47.06^{a-g}	50.98^{a-h}	23.45^{a-m}	37.09^{d-n}	52.75^{a-h}
MT1000	46.64^{a-g}	41.17^{c-k}	8.20^{a-n}	35.31^{e-n}	-57.78^{op}
MT36	46.54^{a-g}	43.49^{c-j}	28.23^{a-j}	42.39^{a-m}	38.21^{a-k}
MT99	46.51^{a-g}	48.35^{b-i}	18.88^{a-m}	64.18^{a}	33.85^{a-l}
MT100	46.14^{a-h}	48.96^{b-i}	13.27^{a-m}	45.82^{a-j}	28.15^{a-l}
MT636	45.80^{a-h}	62.85^{a-c}	64.43^{a}	44.86^{a-k}	54.16^{a-h}
MT89	45.06^{a-i}	45.37^{b-j}	1.63^{d-n}	46.78^{a-i}	-17.28^{k-p}
MT41	43.93^{a-j}	43.31^{c-j}	15.45^{a-m}	49.31^{a-i}	39.70^{a-k}
MT55	43.83^{a-k}	53.01^{a-g}	-50.86^{n-o}	37.66^{c-n}	2.76^{f-n}
MT101	43.63^{a-k}	45.74^{b-j}	-1.10^{e-n}	40.19^{b-n}	34.72^{a-l}
MT81	42.74^{a-l}	34.99^{d-l}	-25.46^{j-o}	47.02^{a-i}	-13.22^{j-o}
MT180	42.54^{b-l}	56.72^{a-d}	48.13^{a-g}	46.11^{a-i}	35.71^{a-l}
MT754	42.40^{b-l}	34.63^{d-l}	-1.21^{e-n}	22.98^{k-n}	-2.47^{h-o}
MT620	41.99^{b-l}	49.35^{a-i}	36.14^{a-h}	53.00^{a-g}	26.47^{a-m}
MT223	41.41^{b-m}	36.98^{d-l}	9.91^{a-m}	45.30^{a-j}	24.69^{a-m}
MT93	41.23^{b-n}	54.30^{a-f}	32.35^{a-j}	54.69^{a-f}	81.56^{a}
MT326	41.21^{b-n}	51.65^{a-h}	60.98^{abc}	51.08^{a-h}	40.75^{a-j}
MT720	41.14^{b-n}	38.04^{d-l}	-23.38^{h-o}	40.62^{b-n}	-35.04^{np}
MT347	41.08^{b-n}	50.74^{a-h}	41.18^{a-h}	40.96^{b-n}	50.71^{a-i}
MT62	40.82^{b-n}	55.37^{a-e}	26.63^{a-k}	62.07^{ab}	66.75^{a-d}
MT612	40.73^{b-n}	36.78^{d-l}	-7.65^{f-o}	38.26^{c-n}	7.97^{e-n}
MT477	40.69^{b-n}	43.66^{c-j}	27.12^{a-j}	44.98^{a-k}	18.08^{b-n}
MT61	40.52^{b-o}	37.99^{d-l}	26.13^{a-k}	37.82^{c-n}	47.60^{a-i}
MT198	40.36^{b-o}	42.00^{c-k}	9.69^{a-m}	52.67^{a-g}	14.10^{c-n}
MT1291	39.50^{b-p}	32.10^{f-l}	-7.97^{f-o}	40.59^{b-n}	-21.12^{l-p}
MT764	39.02^{c-q}	34.65^{d-l}	20.21^{a-m}	34.21^{g-n}	18.95^{b-n}
MT249	38.64^{d-r}	40.15^{c-k}	10.12^{a-m}	45.17^{a-j}	21.58^{b-n}
MT53	38.52^{d-r}	39.41^{d-l}	-15.55^{h-o}	35.69^{e-n}	32.54^{a-l}
MT32	38.27^{d-r}	29.34^{h-l}	40.49^{a-h}	41.93^{b-m}	53.57^{a-h}
MT27	38.12^{d-s}	37.02^{d-l}	22.16^{a-m}	39.41^{c-n}	62.03^{a-d}
MT221	37.85^{e-t}	48.19^{b-i}	25.19^{a-k}	40.88^{b-n}	26.06^{a-m}
MT241	37.56^{e-t}	33.39^{e-l}	1.01^{d-n}	33.45^{g-n}	14.91^{c-n}

(Table 3.3 continued)

Genotype	Plant height (%)	Fresh shoot wt. (%)	Fresh root wt. (%)	Dry shoot wt. (%)	Dry root wt. (%)
MT11	37.47^{e-t}	62.59^{a-c}	59.40^{a-d}	36.38^{e-n}	59.17^{a-f}
MT247	37.20^{e-u}	72.17^{a}	27.23^{a-j}	48.73^{a-i}	38.21^{a-k}
MT239	37.17^{e-u}	37.39^{d-l}	2.68^{c-n}	41.77^{b-m}	-10.33^{j-o}
MT668	36.51^{f-u}	31.58^{f-l}	-34.13^{l-o}	49.87^{a-h}	12.65^{c-n}
MT52	36.19^{g-u}	43.96^{c-j}	36.87^{a-h}	43.65^{a-m}	13.25^{c-n}
MT212	35.92^{g-u}	36.41^{d-l}	17.61^{a-m}	34.18^{g-n}	10.56^{d-n}
MT199	33.30^{h-u}	45.35^{b-j}	24.24^{a-l}	40.59^{b-n}	58.28^{a-f}
MT68	33.06^{h-u}	38.73^{d-l}	16.55^{a-m}	33.33^{g-n}	41.20^{a-j}
MT281	32.29^{i-u}	41.66^{c-k}	37.12^{a-h}	39.89^{c-n}	66.76^{a-d}
MT257	31.76^{j-u}	36.57^{d-l}	17.13^{a-m}	32.55^{g-n}	9.33^{d-n}
MT242	31.53^{j-u}	19.94^{kl}	-10.60^{g-o}	19.58^{n}	52.79^{a-h}
MT43	30.71^{k-u}	51.61^{a-h}	43.03^{a-h}	49.73^{a-h}	55.19^{a-g}
MT6	30.33^{l-u}	35.92^{d-l}	26.72^{a-j}	47.74^{a-i}	-5.10^{i-o}
MT57	28.78^{m-u}	35.76^{d-l}	25.86^{a-k}	30.43^{h-n}	58.91^{a-f}
MT1219	28.11^{n-u}	29.27^{h-l}	-65.82^{o}	23.80^{j-n}	-72.10^{p}
MT650	27.44^{o-u}	29.77^{h-l}	3.65^{c-n}	42.59^{a-m}	-0.46^{g-o}
MT790	26.91^{p-u}	32.31^{e-l}	35.51^{a-i}	29.02^{h-n}	42.26^{a-j}
MT120	26.20^{q-u}	18.17^{l}	9.61^{a-m}	22.78^{l-n}	68.32^{abc}
MT634	25.78^{r-u}	32.73^{e-l}	35.45^{a-i}	43.71^{a-m}	57.90^{a-f}
MT245	25.72^{r-u}	27.84^{i-l}	-5.14^{e-n}	37.90^{c-n}	-0.26^{g-o}
MT224	25.09^{s-u}	29.08^{h-l}	16.23^{a-m}	21.90^{mn}	53.87^{a-h}
MT48	24.84^{tu}	36.92^{d-l}	40.64^{a-h}	27.57^{i-n}	61.68^{a-d}
MT244	24.24^{u}	30.43^{g-l}	-32.43^{k-o}	22.06^{mn}	19.34^{b-n}

Means with the same letter within each plant parameter do not differ significantly ($P \leq 0.05$, T-grouping).

3.3.2 Correlation of plant parameters

A strong positive correlation among various plant parameters (i.e. percent reduction in plant height, fresh and dry shoot weight, fresh and dry root weight) was observed ($P<0.01$) (Table 3.4). The results showed that the increased sodium ion (Na^+) concentrations in the leaf tissues adversely affected the plant growth parameters ($P<0.01$), but that the potassium sodium ion ratio (K^+/Na^+) had a positive effect on plant parameters ($P<0.01$). There was a strong negative correlation between sodium (Na^+) and potassium (K^+) concentrations in the leaves, suggesting that increased Na^+ concentrations impair the absorption of K^+ ($P<0.01$). However,

there was a no correlation between percent reduction in plant parameters and K^+ concentration in the leaves (P>0.10).

Table 3.4 Correlation among plant parameters and ionic concentrations across salt concentrations in pot culture.

	Plant height	Fresh shoot wt.	Dry shoot wt.	Fresh root wt.	Dry root wt.	Na^+	K^+	K^+/Na^+ ratio
Plant height	1.00	0.95**	0.89**	0.83**	0.72**	0.69**	-0.12	-0.59**
Fresh shoot wt.		1.00	0.97**	0.90**	0.85**	0.75**	-0.18	-0.67**
Dry shoot wt.			1.00	0.91**	0.89**	0.76**	-0.20	-0.67**
Fresh root wt.				1.00	0.84**	0.69**	-0.11	-0.56**
Dry root wt.					1.00	0.84**	-0.39	-0.80**
Na^+						1.00	-0.54**	-0.87**
K^+							1.00	0.77**
K^+/Na^+ ratio								1.00

**=Significance at $P \leq 0.01$.

3.3.3 Plant height

Data from two sets of experiments were combined because time was no significantly different for percent reduction in plant height between two set of treatments. Analysis of variance showed that there were significant differences among cotton genotypes and salt treatments for percent reduction in plant height (P<0.01) (Table 3.5). There was no significant genotype by salt interaction, which suggests that the performance of all genotypes was similar over salt treatments (P=0.94). The data revealed that plant height was significantly reduced as salt concentration increased. Across genotypes, an average reduction in genotype height was 46% at 250 mM NaCl, which was significantly higher than at 125 mM NaCl (30%) (Figure 3.1). Across salt concentrations, MT 11 had the lowest reduction in height (32%) followed by MT43 (34%) and both were significantly lower than MT99 (41%) and FM958 (43%) (Figure 3.2). In

addition to percent reduction in plant height across salt treatments, further analyses within each salt treatment is important for cotton breeders to select the best performance cotton accessions within salt concentrations for developing/improving salt tolerant cotton cultivars. The data showed that there was significantly higher reduction in height for all the cotton genotypes used in this study at 250 mM NaCl than at 125 mM NaCl (Figure 3.3). At 250 mM NaCl, MT11 had the lowest reduction in plant height (38%) followed by MT45 (42%) and both were significantly lower than FM958 (52%). At 125 mM NaCl, MT43 had the lowest reduction in plant height (26%) than MT224 (34%) (Figure 3.3).

Table 3.5 Effect of salt concentration and genotype on percent reduction in plant height.

Source	df	Mean square	F value
Salt	1	7715.02	151.60**
Genotype	10	123.80	2.43**
Salt *Genotype	10	20.46	0.40

**=Significance at $P \leq 0.01$.

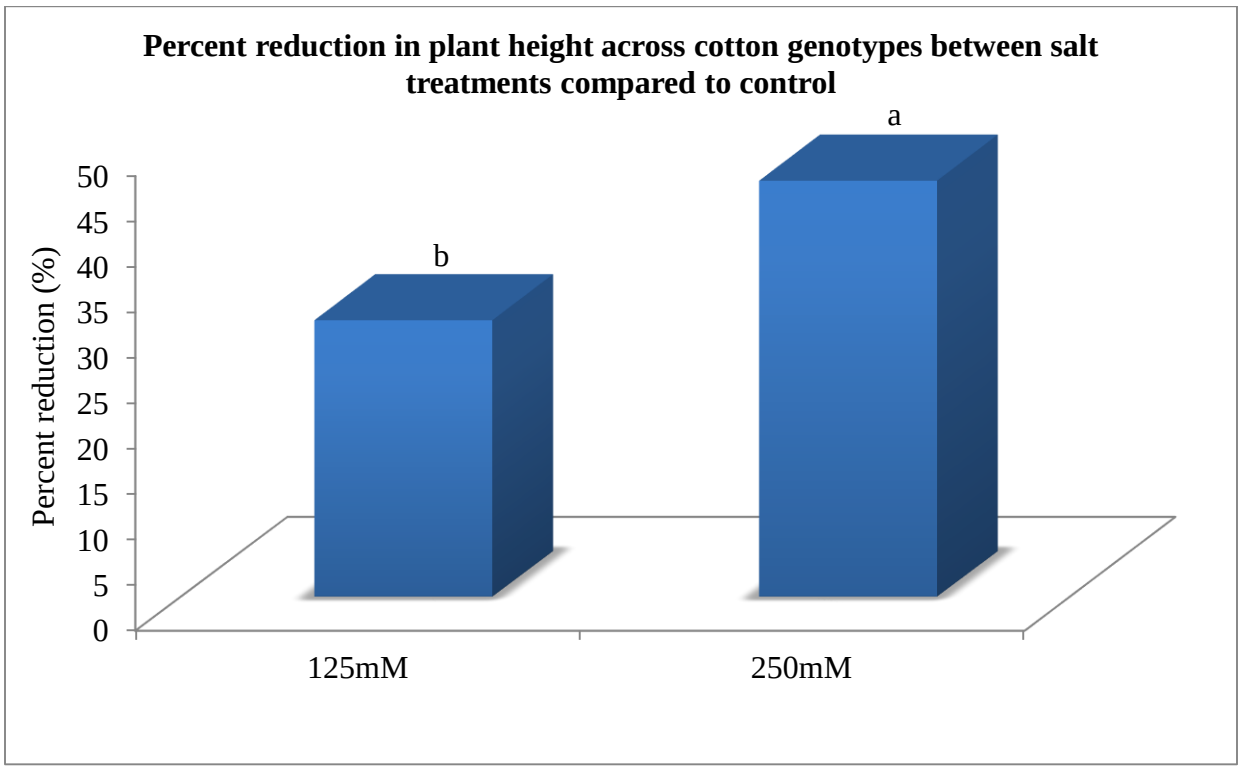

Figure 3.1 Percent reduction in plant height (cm) between salt treatments across cotton genotypes compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

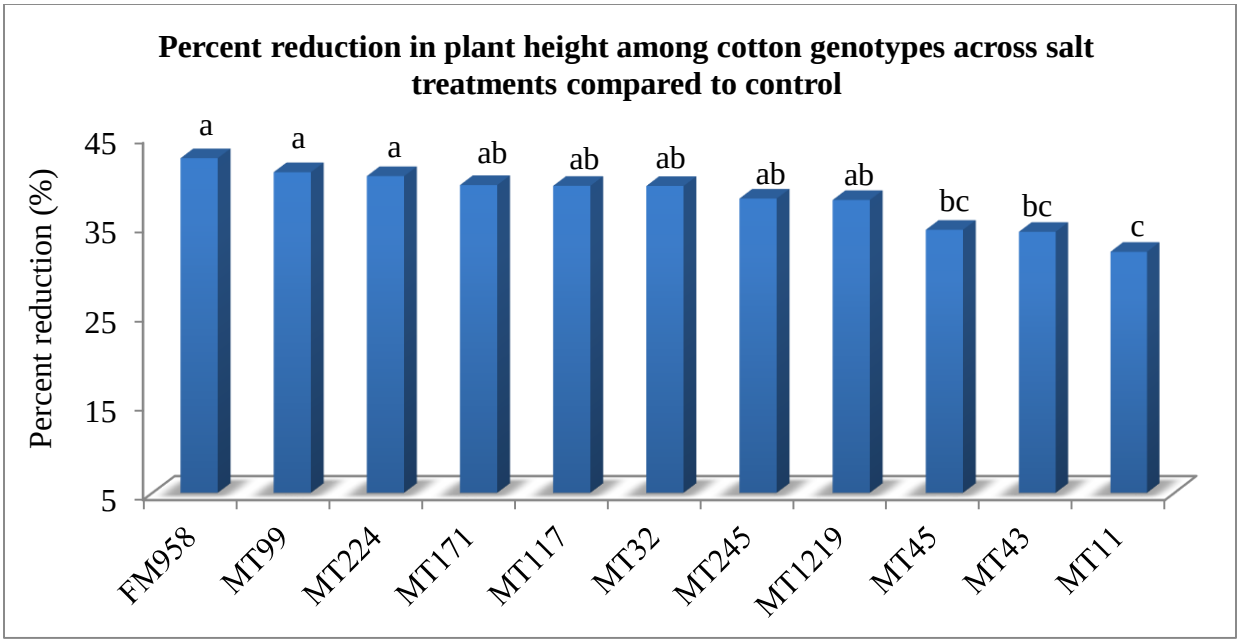

Figure 3.2 Percent reduction in plant height (cm) of cotton genotypes across salt concentrations compared to control. Means with same letter do not differ significantly (P ≤ 0.05, T-grouping).

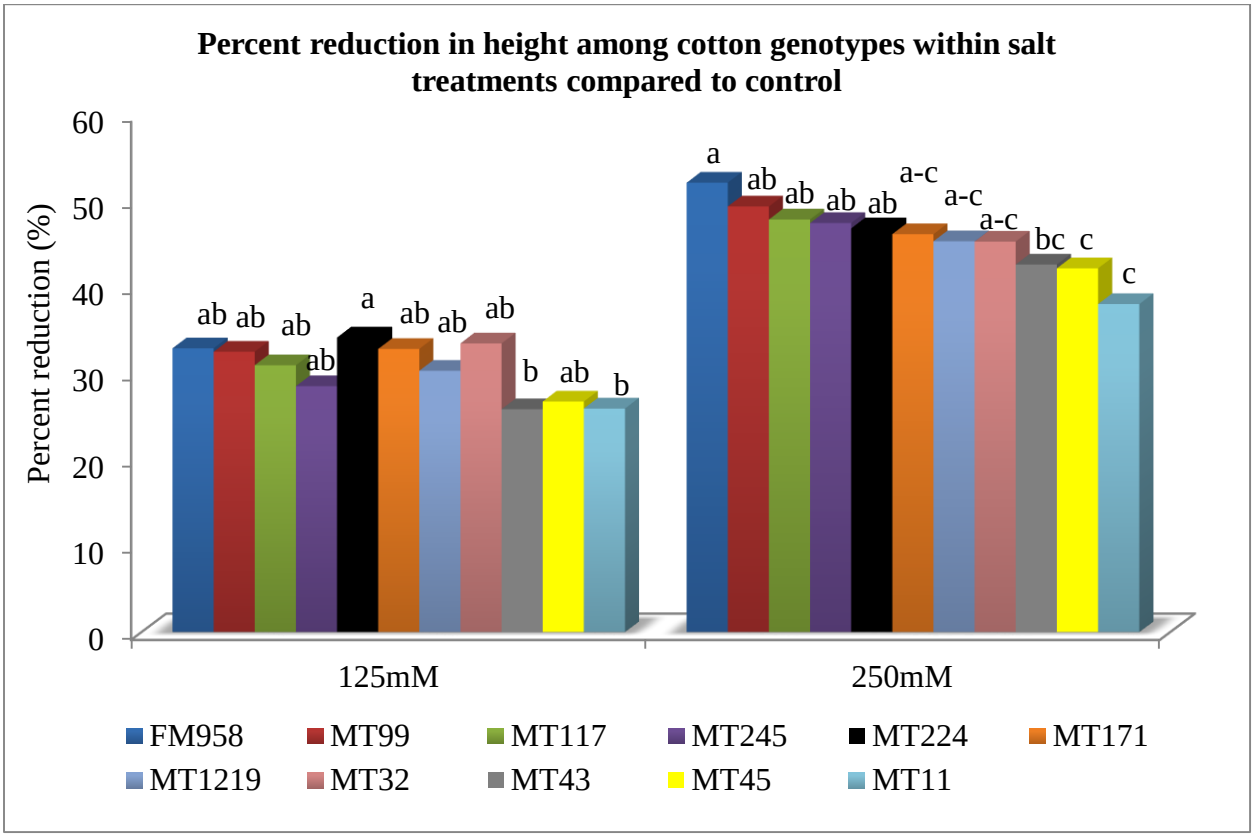

Figure 3.3 Percent reduction in plant height among cotton genotypes within salt treatments compared to control. Within salt treatments, means with same letter do not differ significantly (P ≤ 0.05, T-grouping).

3.3.4 Fresh and dry shoot weight

Analysis of variance showed that there were significant differences among genotypes and salt treatments for fresh and dry shoot weight (P<0.01) (Table 3.6). There was no significant genotype by salt interaction for fresh and dry shoot weight, which suggests that the trend of genotype response was similar across salt treatments (P=0.99 and 0.68, respectively). The data also revealed that increased salt concentration significantly decreased fresh shoot weight across the genotypes. At 250 mM NaCl, an average percent reduction in shoot weight was 72%, which was significantly higher than at 125 mM NaCl (50%) (Figure 3.4). Across salt concentrations, MT11 had the lowest reduction in fresh shoot weight (53%), followed by MT1219 (56%), while the largest reduction in fresh shoot weight was observed in FM958 (67%) (Figure 3.5).

In addition to percent reduction in plant height across salt treatments, it is also important to determine the percent reduction in plant height across primitive cotton accessions within salt treatments so that plant breeder can select the best salt tolerant cotton genotypes within salt treatments. The data showed that highest level of salt treatments (250 mM NaCl) reduced the fresh weight by at least 60%, while modest level of salt treatments (125 mM NaCl) causes at least 40% reduction in fresh shoot weight for the primitive cotton accessions used in this study (Figure 3.6). MT 11 had the significantly lowest reduction in fresh shoot weight (42% and 65%, respectively) than FM958 (58% and 76%, respectively) at 125 and 250 mM NaCl (Figure 3.6).

Table 3.6 Effect of genotype and salt concentration on percent reduction in fresh and dry shoot weight.

		Fresh shoot weight		Dry shoot weight	
Source	df	Mean square	F value	Mean square	F value
Salt	1	16412.00	273.54**	15690	118.84**
Genotype	10	273.54	3.42**	434.82	3.28**
Salt*Genotype	10	16.53	0.28	98.58	0.74

**=Significance at P ≤ 0.01.

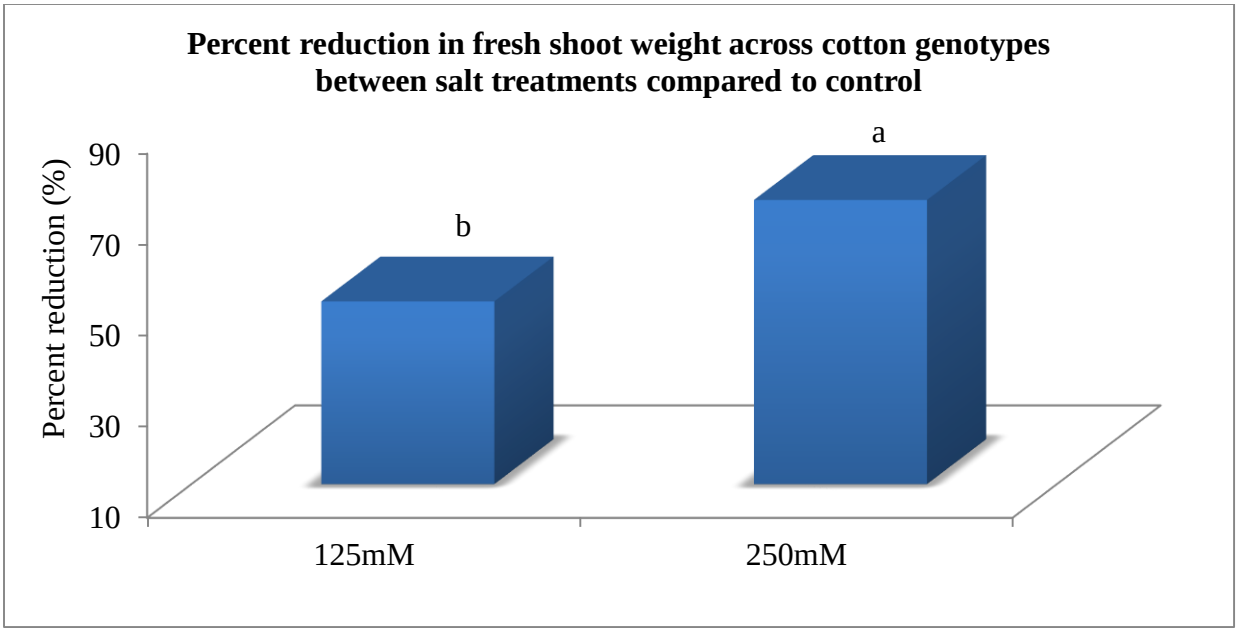

Figure 3.4 Percent reduction in fresh shoot weight (g) between salt treatments across cotton genotypes compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

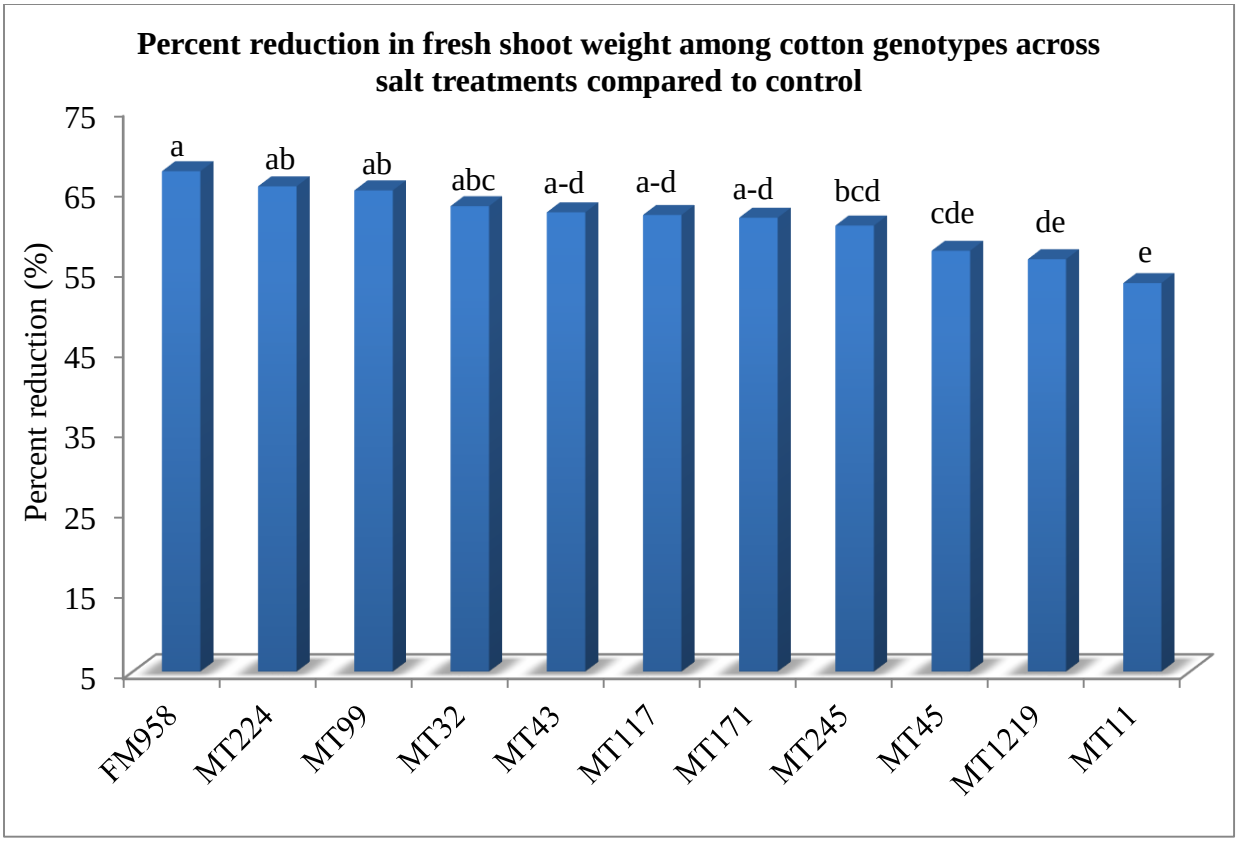

Figure 3.5 Percent reduction in fresh shoot weight (g) among cotton genotypes across salt concentrations compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

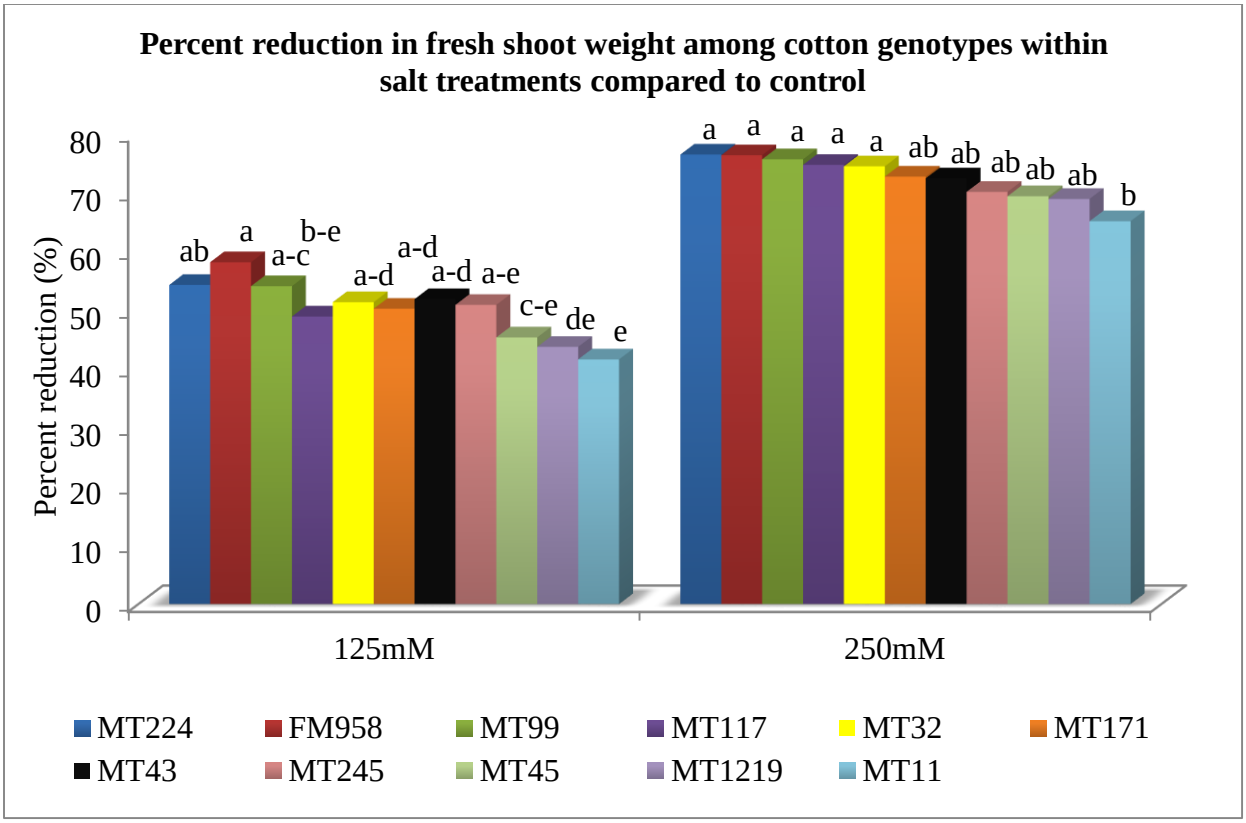

Figure 3.6 Percent reduction in fresh shoot weight among cotton genotypes within salt treatments compared to control. Within salt treatments, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

There was also a significant decrease in dry shoot weight as salt concentration increased. An average reduction in dry shoot weight at 250 mM NaCl was 69%, which was significantly higher than at 125 mM NaCl (48%) (Figure 3.7). Across salt concentrations, MT11 had the lowest reduction in dry shoot weight (47%), followed by MT1219 (51%) and both were significantly lower than and FM958 (66%) (Figure 3.8). Similarly, the data showed that at least 40% and 60% reduction in dry shoot weight was observed in all the cotton genotypes at 125 and 250 mM NaCl, respectively. Although MT1219 had the lowest reduction in dry shoot weight at 250 mM NaCl (62%), it was not significantly different than the MT224 (75%) (Figure 3.9). At 125 mM NaCl, MT11 had the significantly lowest reduction in dry shoot weight (31%) than FM958 (59%).

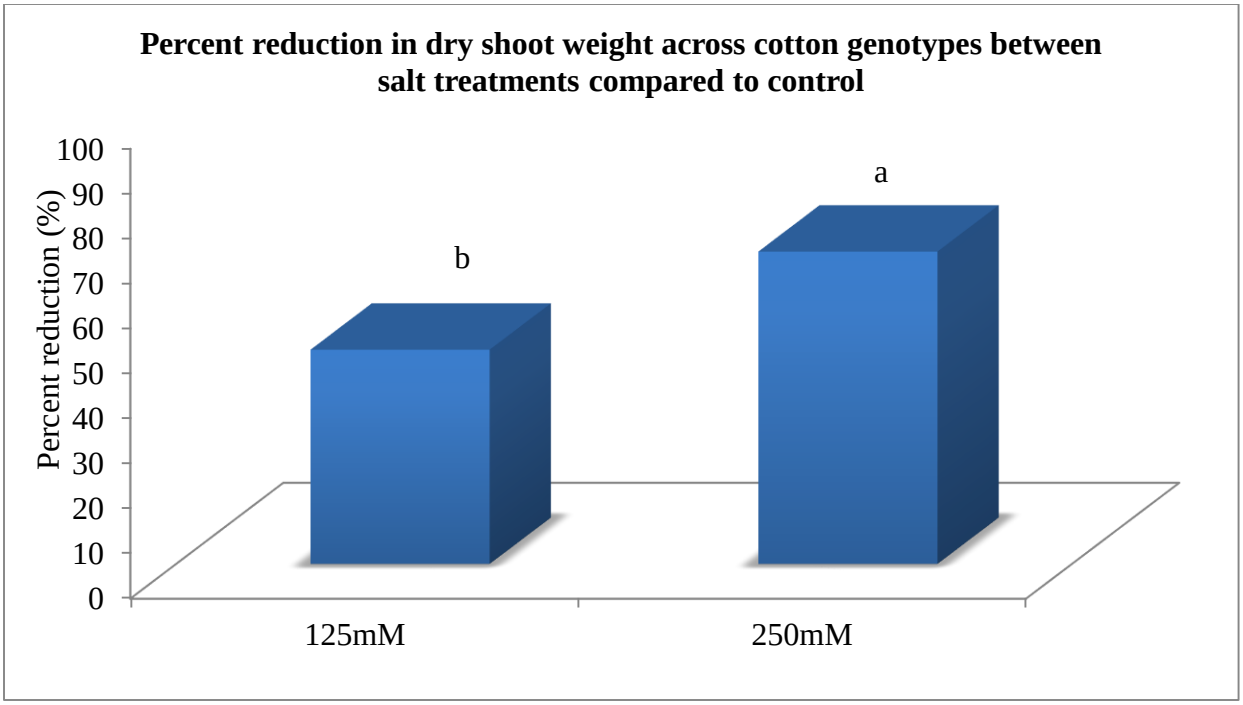

Figure 3.7 Percent reduction in dry shoot weight (g) between salt concentrations across cotton genotypes compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

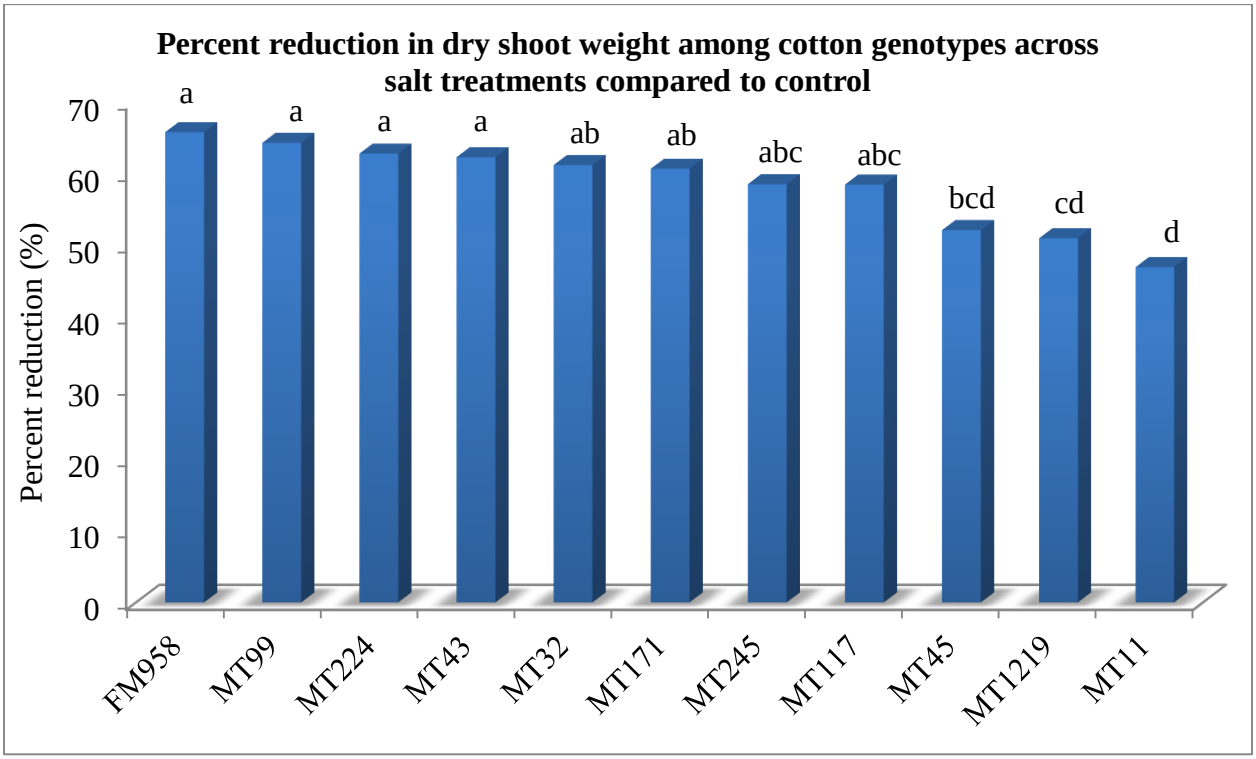

Figure 3.8 Percent reduction in dry shoot weight (g) among cotton genotypes across salt concentrations compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

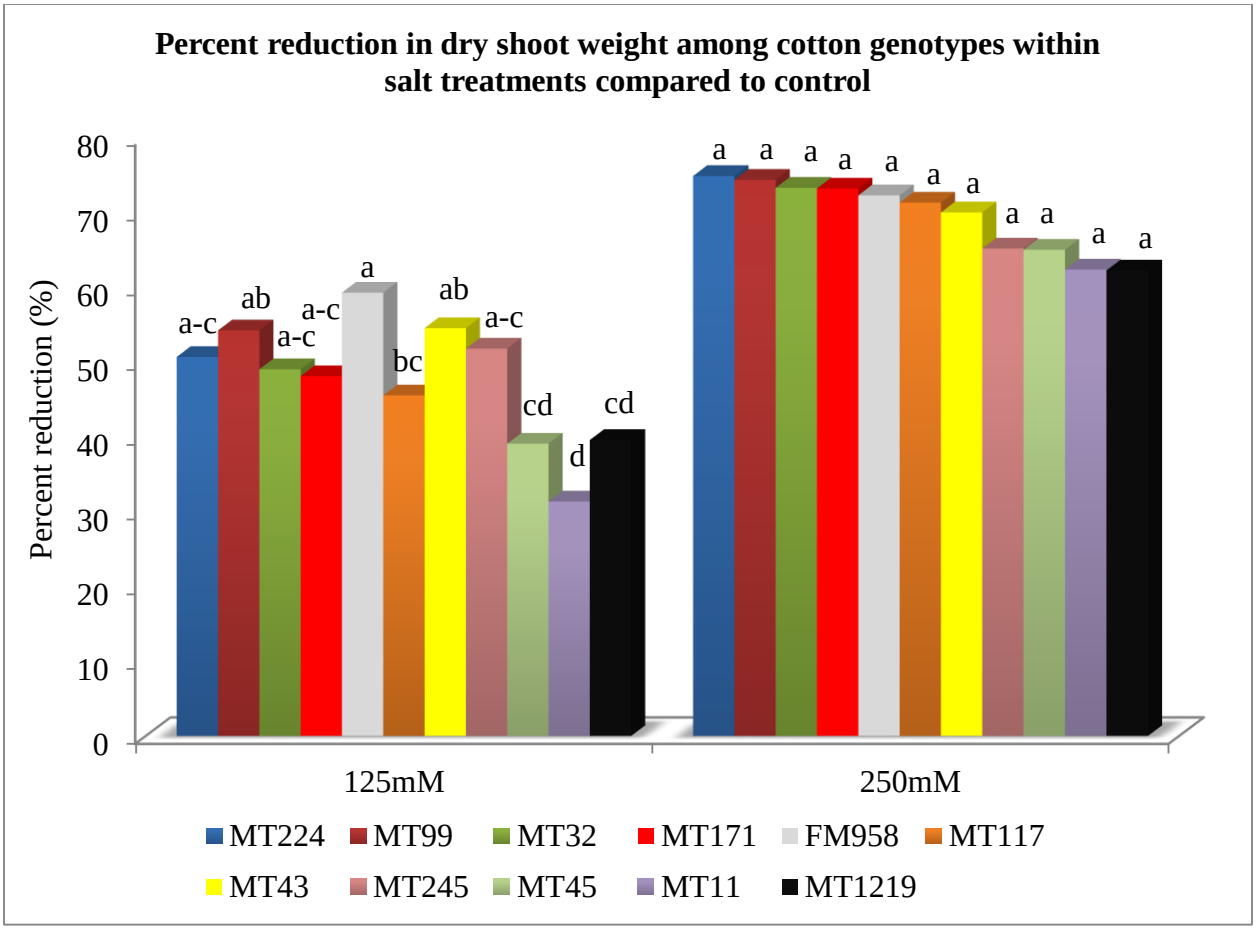

Figure 3.9 Percent reduction in dry shoot weight among cotton genotypes within salt treatments compared to control. Within salt treatments, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

3.3.5 Fresh and dry root weight

There were significantly different among genotypes and salt treatments for percent reduction in fresh and dry root weight ($P<0.01$), but no significant genotype by salt interaction was observed for both fresh and dry root weight ($P=0.98$ and 0.59, respectively). The lack of interaction between genotypes and salt treatments for percent reduction in fresh and dry root weight suggests the genotypes performed with similar trends over salt treatments (Table 3.7). An average percent reduction in fresh root weight of genotypes at 250 mM NaCl was 47%, which was significantly higher than at 125 mM NaCl (21%) (Figure 3.10). Across salt treatments, MT45 had the lowest reduction in fresh root weight (14%), followed by MT1219 (24%), and

both were significantly lower in reduction than MT117 (41%) and FM958 (69%) (Figure 3.11). Although MT45 had the lowest reduction in fresh root weight (30%), it was not significantly different than all the genotypes in this study at 250 mM NaCl. The data showed that 125 mM NaCl increased the root growth by 3% on MT45, while FM958 had the highest reduction in fresh root weight at 125 mM NaCl (39%) (Figure 3.12).

Table 3.7 Effect of genotype and salt concentration on percent reduction in fresh and dry root weight.

		Fresh root weight		Dry root weight	
Source	df	Mean square	F value	Mean square	F value
Salt	1	21587.00	42.27**	16919.00	54.17**
Genotype	10	1037.20	2.05*	1232.04	3.95**
Salt*Genotype	10	149.40	0.30	263.03	0.84

* and **=Significance at P ≤ 0.05 and P ≤ 0.01, respectively.

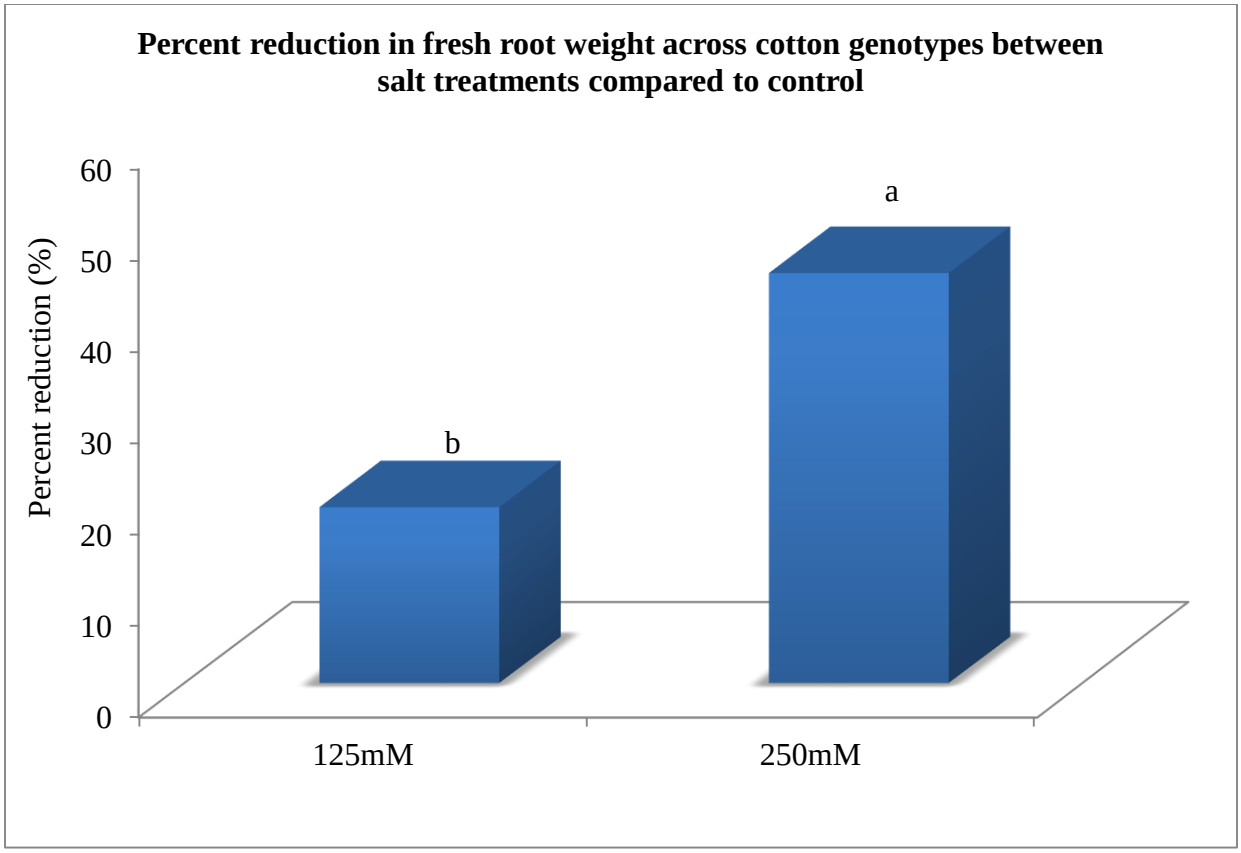

Figure 3.10 Percent reduction in fresh root weight (g) between salt treatments across cotton genotypes compared to control. Means with same letter do not differ significantly (P ≤ 0.05, T-grouping).

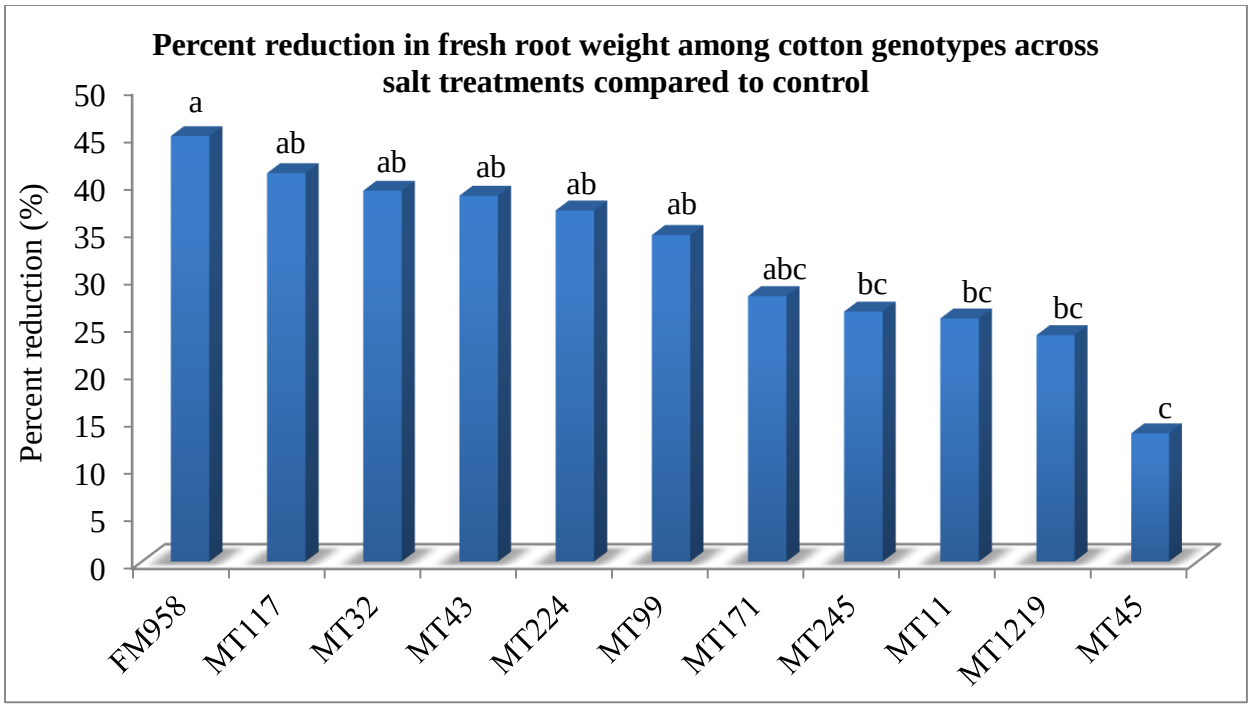

Figure 3.11 Percent reduction in fresh root weight (g) among cotton genotypes across salt concentrations compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

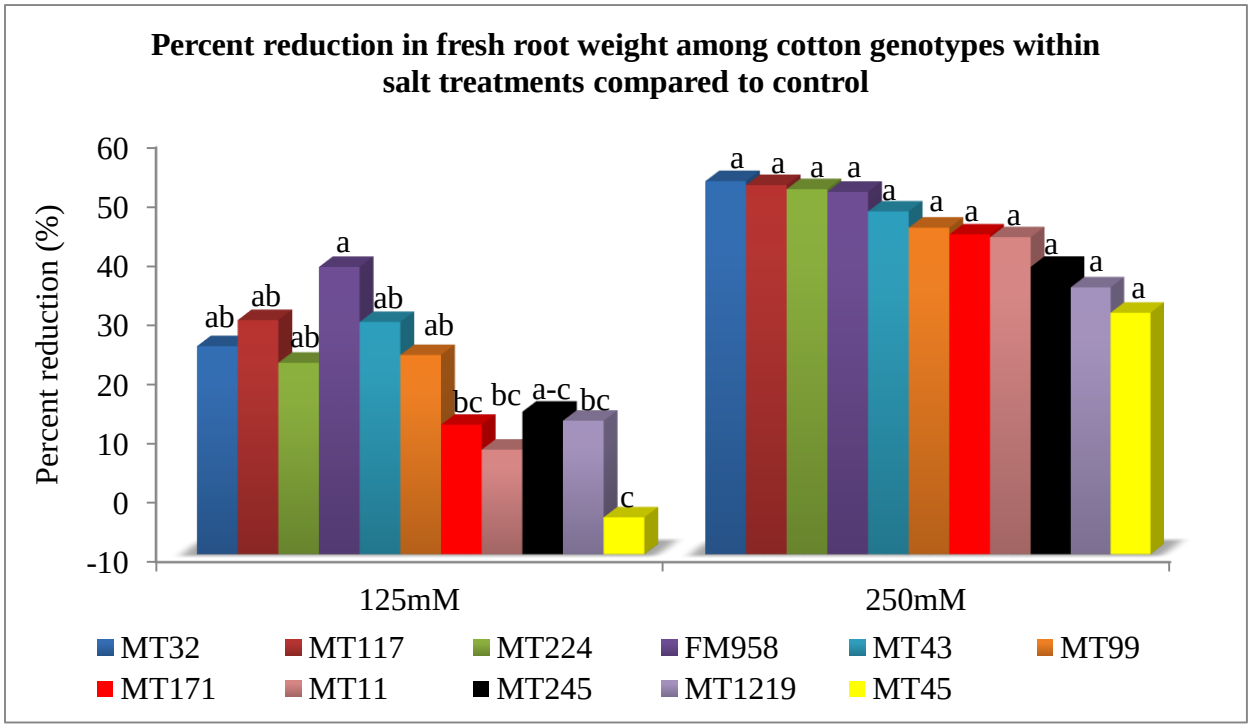

Figure 3.12 Percent reduction in fresh root weight among cotton genotypes within salt treatments compared to control. Within salt treatments, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

A similar trend in the dry root weight among genotypes across salt concentrations was observed. At 250 mM NaCl, the average reduction in dry root weight (53%), was significantly higher than at 125 mM NaCl (30%) (Figure 3.13). Across salt concentrations, MT1219 had the lowest reduction in dry root weight (17%) and was significantly lower than all other genotypes, while the highest reduction in dry root weight was observed in FM958 (54%) (Figure 3.14). At 250 mM NaCl, MT1219 had the significantly lowest reduction in dry root weight (34%) than FM958 (63%). The data showed that 125 mM salt concentration had the positive effect on MT1219 for dry root weight (increased by 1%), while MT43 had the highest reduction in dry root weight (46%) (Figure 3.15).

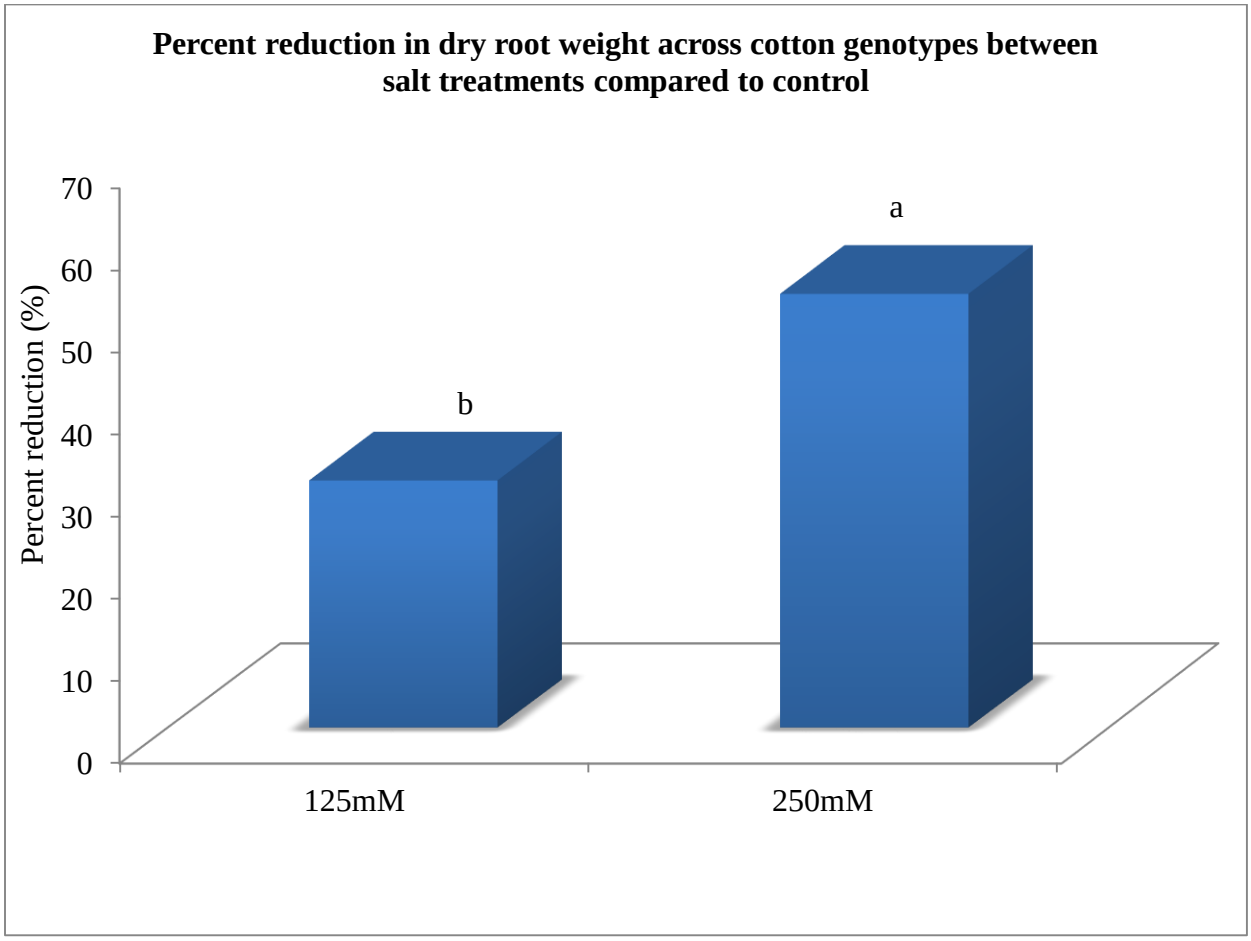

Figure 3.13 Percent reduction in dry root weight (g) among salt treatments across cotton genotypes compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

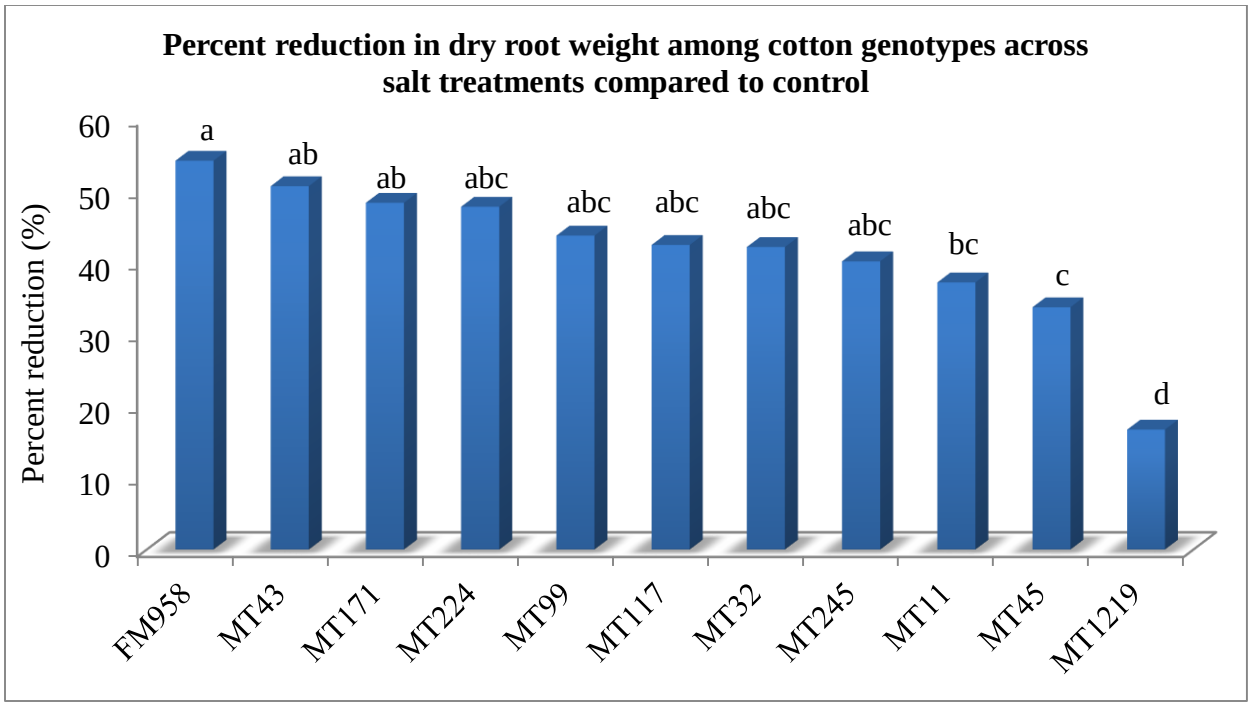

Figure 3.14 Percent reduction in dry root weight (g) among cotton genotypes across salt concentrations compared to control. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

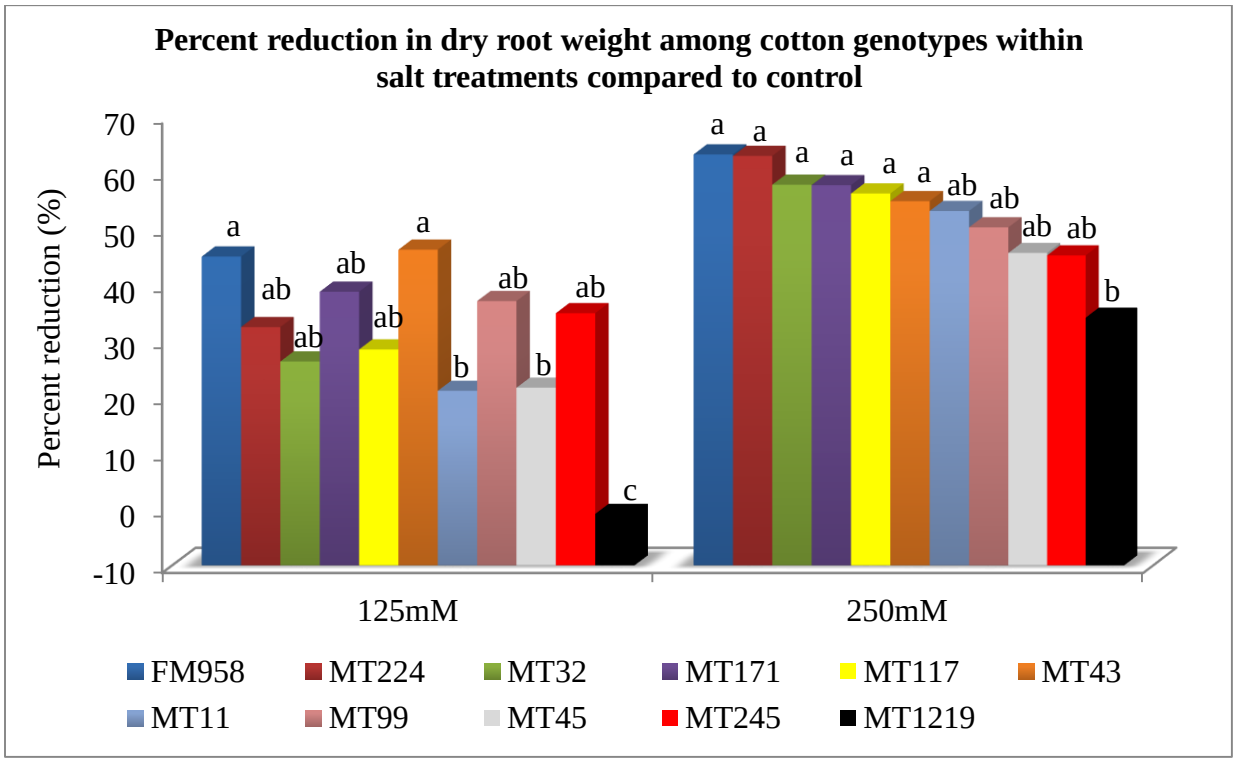

Figure 3.15 Percent reduction in dry root weight among cotton genotypes within salt treatments compared to control. Within salt treatments, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

3.3.6 Sodium and potassium concentrations

For Na^+ concentration, there were significant differences between salt treatments, genotypes, and there was a significant salt by genotype interaction, which implies that the performance of genotypes did not exhibit a similar trend across salt concentrations ($P<0.01$) (Table 3.8). The Na^+ accumulation in the leaves significantly increased as salt concentrations increased. Averaged across genotypes, leaf Na^+ concentration on a dry weight (DW) basis in the control was 211.50 mM/Kg, which was significantly lower than its concentration at 125 mM (1,304.41 mM/Kg) and at 250 mM (1753.40 mM/Kg) (Figure 3.16). Across salt treatments, MT1219 had the lowest accumulation of Na^+ in leaf tissues (656.69 mM/Kg, DW), and was significantly lower than all other genotypes in this study. Although MT1219 had the lowest accumulation in salt in the leave tissues (158.43 mM/Kg, DW), it was not significantly different than all cotton genotypes in this study. At 250 mM NaCl, MT1219 had the significant lowest Na^+ concentration (1,026.37 mM/Kg, DW), followed by MT45 (1,573.99 mM/Kg, DW), and both were significantly different from each other. The highest Na^+ concentration was observed in FM958 (2,135.39 mM/Kg, DW) at 250 mM NaCl, which was two times higher than salt tolerant genotype MT1219 (Figure 3.17). Similarly, MT1219 had the significantly lowest Na^+ concentrations in the leave tissues (785.27 mM/Kg, DW), followed by MT245 (1093.53 mM/Kg, DW) at 125 mM NaCl, and both were significantly different from each other.

Table 3.8 Effect of salt concentration and genotype on Na^+, K^+ and K^+/Na^+ ratio in the leaf tissues.

		Na^+ concentrations		K^+ concentrations		K^+/Na^+ ratio	
Source	df	Mean square	F value	Mean square	F value	Mean square	F value
Salt	2	41508241.00	615.40**	194250.00	14.35**	2901.93	28.60**
Genotype	10	664241.00	9.85**	97067.00	7.13**	216.49	2.13*
Salt*Genotype	20	182752.00	2.71**	8906.21	0.65	198.58	1.96*

* and **=Significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

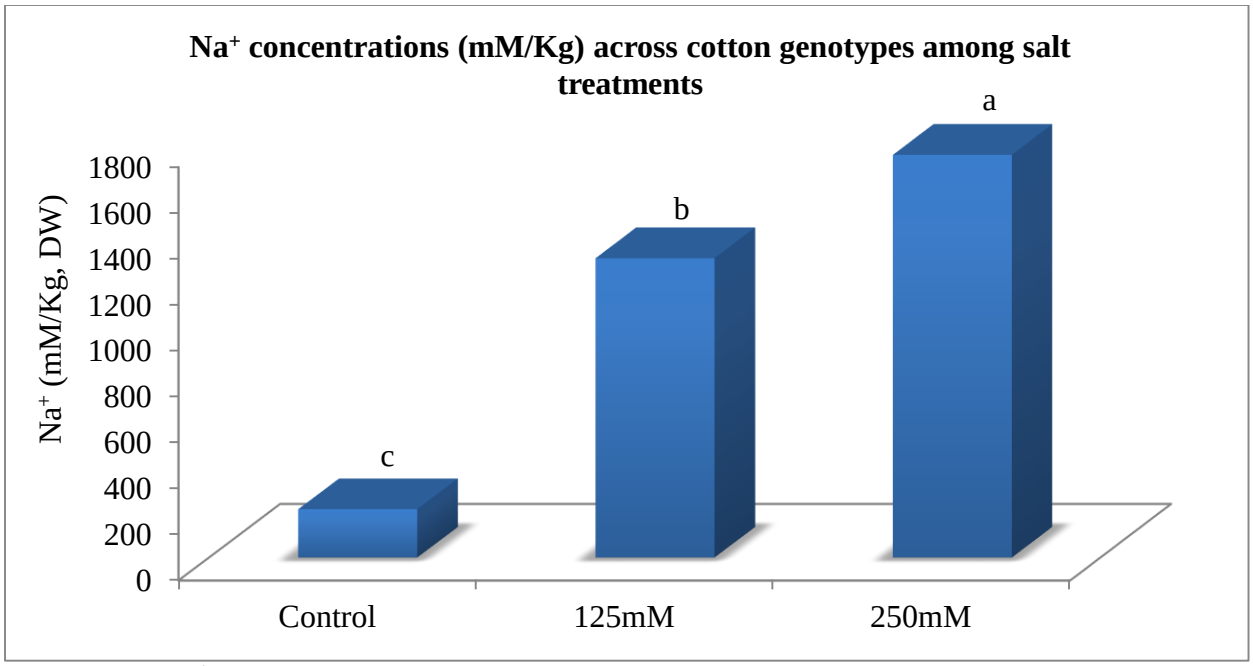

Figure 3.16 Na^+ concentrations (mM/Kg, DW) among salt treatments across cotton genotypes. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

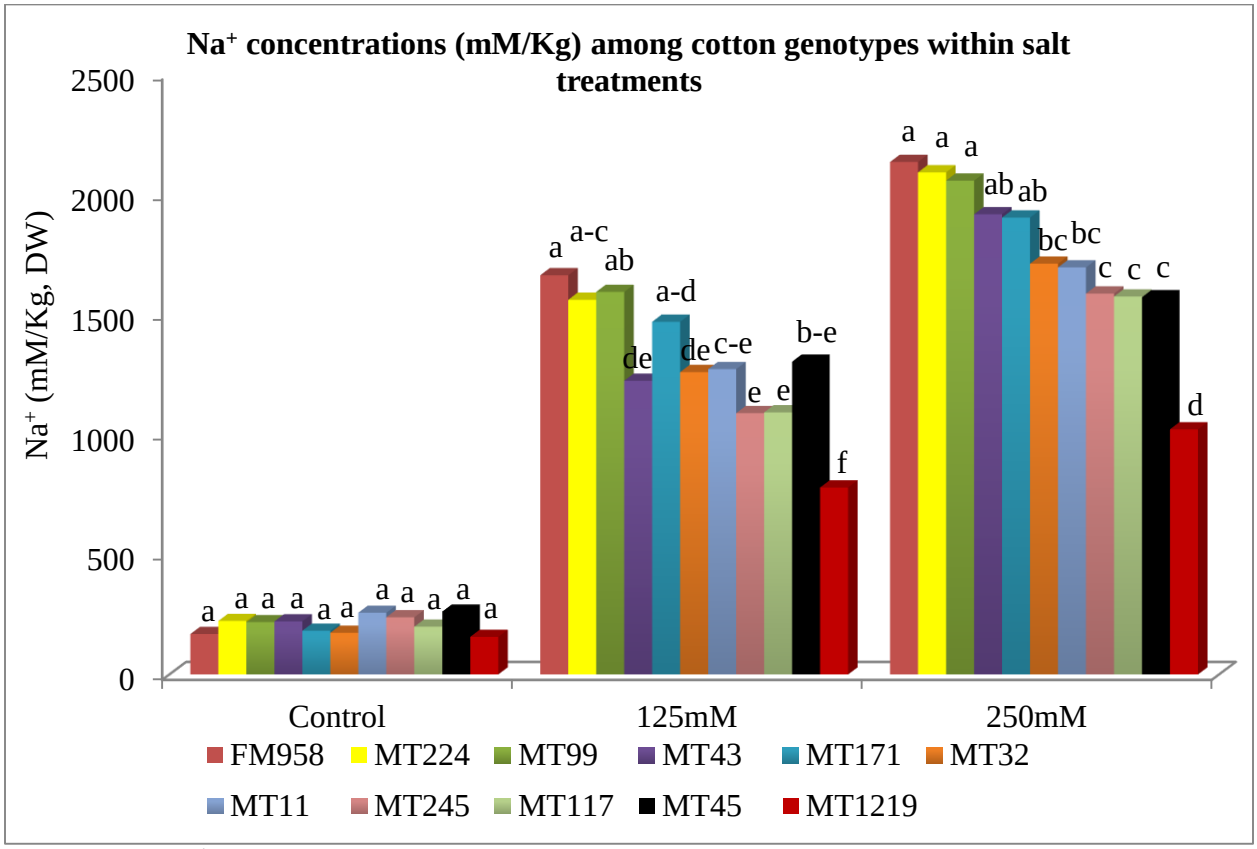

Figure 3.17 Na^+ concentrations (mM/Kg, DW) among cotton genotypes within salt treatments. Within salt treatments, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

The trend of K^+ accumulation was contrary to Na^+ accumulation in the leaf tissues. K^+ concentration, averaged across genotypes, at control was 1019.25 mM/Kg (DW) of leaf tissue, and was significantly higher than at both 125 mM NaCl (931.25 mM/Kg, DW) and 250 mM NaCl (919.25 mM/Kg, DW); there was no significant difference between 125 mM and 250 mM NaCl (Figure 3.18). Across salt treatments, MT1219 had the highest K^+ accumulation in the leaves (1145.60 mM/Kg, DW), which was significantly higher than all the cotton genotypes included in this study, while the lowest K^+ concentration was reported in MT32 (863.06 mM/Kg, DW) (Figure 3.19). It showed that MT1219 had significantly highest accumulation of K^+ in the leaves (1215.39, 1168.14, and 1053.27 mM/Kg, DW, respectively) than MT32 (943.04, 818.90 and 826.31 mM/Kg, DW respectively) at three salt treatments; control, 125 mM and 250 mM NaCl (Figure 3.20).

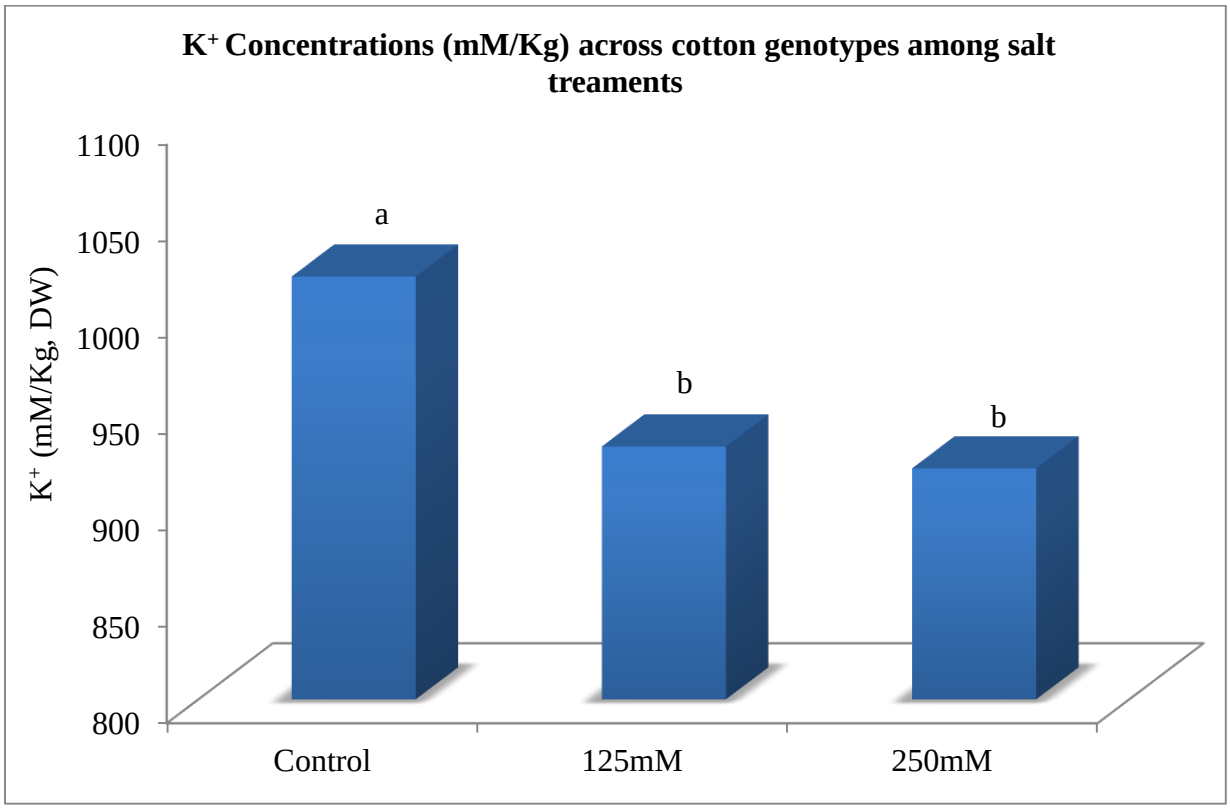

Figure 3.18 K^+ concentrations (mM/Kg, DW) across cotton genotypes among salt treatments. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

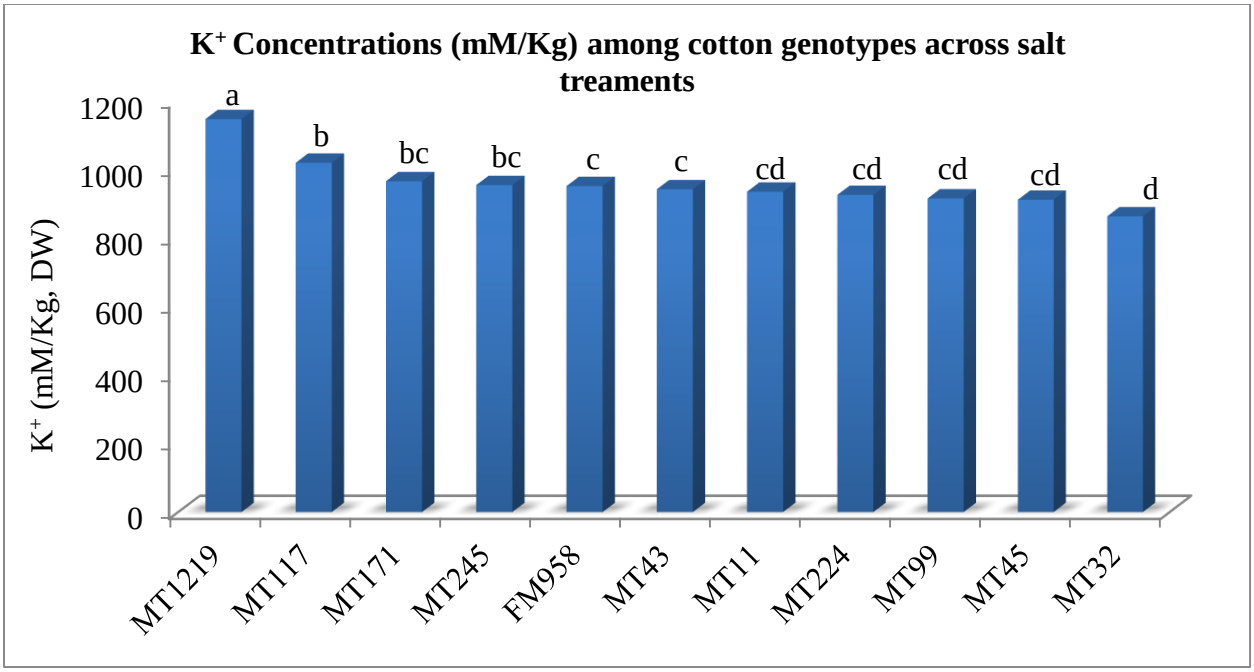

Figure 3.19 K^+ concentrations (mM/Kg, DW) among cotton genotypes across salt concentrations. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

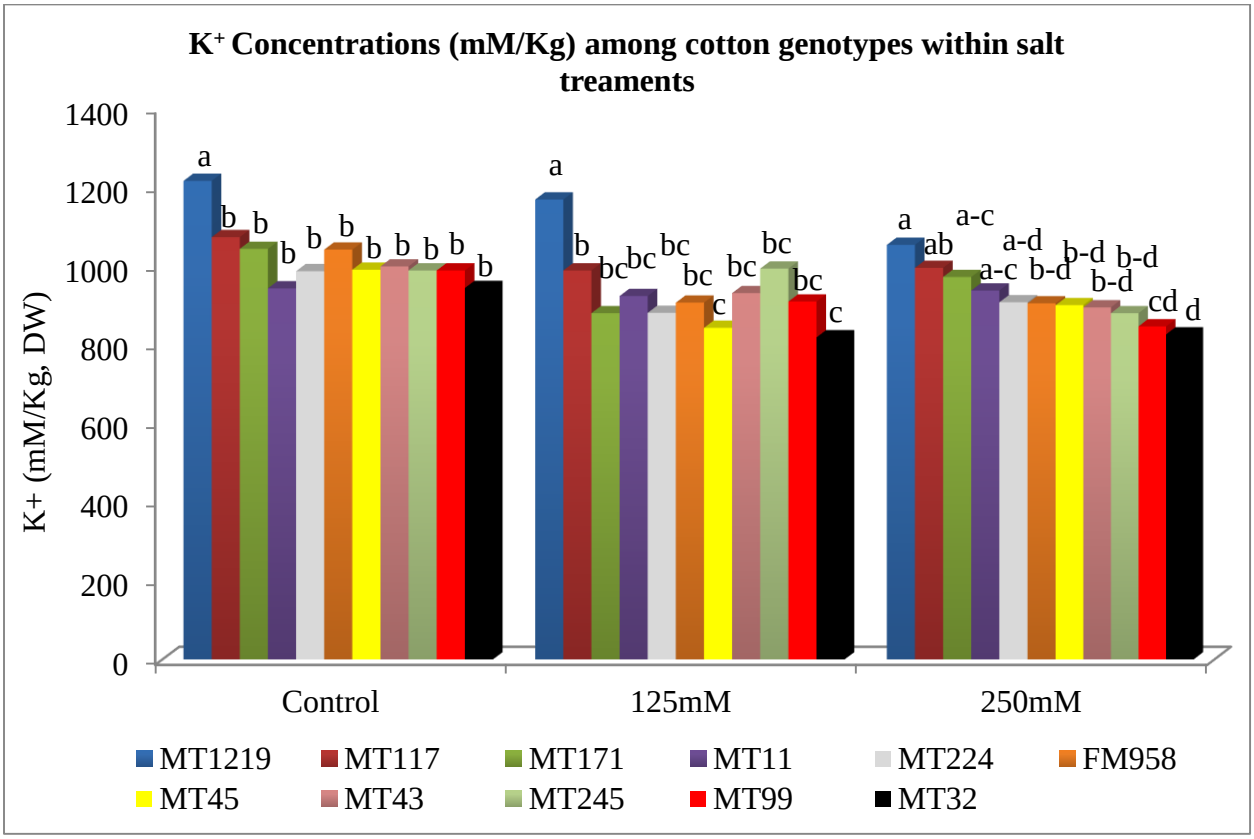

Figure 3.20 K^+ concentrations (mM/Kg, DW) among cotton genotypes within salt treatments. Within salt treatments, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

The K^+/Na^+ ratios drastically decreased as salt concentration increased. There was a significant interaction between genotypes and salt treatments for K^+/Na^+ ratio, which suggests that the performance of cotton genotypes was different for K^+/Na^+ ratio across salt treatments. The K^+/Na^+ ratio at 250 mM NaCl was 0.56, which was significantly lower than the control (12.18), but was not significantly different from than at 125 mM NaCl (0.83) (Figure 3.21). Although MT1219 had the highest K^+/Na^+ ratios: 1.44 and 0.91 at 125 and 250 mM NaCl, respectively, it wasn't significantly higher than that of all the other genotypes (Figure 3.22).

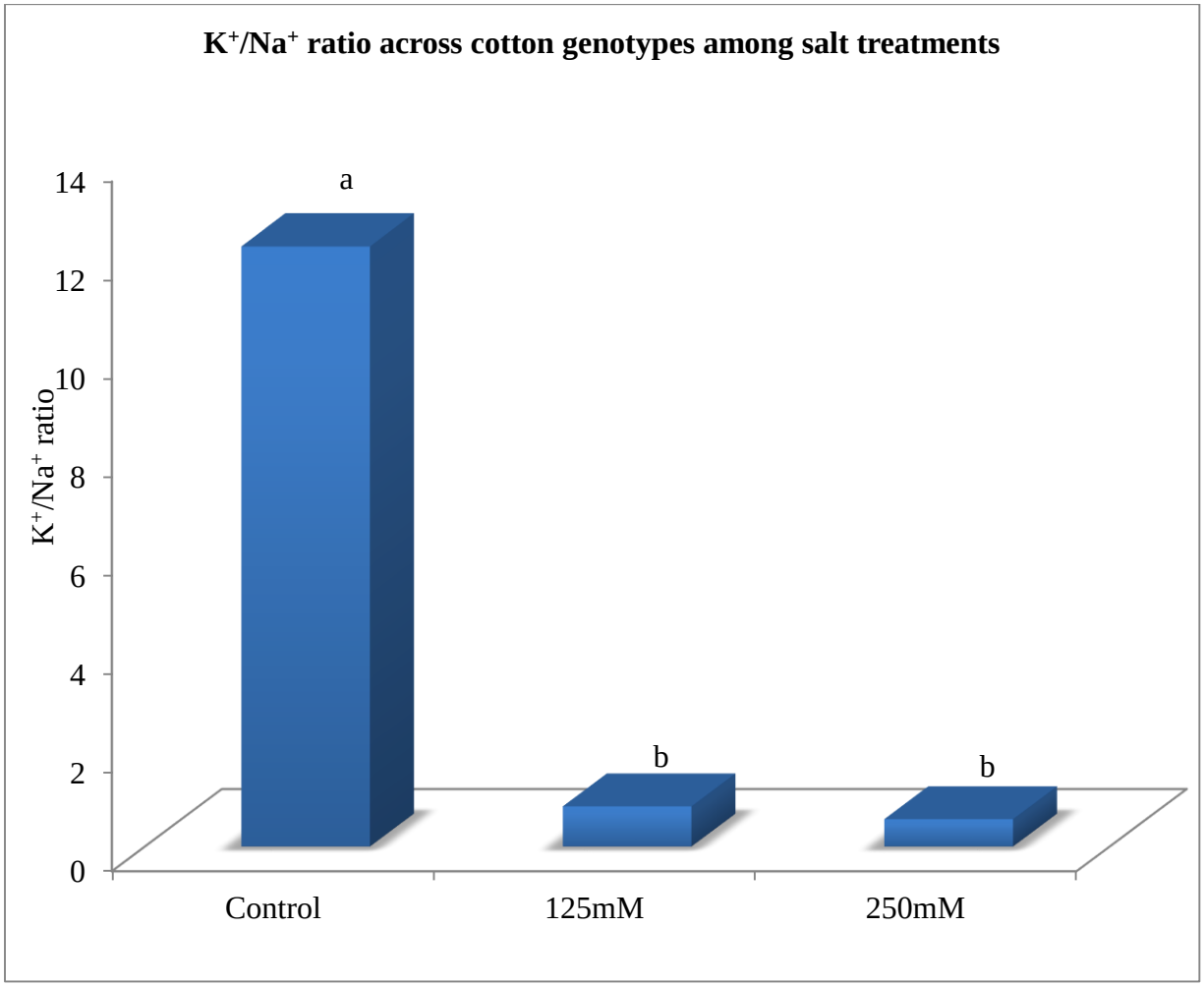

Figure 3.21 K^+/Na^+ ratio across cotton genotypes among salt treatments. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

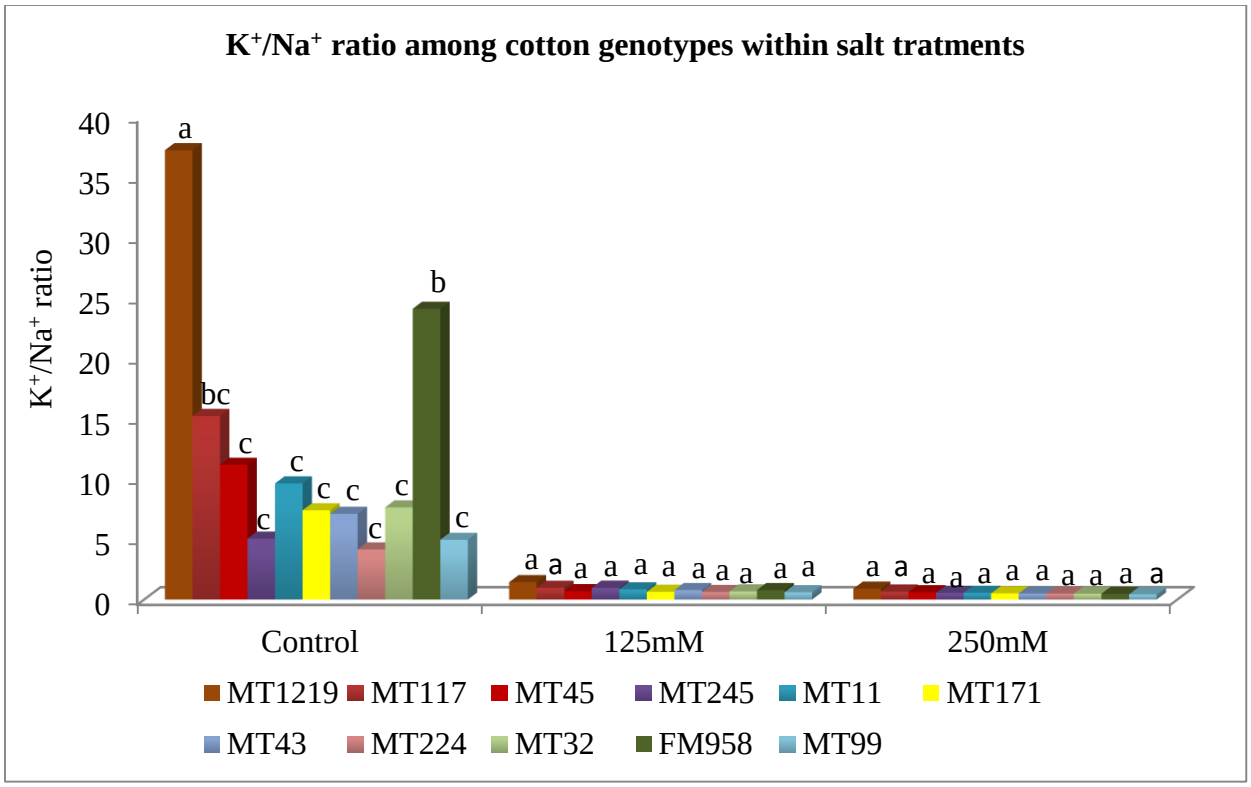

Figure 3.22 K^+/Na^+ ratio among cotton genotypes within salt treatments. Within salt treatments, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

3.4 DISCUSSION

A preliminary test was conducted in the greenhouse under a hydroponic technique using 0, 125, and 250 mM salt concentration treatments for 14 days on cotton seedlings. It was observed that seedlings wilted rapidly and died within 2 and 5 days at 250 mM and 125 mM NaCl, respectively. The rapid death may be due to osmotic shock, which may result in rapid loss of water from leaves cells, ultimately leading to cell collapse (Yeo et al., 1991). Therefore, the experimental protocol was revised and an increment of salt concentrations over time was used to acclimate the seedlings to elevated salt concentrations until final concentrations were reached. Timing of salt application is an important factor in the experiment since *Gossypium* spp. seedlings are most vulnerable at early stages of development before the emergence of the first true leaves after 10-14 days. Application of salt treatments before the emergence of first true

leaves is not recommend because the salt might suppress or kill the first true leaves and mask genotypic differences in salt tolerance parameters. Using our recommended protocol, we delayed the initiation of the salt treatments until the attainment of the first true leaf stage (14 days after seed sowing) and only then applied the salt concentration treatments in increments of 62.50 mM in each day to prevent salt stress shock.

Researchers have developed a number of salt screening techniques to evaluate the salt tolerant cotton genotypes. Due to spatial and temporal variations in soil salinity across the field, hydroponic and pot cultures have been widely used for salt screening because they are rapid and reliable (Akhtar et al., 2010; Munns et al., 2002). Akhtar et al. (2010) reported that hydroponic and soil based screening techniques are both effective in salt screening for cotton genotypes. However, Tavakkoli et al. (2012) reported that salt screening using hydroponic techniques did not truly replicate field conditions because seedlings are exposed to salt stress for only a short period of time. In contrast to hydroponics, a strong correlation between a pot culture based salt screening technique and field screening was observed (Tavakkoli et al., 2012). In this study, the hydroponic technique had presented a limitation due to its inability to provide adequate physical support to seedlings beyond 14 days after salt treatments. The hydroponic technique did have the advantage of using less space but after the initial screening a pot based protocol was used.

Based on reduction in plant height, there was a similarity in genotype ranking for salt tolerance across salt treatments between hydroponic and pot culture except for MT45 and MT224. In the hydroponic technique, MT45 and MT224 had one of the highest and lowest reduction in plant height across salt concentrations, but the opposite was observed for MT45 and MT224 in the pot culture, respectively. There were also some discrepancies among the measured plant parameters between the hydroponics and pot culture. These discrepancies for genotype

rankings against elevated salt concentrations might be due to fundamental differences in nature between the hydroponic and pot culture (Tavakkoli et al., 2012). Compared to pot culture, smaller and thicker leaves were observed in the medium and high salt concentrations (125 and 250 mM) under hydroponic technique. The rate of reduction (percent reduction) in plant parameters measured over time was faster in hydroponics than pot culture.

Identification of variation in genotype response to elevated salt concentrations is a first step to identify and breed for more salt tolerant cotton. Researchers have been using a variety of phenotypic, physiological, and biochemical criteria to identify the salt tolerant genotypes in many crop species (Higbie et al., 2010; Parida and Das, 2005). Phenotypic criteria, such as percent reduction in plant height and dry shoot weight under salt stress are easy and efficient screening criteria to identify salt tolerant cotton genotypes. Reduction in plant height under salt stress in the field condition might be an easy, reliable, and non-destructive selection criteria because the impact of increasing salt concentration on plant height is prominent (Higbie et al., 2010; Lashin and Atanasiu, 1972). In contrast, Abbas et al. (2011) argued that percent reduction in dry shoot weight was a more viable criteria to select salt tolerant genotypes because it is highly correlated with Na^+ concentration in the leaves. This study showed that reduction in plant height and dry shoot weight were both equally effective in screening for salt tolerance of any cotton genotypes because they were highly correlated with each other (Table 3.4). This study also supports the finding that reduction in plant height is an easy, rapid, and reliable criteria for screening salt tolerance in both greenhouse and field conditions.

Although cotton is relatively tolerant to salt (7.7 dSM^{-1}) compared to many other row crops, a significant and rapid decrease in plant height and dry shoot weight was observed across elevated salt concentrations. Out of 11 genotypes, the performance of MT1219 and MT245 were

phenotypically consistent and better than other genotypes in both hydroponic and pot culture. Based on pot based screening method, FM958, MT99, and MT224 had the greatest reduction in plant height, fresh and dry shoot weight and should be considered as salt sensitive genotypes. Compared to other genotypes, MT11, MT1219, MT45, and MT245 had the lowest reduction in plant height, fresh and dry shoot weight and should be considered as salt tolerant genotypes.

Physiologically, the accumulation of Na^+ in leaves is the primary response of genotypes against elevated salt concentrations (Cramer, 2002; Meneguzzo et al., 2000). It is well established that a low Na^+ concentration in leaf tissues is positively correlated with salt tolerance in many crops species (Basel, 2011; Munns et al., 2003). There was a significant positive correlation between the percent reduction in plant parameters (plant height, fresh and dry shoot weight, fresh and dry root weight) and Na^+ accumulation in this study, supported by the observation that the lowest reduction in plant height and dry shoot weight was found across genotypes with the smallest accumulation of Na^+ in the leaf tissues (Table 3.4). The data revealed that there was an increase in Na^+ concentration among genotypes when salt treatments increased from 0 to 250 mM NaCl, but the rate of Na^+ accumulation in the leaves varied across the genotypes (Figures 3.16 and 3.17). Compared to the control, a thin stem with small leaves was observed across genotypes under salt stress. This might be due to a rapid accumulation of Na^+ on the leaf tissue under elevated salt concentrations which interferes with cellular metabolic activities, and ultimately reduces cell division and elongation (Cramer, 2002; Fricke and Peters, 2002). Under both low and high salt concentrations (125 mM and 250 mM NaCl), chlorosis and necrosis was observed in the older leaves across all genotypes. Such symptomology is likely due to longer exposure to salt stress in old vs. young leaves (Munns and Tester, 2008). Across salt

concentrations (250 mM), the lowest Na^+ accumulation in the leaf tissues was observed in MT1219 followed by MT45, MT245, and MT11 and all are considered as salt tolerant varieties.

The pattern of Na^+ accumulation in various plant tissues under salt stress may be due to both inter- and intra-specific variation among genotypes, which allows for the discrimination between salt tolerant and susceptible genotypes (Ashraf, 2002; Flowers et al., 1977). Exclusion of sodium ion entry and control of its transport through the root system is one of the important physiological mechanisms to limit the accumulation of Na^+ and prevent it from reaching toxic levels in leaf tissues. Such exclusion can be achieved by low net Na^+ uptake by the root cortex and by tight control of ion transport into the xylem through the parenchyma cells in the roots (Davenport et al., 2005). Previous studies reported that Na^+/H^+ and K^+/Na^+ anti-transporter are the two important transport systems, which regulate the flow of Na^+ in, between, and within plant cells (Apse et al., 1999; Zhu et al., 1993). Compartmentalization of Na^+ into vacuoles in the cytosol, regulation of K^+, and maintaining a high K^+/Na^+ ratio in the cytosol are features, also observed in many salt tolerant crop species (Munis et al., 2010; Munns and Tester, 2008).

The increased concentration of Na^+ in the medium competes with K^+ for absorption, which leads to a significant decreased in K^+ and an increase in Na^+ concentration in the leaves (Ashraf and Ahmad, 2000; Higbie et al., 2010; Qadir and Shams, 1997). The decrease in K^+ concentration in the leaves may also be responsible for reduction in the leaf expansion and plant growth (Higbie et al., 2010). In this study, K^+ concentrations were significantly decreased in the leaf tissues at elevated salt concentrations (Figure 3.18). Previous studies have shown that K^+/Na^+ ratio significantly decreases with increased salt concentration (Ahmad et al., 2002; Khan et al., 2009). The high K^+/Na^+ ratio in the leaves is important for normal cellular function and might be considered another useful indicator for salt tolerance in many crop species (Zhu, 2003).

This study also showed that high K^+/Na^+ ratio in the leaf tissues decreases the rate of reduction in plant growth under salt stress conditions (Table 3.4). Although there were no significant differences among genotypes at 250 mM, salt tolerant genotypes, i.e. MT1219, MT45, MT245, and MT11 had slightly higher K^+/Na^+ ratios than the salt susceptible genotypes MT224, FM958, and MT99. A high K^+/Na^+ ratio in the leaf tissues in the salt tolerant genotypes might be due to selective uptake of K^+ over Na^+ and exchange of K^+ over Na^+ during ion transport in the plasma lemma of root cortex (Jeschke and Wolf, 1988).

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CHAPTER 4: PREDICTION OF SPINNING VALUES OF COTTON FIBERS

4.1 INTRODUCTION

Cotton is the leading natural textile fiber as well as one of the most important oilseed crops in the world. It is important in agricultural trade and is grown in more than 80 countries. The goal of cotton breeders from public and private institutions is to develop high yielding cotton varieties with improved fiber qualities to meet the requirements of standard yarn properties. The development of new cotton varieties takes about 8-10 years and requires meticulous effort and time (Poehlman, 1987; Russell, 1978). Knowledge of the relationship between yarn and fiber properties is important for cotton breeders to select high quality genotypes/offspring in the breeding program. Yarn is a long twisted strand of the cotton fibers prepared by using various spinning techniques. In the textile industry, yarn quality is a vital component which determines the quality of fabric and clothes (Zhu and Ethridge, 1996).

Before the introduction of High Volume Instrument techniques, cotton breeders used hand grading and staple length of fibers to select the cotton fibers for the spinning industry (Majumdar et al., 2004). In 1969, the High Volume Instrument (HVI) was developed by the United States Department of Agriculture (USDA) to mechanically measure the fiber properties. HVI provides information about upper half mean length (UHML), micronaire, strength, uniformity, elongation, reflectance, and yellowness of fibers (Sasser, 1981). HVI is currently used as the marketing tool for textile mills to evaluate the fiber properties in the bales of cotton (Suh and Sasser, 1996). It is very popular tool for cotton breeders because a large number of fiber samples can be processed in short periods of time at low cost. One of the problems with using HVI data directly, is that multiple, individual data points are generated. The interrelationship between the various HVI parameters is not represented and it is their interplay,

along with spinning equipment variables that lead to the production of usable yarn. In essence, some sort of selection index could be useful if it was able to reasonably and reliably predict yarn quality. Two recent attempts to develop such an index, based on the HVI data and consultation with the textile professionals, are the fiber quality indices: Qscore 1 and Qscore 2. These were developed as a single index incorporating four different fiber properties (length, strength, uniformity, and micronaire) (Bourland et al., 2010). Most cotton breeders hesitate to use this score in their breeding program because this algorithm gives an arbitrary weight for each fiber property and the optimum weight of each fiber property in relation to yarn quality is still unknown (Bourland et al., 2010).

Though HVI provides the overall properties of fiber, textile professionals are also interested in the variability of individual fiber properties within a sample because uniform individual fiber properties enhance spinning efficiency and control the quality of fabric products (Hearle and Morton, 2008). In 1990, the Advanced Fiber Information System (AFIS) was developed to measure twenty different fiber properties and distribution of fiber length in a sample used for processing (Shofner et al., 1990). Although various fiber properties are determined by using HVI and AFIS, it is still challenging to give priority to a parameter or group of parameters to select the best fibers for industrial uses (Majumdar, 2010). Since various properties of cotton fibers largely influence the final quality of yarn, researchers have developed mathematical, regression, and other computation models using various properties of fibers obtained from HVI and AFIS technology to predict the yarn properties.

With an advancement of computational and analytical tools, a number of data mining and machine learning techniques are increasingly popular and widely used to develop predictive models for simple to complex data in many scientific disciplines. Generalized linear models are

the simplest and the most widely used tools to determine the functional relationships between independent and dependent variables. This technique enables us to quantify the effect of each independent variable on the dependent variable (Tranmer and Elliot, 2008). Multiple linear regression is represented by the following expression (Kutner et al., 2004).

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{\varepsilon}$$

In the model, Y is represented by the n × 1 matrix of the dependent observations, X and β are represented by n × p matrix of the independent observations and p × 1 vectors of the unknown regression coefficients, respectively. The error term (ε) is represented by n × 1 matrix of the errors and assumed to be independent and normally distributed (N (0, σ^2)) (Kutner et al., 2004). The least square estimation is most commonly used in the regression analysis such that it minimizes sum of square deviations between the actual observation and the regression (Matthews, 2005). The least square estimator for β is given by

$$\widehat{\boldsymbol{\beta}} = (\boldsymbol{X'X})^{-1}\boldsymbol{X'Y}$$

The individual coefficient ($\hat{\beta}i$) determines the partial effect of X_i on Y holding all the regressors constant (Matthews, 2005; Myers, 2000). In cotton breeding, it has been used to determine the functional relationship between fiber and yarn properties (Üreyen and Gürkan, 2008; Zhu and Ethridge, 1996).

Path analysis is a form of standardized linear regression, which has been widely used in agriculture (Bhatt, 1973; Wullschleger et al., 2010). Path analysis determines the interrelation among the variables, which affect the dependent variable. In other words, it allows us to determine the direct and indirect effects of each explanatory variable on the response variable in the system (Wright, 1921). Path analysis is applicable in highly correlated agronomic and genetic traits (Kang et al., 1983).

Regression/decision tree methods are more complex and flexible analytical tools than classical linear regression, and can be applied to determine the simple linear to complex non-linear relationships among multiple traits. This technique splits data sequentially into two distinct and exclusive sets by applying a recursive binary splitting approach and building the trees (Loh, 2002). This approach splits the traits in such a way that minimizes the residual sum of squares (RSS) and maximizes the homogeneity within each resulting group. The cost complexity pruning method is used to optimize the size of the trees by pruning the trees and prevent over fitting (James et al., 2014). Compared to linear modeling, the decision tree method is more graphically representative, similar to a flow chart and is easier to interpret, but it might not have the same prediction accuracy (De'ath and Fabricius, 2000). Boosting and random forest are both ensemble methods, which combine a large number of regression trees obtained from the bootstrapped dataset (Breiman, 1996; Büchlmann and Yu, 2002; James et al., 2014). These ensemble techniques improve the stability of the regression tree (Breiman, 1996; Witten et al., 2011). Boosting uses prior tree information to build the new trees and reduces the correlation between bagging trees (James et al., 2014). In contrast, random forest uses a random subset of predictors from all available predictors in each split so that this technique might reduce the correlation among the bagging trees (Breiman, 2001). Although ensemble techniques minimize the variance and improve the prediction accuracy, the interpretation is very difficult compared to regression trees (James et al., 2014).

Artificial neural networks (ANN) is a complex and highly flexible data mining and machine learning technique, which is widely applied in the modeling of highly correlated, complex, linear to non-linear, and multidimensional data (Altun et al., 2007). ANN data processing systems mimic biological nervous systems and seek to establish the complex

relationship of between the input and output parameters (Gurney, 1997). In ANN models, input layers receive the information in the form of explanatory variables and process them through 'hidden' layers using the sigmoid activation function and predict the output (Goh, 1995; Karayiannis and Venetsanopoulos, 1992; Priddy and Keller, 2005). The predicted output is compared with actual output to determine an error. An error signal is back propagated from the output layers towards the input layers through a neural network. With ANN, the steepest descent method is used to adjust the weight in each iteration so that the error signal is decreased and this process is continued until a minimal difference between two outputs is achieved (Khazaei et al., 2008; Majumdar, 2010).

Since as early as 1980, cotton breeders have investigated two data mining and machine learning techniques, such as classical linear regression and artificial neural network (ANN) to determine the functional relationship between yarn and fiber properties (Cheng and Adams, 1995; Ramesh et al., 1995). The varieties used in these older experiments and their limited data sets may no longer be relevant. Additionally, none of the published classical linear regression and ANN models used AFIS data. In addition, there are limited studies in the application of other data mining tools and techniques in cotton breeding. From HVI it is possible to calculate a spinning consistency index (SCI), which suggests the overall quality and spinning ability of cotton fibers and can be used to evaluate the technological value of cotton fibers. Unfortunately, this index is a "black box" for the cotton breeder, as the research that led to its development provides little rationale about how and what fiber parameters were considered in its development and SCI's ability to predict yarn properties. Therefore, the objective of this research is to develop a number of statistical models using data mining and machine learning tools to identify the

important fiber properties, which affects SCI and to compare this index with yarn strength to determine its applicability in the textile industries.

4.2 METHODS AND MATERIALS

4.2.1 Data collection

The yarn strength, HVI, and AFIS fiber data were obtained from the Southern Regional Research Center, New Orleans. The data consists of US National Cotton Variety Trial (NCVT) fibers samples collected over a two year period (2012 and 2013 growing seasons). The NVCT is a collaborative testing program wherein adapted high yielding varieties are compared across their larger target environments. A subset of the NCVT is the Regional High Quality Test where varieties of superior quality are compared across their target environments. All varieties grown are harvested for yield and boll sampling is used to analyze fiber quality prior to small scale yarn spinning. This NCVT data set provides the information about fiber traits of cotton cultivars grown in 22 locations across 12 US states in 2012 and 2013 growing seasons. In this study, we used 1610, 1539, and 1552 HVI, AFIS and yarn strength observations for statistical modeling, respectively. High volume instrument (HVI) provides the following fiber properties (Sasser, 1981):

1. Fiber length expressed as upper half mean length (UHML): It is the average of the longest 50% of the fibers.
2. Fiber uniformity: It is the ratio of average length to UHML of fibers.
3. Micronaire (Mic): It is determined by measuring the permeability of air passing through cotton samples. It is an indirect measure of fineness and maturity of the fibers.
4. Fiber strength: It measures the amount of force (g) required to break one tex of fiber bundle.

5. Fiber elongation: It refers to the elasticity of fibers.
6. Reflectance (whiteness) of fibers.
7. Yellowness of fibers.

HVI data also provides the information necessary to calculate a SCI, which determines the spinning ability of fibers for ring spun in the textile industry (Majumdar et al., 2004).

AFIS provides twenty different fiber properties. Out of them, the following are the most important fiber properties in cotton breeding program (Kelly et al., 2012):

1. Neps: Refers to clumps of the immature fibers. AFIS measures the number of neps in the sample.
2. Fiber length expressed as upper quartile length (UQL): AFIS measures the length that exceeded by 25% of the fibers by weight.
3. Fineness: AFIS measures the cross-section area of individual fiber by penetrating the near infrared spectrum to determine the fineness.
4. Immature fiber contents (IFC): AFIS measures the number of immature fibers in the sample.
5. Short fiber content (SFC): It measures the amount of short fiber contents by weight (length less than 12.5 mm) in the sample.
6. Trash content: It measures the total amount of trash, such as leaf, bark, seed coats per gram in the fiber sample.

For the fiber data used in this research, HVI data was determined using a Uster® HVI 1000 (Uster Technologies AG, Switzerland). AFIS data was determined using a Uster® AFIS PRO (Uster Technologies AG, Switzerland). Yarn strength measurements were determined using a Uster® TENSORAPID (Uster Technologies AG, Switzerland).

4.2.2 Model development

The data mining and machine learning tools available in R software (www.r-project.org) were used to develop the statistical modeling for spinning consistency index except the artificial neural network. The artificial neural network model was developed in JMP Pro 11.2 (SAS Institute Inc., Cary, NC). The dataset was randomly split into a 70:30% training to validation data. The lm and lm.beta functions were used for classical multiple linear regression and path analysis, respectively. The rpart library package was used for building the regression/decision trees. The ensemble methods, such as random forest and boosting were developed by using randomforest and gbm library packages, respectively. Each ensemble method combined 5,000 trees together to determine the relative importance of parameters for spinning value of cotton fibers. In artificial neural network, the number of hidden layers and number of neurons in each hidden layer were adjusted to improve the best fitting model.

After developing the models on the training data set, they were validated on the validation data set to determine the reliability and accuracy of the models. The best models were selected based on the coefficient of determination (R^2) obtained from validation data set.

4.3 RESULTS

4.3.1 Multiple linear regression

Fiber length and uniformity index were highly correlated (>0.75). This is not surprising because the estimate of uniformity index is based on fiber length. Consequently, uniformity index was dropped as a independent variable from the statistical models to avoid over fitting, except in path analysis. Multiple linear regression resulted in all fiber parameters being highly significant ($P<0.01$) (Table 4.1). This suggests that any improvement in fiber length, strength,

and elongation significantly increases the SCI, while increasing micronaire reduces the spinning ability of fibers.

Table 4.1 Regression coefficients from multiple linear regression of HVI fiber properties on spinning consistency index.

Parameters	Estimate	Standard error	t value
Intercept	-113.03	3.69	-30.62**
Mic	-5.03	0.45	-11.19**
Length	142.44	3.20	44.45**
Strength	3.04	0.07	44.25**
Elongation	2.00	0.19	11.23**

**=Significant at $P \leq 0.01$.

Although nep was not significant (P=0.73), it showed that it reduced the spinning values of cotton fibers (Table 4.2). Higher values for measurements of fiber fineness represent coarse fibers, whereas lower values represent finer fibers. The model indicates that as fiber fineness values increase, fibers are more coarse, and this has the significant effect of reducing the spinnability (P<0.01). Thus, fibers with a lower fineness values demonstrated superior spinnability. Similarly, upper quartile length (UQL) significantly affected spinnability as longer fiber samples had a superior spinnability (P<0.01). The amount of short fiber content (SFC), immature fiber content (IFC), trash content (TC) decreased the spinnability of cotton fibers (P<0.01) (Table 4.2).

Table 4.2 Regression coefficients from multiple linear regression of AFIS fiber properties on spinning consistency index.

Parameters	Estimate	Standard error	t value
Intercept	-10.21	11.99	-0.85
Nep	-0.002	0.008	-0.34
SFC	-1.50	0.28	-5.22**
UQL	169.70	5.59	30.36**
Fineness	-0.22	0.03	-6.10**
IFC	-1.80	0.36	-4.88**
TC	-0.006	0.0006	-10.83**

** = Significance at $P \leq 0.01$.

4.3.2 Path analysis

Path analysis provided detailed information how the predictors were correlated with each other and how they collectively affected the spinning consistency index (Figure 4.1). Each predictor had its own direct effect (partial standardized coefficient) and the indirect effects (association with other predictors). Based on partial standardized coefficients, the HVI fiber data showed that fiber strength and uniformity index had the highest direct effect (0.52 and 0.42, respectively). Although the fiber length had the modest direct effect on spinning values of cotton fibers (0.18), it had the highest indirect effects through all predictors (0.69, 0.76, -0.14, -0.10). The lowest direct and indirect effects were observed for micronaire and fiber elongation. Based on the path analysis, any improvement on strength and uniformity index improved the spinning ability of fibers, while length had a modest direct (but a substantial indirect effect) on the spinning ability of fibers.

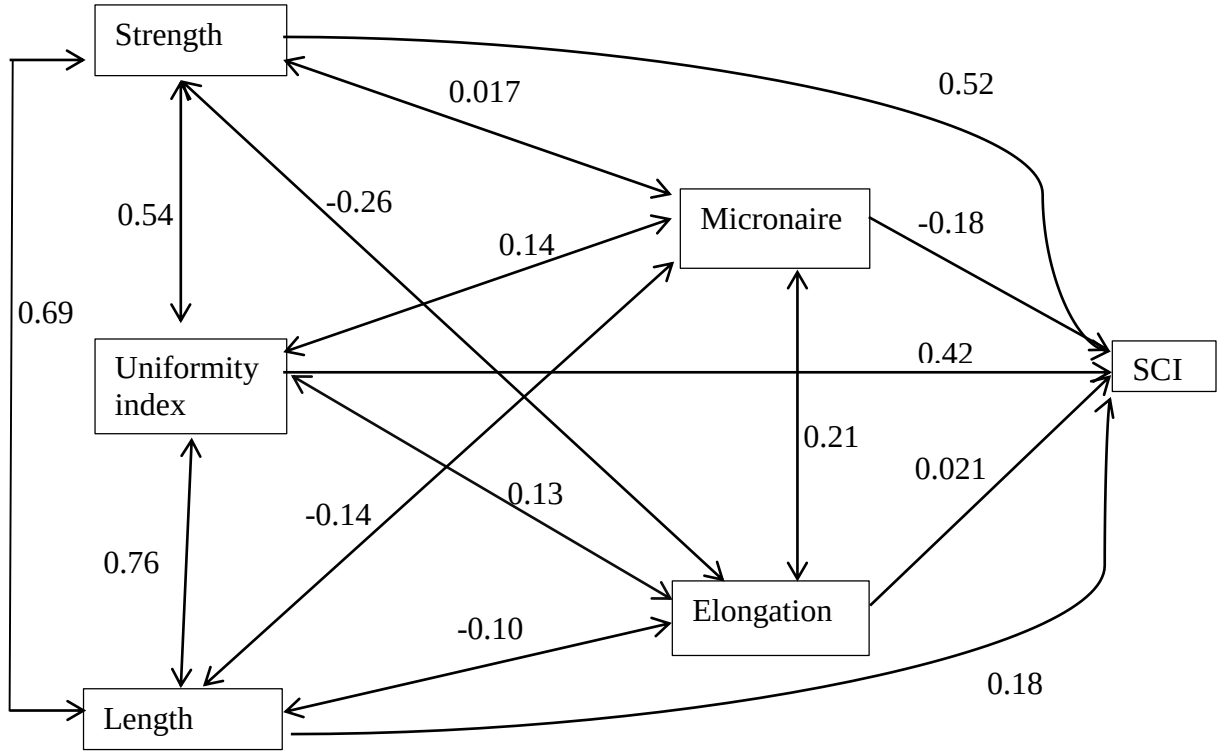

Figure 4.1 A path diagram showed the direct and indirect effects of HVI fiber traits on SCI.

The AFIS data showed that upper quartile length (UQL) had the highest direct effect (0.71) and modest indirect effects through all fiber traits (-0.66, -0.20, -0.01, -0.09) on the spinning values of fibers (Figure 4.2). The short fiber content, fineness, and amount of trash content had the modest direct effect on spinning ability of cotton fibers (-0.15, -0.11, and -0.16, respectively). The short fiber content and fiber fineness had the highest indirect effects through all fiber traits (-0.66, 0.55, -0.35, and 0.10, and -0.35, -0.50, -0.20, and -0.09, respectively) on spinning value of cotton fibers (Figure 4.2).

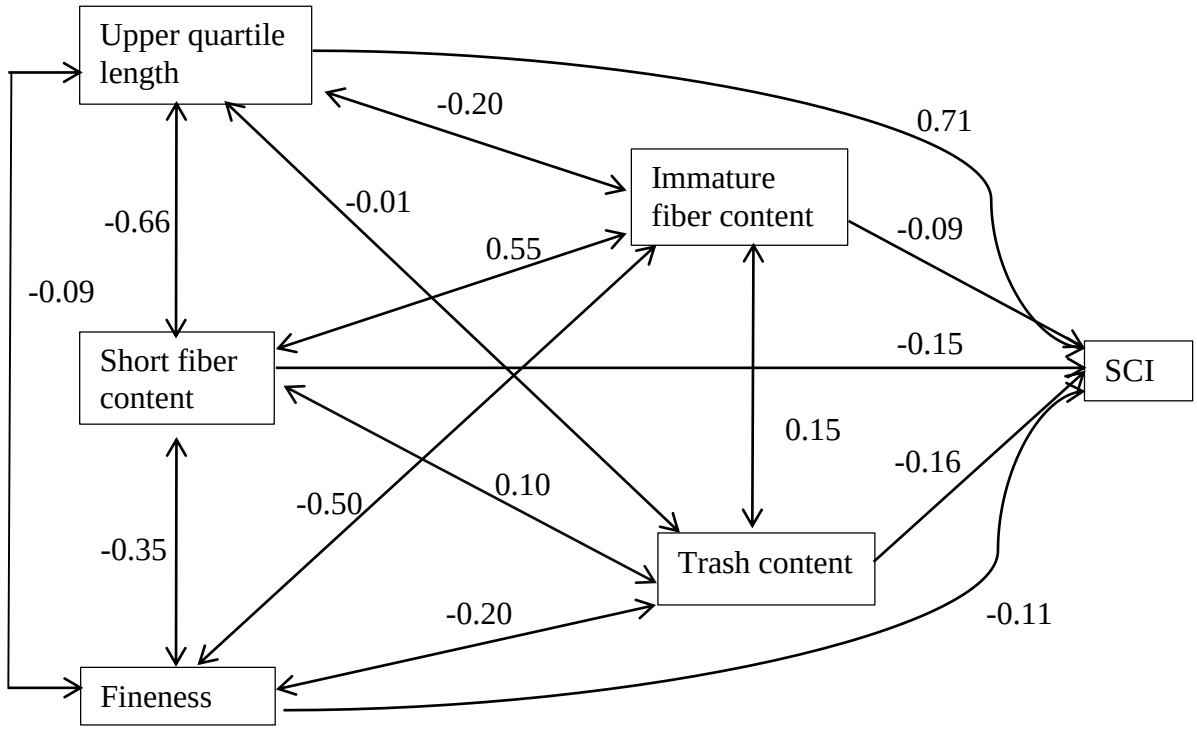

Figure 4.2 A path diagram showed the direct and indirect relationship of AFIS fiber parameters on SCI.

4.3.3 Regression/decision trees

The regression/decision tree analysis of HVI data indicates that fiber length was the most important parameter, followed by fiber strength, and both together had the highest effect on the spinning ability of cotton fibers, considerably more so than other HVI parameters (Figure 4.3). It

can be concluded that cotton fiber of length less than 0.93 inches had the lowest spinning consistency index. The highest spinning index was achieved by selecting for fiber which had a length and strength greater than 1.36 inches and 44.07 g/tex, respectively. The decision tree makes it easier for cotton breeders to select those fiber traits, which have the most effect on spinning value of cotton fibers to improve fiber quality.

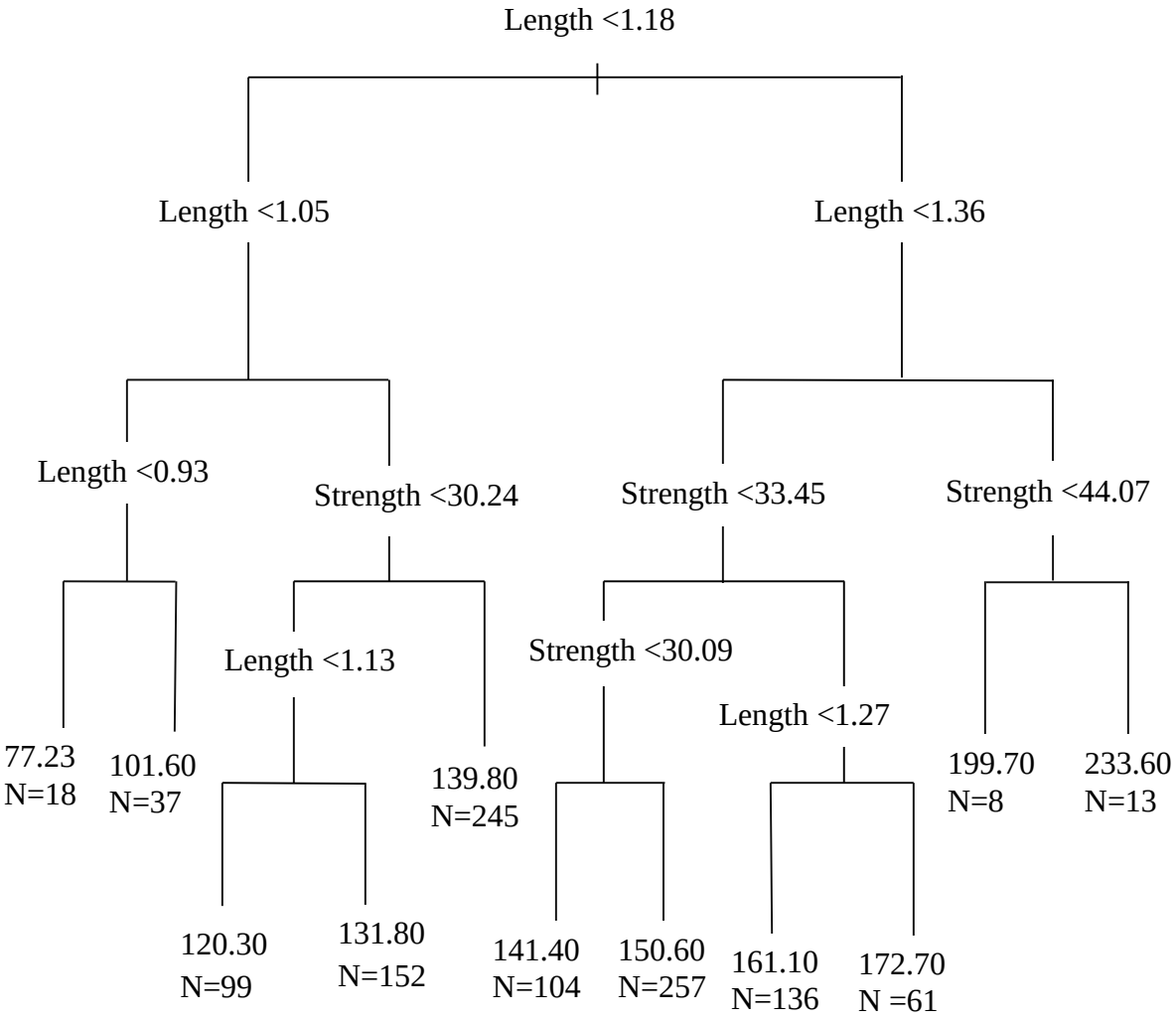

Figure 4.3 Regression/Decision trees showing impact of HVI fiber length and strength at each split on spinning consistency index.

The AFIS fiber data again indicate that upper quartile length (UQL) is the most important parameter to improve the spinning value of cotton. The cotton fibers with an UQL of less than 1.01 inch had the lowest spinning value, while UQL values greater than 1.43 inch had the highest

spinning value of cotton fibers (Figure 4.4). The amount of trash content and short fiber content had the modest effect on spinning values of cotton than other parameters. It suggests that the increased trash content and short fiber content in the fiber bundles decreased the spinning values of cotton.

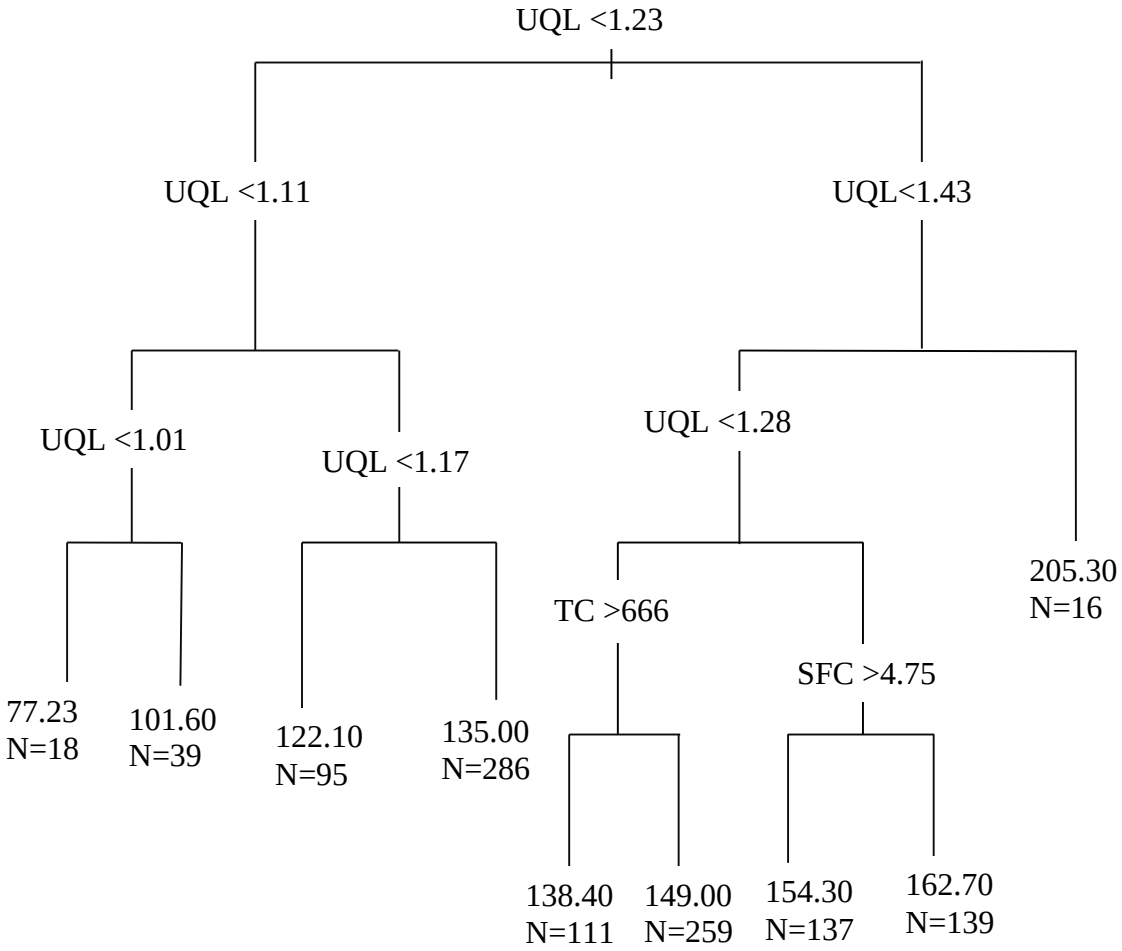

Figure 4.4 Regression/Decision trees showing the impact of AFIS UQL at top split, and TC and SFC at bottom splits on spinning consistency index.

4.3.4 Random forest and boosting

The ensemble methods results, for both random forest and boosting, suggest that fiber length and strength are the most important traits for maximizing SCI. There were, however, some discrepancies between the two methods in their calculation of the relative importance of

micronaire and elongation (Figure 4.5). In random forest, the mean decrease in prediction accuracy (or %IncMSE – percent increase in mean square error) was calculated for each fiber trait, suggesting that fiber traits with highest values are the most important parameters for improving the spinning consistency index.

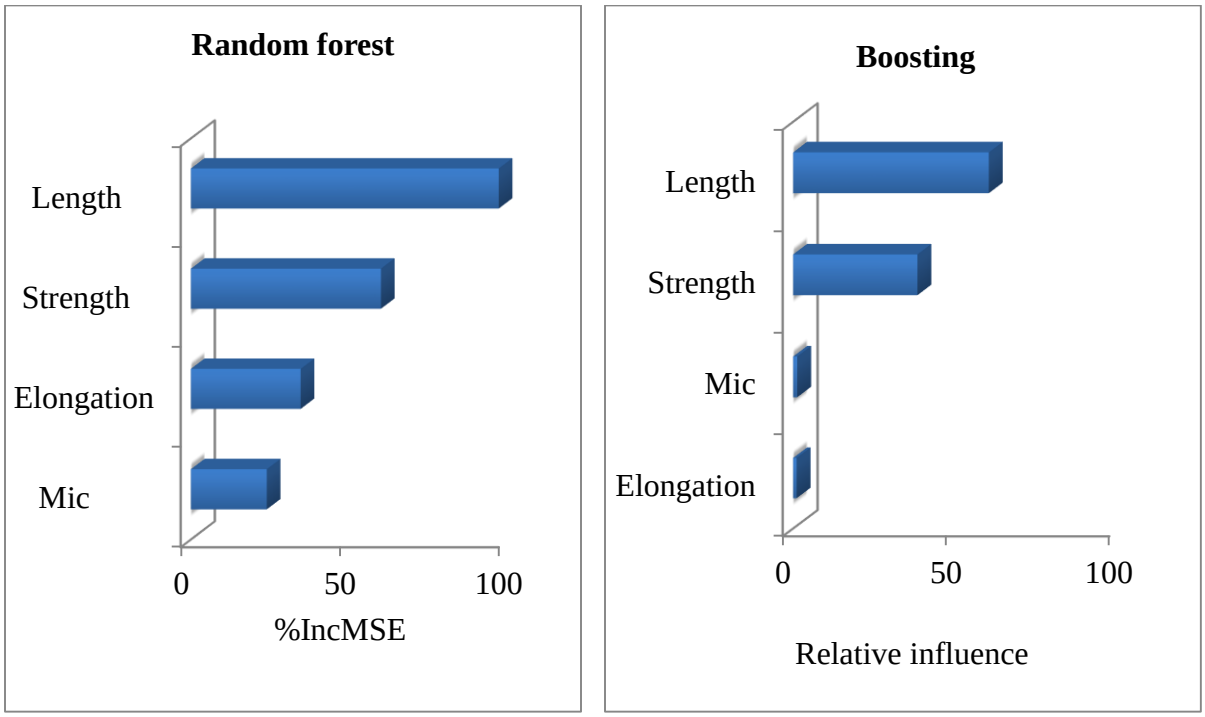

Figure 4.5 Relative importance of HVI parameters on spinning consistency index as determined by random forest (left) and boosting (right).

Analysis of AFIS data for both methods (random forest and boosting) clearly demonstrates that upper quartile length (UQL) is the most important parameter to improving the spinning ability of cotton fibers (Figure 4.6). Although there were some discrepancies in ranking of the other fiber parameters, short fiber content and trash content were consistently ranked as important and only modest effect estimates were detected for fiber fineness and immature fiber content on the spinning ability of cotton fibers. Nep counts (Nep) had the lowest effect on spinning values of cotton fibers (Figure 4.6).

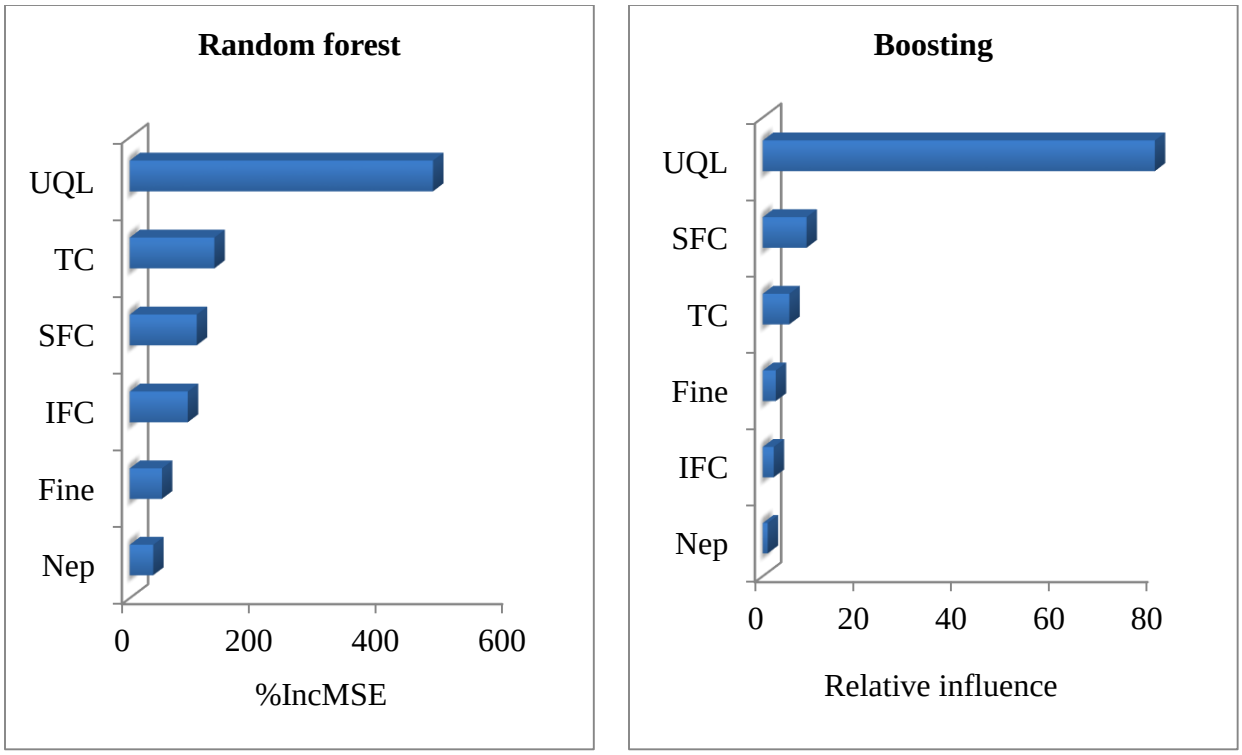

Figure 4.6 Relative importance of AFIS fiber parameters on spinning consistency index as determined by random forest (left) and boosting (right).

4.3.5 Artificial neural network

Using HVI fiber properties the spinning consistency index (SCI) was best predicted by a 4:6:4:1 neural network model. The 4:6:4:1 neural network refers to four inputs, 6 and 4 neurons in hidden layers 1 and 2, and one output, respectively as shown in the Figure 4.7. Fiber length was the most important fiber trait, followed by fiber strength, in determining the spinning value of cotton fibers (Figure 4.8).

The AFIS fiber properties resulted in a 6:3:4:1 artificial neural network as being the best model for predicting spinning consistency index (Figure 4.9). The artificial neural network showed that upper quartile length and short fiber content were the most important fiber properties, while nep was the least important fiber property in predicting the spinning value of cotton fibers (Figure 4.10).

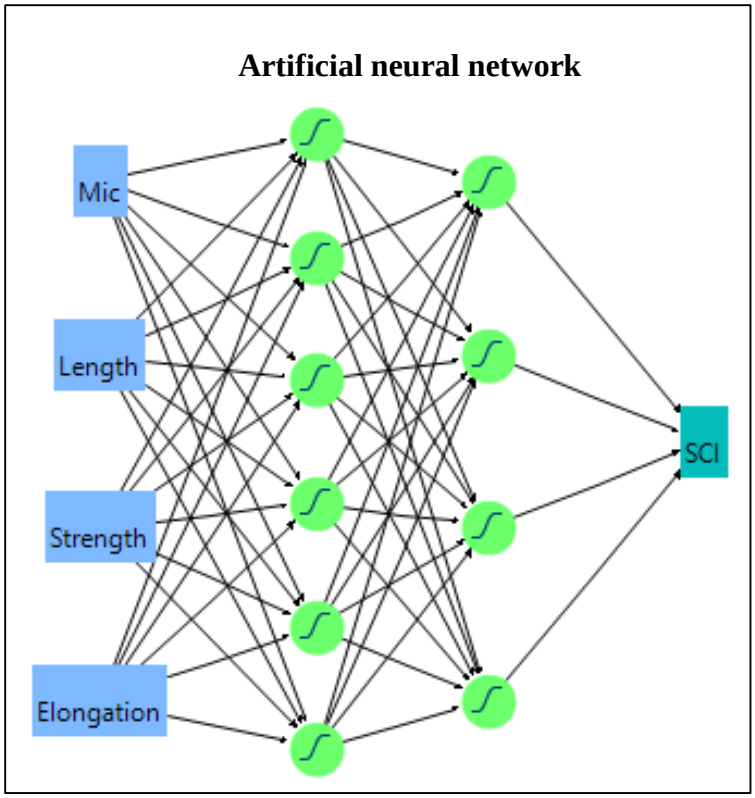

Figure 4.7 A 4:6:4:1 Artificial neural network model for predicting SCI (HVI).

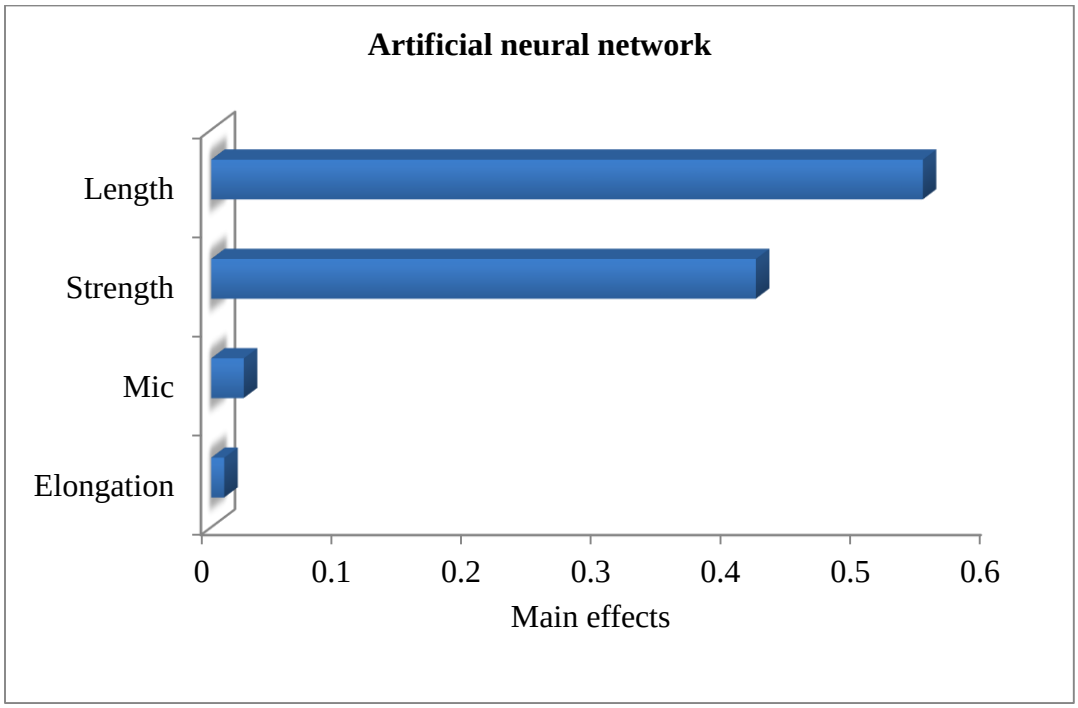

Figure 4.8 Relative importance of HVI fiber traits on spinning consistency index as determined by artificial neural network analysis.

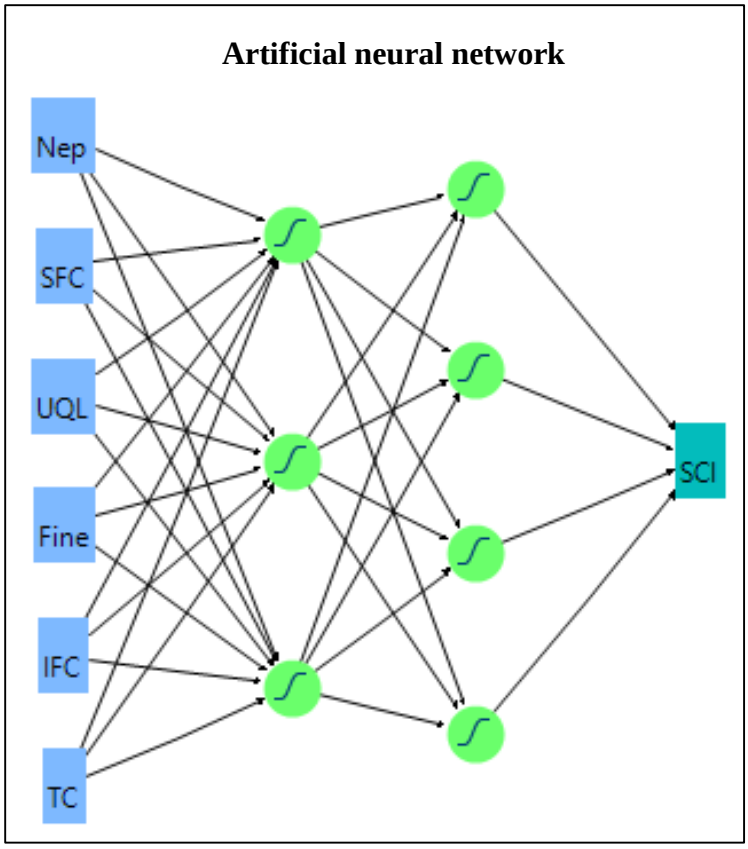

Figure 4.9 A 6:3:4:1 Artificial neural network model for predicting SCI (AFIS).

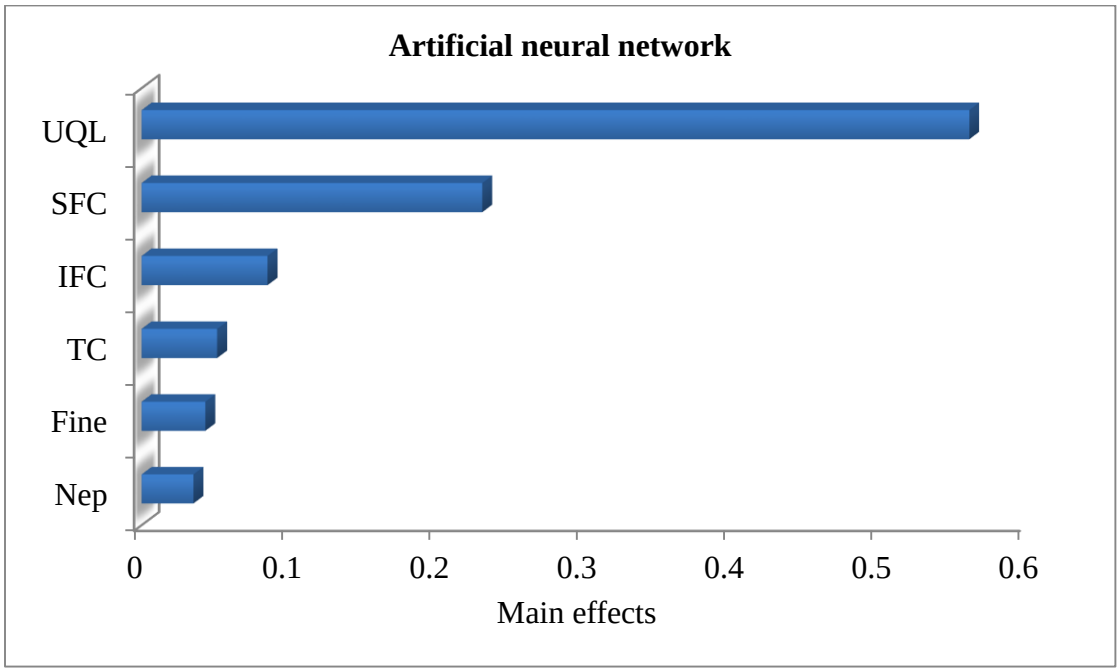

Figure 4.10 Relative importance of AFIS fiber traits on spinning consistency index as determined by artificial neural network analysis.

4.3.6 Correlation between SCI and yarn strength

The Pearson correlation showed that spinning consistency index (SCI) and yarn strength were significantly correlated with each other ($r=0.59$, $P<0.01$). The positive correlation suggested that higher spinning consistency index (SCI) significantly improved the yarn strength (Table 4.3).

Table 4.3 Pearson correlation coefficient.

	Qscore1	Qscore2	SCI	Yarn strength
Qscore1	1.00	0.86**	0.62**	0.42**
Qscore2		1.00	0.72**	0.48**
SCI			1.00	0.59**
Yarn strength				1.00

** = Significance at 0.01.

4.3.7 Comparisons of statistical models

All the statistical models developed from HVI fiber parameters predicted the spinning value of cotton well (Table 4.4). Based on the coefficient of determination (R^2), all the statistical models could be considered as competing models. In comparison to the other evaluated models in this study, regression tree had the lowest R^2 values for both training and validation data, to predict the spinning consistency index. The random forest model seems to over predict in the training data, but it predicted well in the validation data. Path analysis had the highest R^2 values for both training and validation datasets because of inclusion of uniformity index for model development. It must be noted that uniformity index was dropped in other models due to a high collinearity with upper half mean length (UHML).

Table 4.4 The coefficient of determination (R^2) of statistical models (HVI).

Statistical models	Training data	Validation data
1. Multiple linear regression	0.93	0.93
2. Path analysis	0.98	0.98
3. Regression trees	0.86	0.84
4. Random forest	0.98	0.92
5. Boosting	0.92	0.91
6. Artificial neural network	0.93	0.92

The developed models based on the AFIS fiber properties successfully predicted the spinning consistency index (SCI) of cotton fibers (Table 4.5). The lack of fiber strength data in this dataset is likely a culprit for this. As was the case for HVI fiber data, regression trees had the lowest R^2 values for both datasets, while random forest over predicted in the training data, but had a lower R^2 in the validation data. Although artificial neural network had relatively higher R^2 value in validation data, all the developed models could be considered as competing models for prediction of spinning consistency (SCI) index of cotton fibers.

Table 4.5 The coefficient of determination (R^2) of statistical models (AFIS).

Statistical models	Training data	Validation data
1. Multiple linear regression	0.76	0.72
2. Path analysis	0.76	0.72
3. Regression trees	0.70	0.66
4. Random forest	0.95	0.72
5. Boosting	0.79	0.73
6. Artificial neural network	0.77	0.78

4.4 DISCUSSION

Except for the regression tree analysis, all the developed models agree that fiber length, strength, micronaire, and elongation were important fiber parameters in determining the spinning value of cotton fibers as calculated using the SCI formula. Among them, upper half mean length and fiber strength were the most important parameters that largely affected the spinning value of fibers. Previous studies have also shown that long fibers increased the efficiency of ring spinning yarn than short and medium fibers (Long et al., 2010). The importance of length and strength to maximizing SCI was also supported by path analysis, with a suggestion that they are positively associated with each other both directly and indirectly (Figure 4.1). The path analysis also showed that there was a significant positive correlation between fiber length and uniformity index. A high uniformity in fiber length reduces the amount of defective yarn, increases the efficiency of ring spinning, and enhances uniform dyeing, all ultimately leading to a high quality

end fabric products (Bradow and Davidonis, 2000). Fiber length is highly heritable trait, governed by four to six major genes, but genotype in response to growing environment is also partly responsible for fiber length (Paterson et al., 2003; Zhang et al., 2009). Over 50 years, cotton breeders have been incorporating this trait in cotton breeding program to improve the fiber quality, which also ultimately increases the spinning performance of cotton fibers.

These models also showed that increasing micronaire had a significant negative impact on the spinning ability of cotton fibers. The USDA Agricultural Marketing Service classifies cotton fibers based on the micronaire: values from 3.7-4.2 refer to premium category, the acceptable category ranges from 3.2-3.6 and 4.3-4.9, while a discount rate is applied to values outside of these ranges (El Mogahzy and Gowayed, 1995). The relative magnitude of micronaire compared to other traits in the models predicting SCI is, however, indicative of its lower rank amongst fiber quality selection criteria. The relatively broad acceptable micronaire range lacks comparative focus; fiber length and strength are clearly more directional. Within its range, increasing micronaire can be seen as a tradeoff to increasing yield. Consequently, cotton breeders have placed more emphasis on other fiber traits. Micronaire is also a dimensionless proxy value for information about the fineness and maturity of fibers, and is highly influenced by environment (Long et al., 2010). What is clearly demonstrable is that high micronaire is caused by mature and coarse fibers, and significantly reduces ring spinning performance, yarn evenness, and the number of fibers in yarn cross-section (Bradow and Davidonis, 2000; Kloth, 1998). Conversely, fine fibers and the amount of immature fiber are both responsible for low micronaire (Bradow and Davidonis, 2000). The AFIS models showed that fine fibers increased the spinning ability of cotton, while immature fibers decreased the spinning value of fibers (Table 4.2). Compared to discount range, premium and acceptable ranges have relatively a small amount of

immature and coarse fibers. As expected, micronaire does not have a huge impact on spinning performance of fibers because premium and acceptable ranges are most widely used in the selection of breeding progenies, cultivated varieties, and bales for yarn spinning.

All the models agreed that fiber strength is one of the important fiber traits, which influences the spinning value of cotton fibers. Munro (1987) reported that the individual fiber strength directly affects the yarn strength. With the advancement of high speed spinning technology, textile professionals prefer highly elastic, strong fibers because they minimize yarn breakage, and improve the spinning efficiency at low cost and also increase the elasticity of the fabrics (Bradow and Davidonis, 2000; Cheng and Adams, 1995). Here the developed models showed that fiber elongation had a very insignificant effect on the spinning consistency index. This may be due to the fact that all of the varieties used in this study would be considered to have good to excellent fiber elongation values to begin with, giving the models little variation to utilize. The path analysis agreed with previous studies and suggested that there was no significant correlation between fiber strength and elongation (Chee et al., 2005; Riley, 1997). Fiber strength is also genetically controlled by two to four major genes and highly heritable from generation to generation (Kohel et al., 2001; Zhang et al., 2009). Therefore, it can be successfully incorporated into breeding programs to improve the fiber quality. However, fiber elongation was controlled by twenty two minor genes and hard to incorporate all of them into a breeding program (Chee et al., 2005).

Using AFIS fiber property data, all the models agreed that upper quartile length (UQL) was the most important fiber property and positively influenced the spinning performance of fibers as calculated by SCI. Although there were some discrepancies in the ranking of the other fiber traits, fiber fineness was one of the more important traits for increasing the spinning value

of cotton fibers. Compared to coarse fibers, fine fibers increase yarn strength and uniformity leading to the production of strong and uniform fabrics largely by fitting a large number of fibers per unit cross-section (Hearle and Morton, 2008; Munro, 1987). The models also showed that the amount of short fiber content (SFC) reduced the spinning ability of fibers. Previous studies have also reported that increased short fiber contents increases the rate of yarn breakage and irregularities, and also reduces yarn uniformity, strength, and appearance of fabric products (Backe, 1986; Thibodeaux et al., 2008). Although the fiber length is largely determined by 2-4 major genes, short fiber content is determined by an interaction between genotypes by environment, harvesting techniques, and processing (e.g. ginning) (Bradow et al., 1999; Cui et al., 2003). Although nep was not significantly different, both nep and immature fiber contents reduced the spinning performance of cotton fibers. These factors are somewhat related in that immature fibers are more likely to entangle and lead to the formation of neps in yarn. These are important traits from the dyeing point of view, with elevated levels of both drastically reducing the uniformity dyeing (Smith, 1991; Thompson and Hsieh, 1998). Fortunately, the path analysis results imply that any improvement in the upper quartile length of fibers drastically reduced the amount of short and immature fiber contents in the bales. All the developed models agreed that the amount of trash in the cotton bales reduced the spinning performance of fibers. The presence of large leaf, bark, and pin trash reduces the marketing value of cotton and processing to remove them is very expensive (Kang and Kim, 2002).

In the process of variety development the selection of progenies with highly elastic, long, strong and fine fibers is a key to success in the competitive global textile industries. However, a complex association among the fiber traits makes the task of combining all these desirable traits into a single variety difficult for cotton breeders. And therein lies one of the paradoxes that this

research and other (e.g. Qscore) seek to address. The immediate clientele for a variety is a producer whose marketing is influenced by a few specific traits: first and foremost yield, then fiber quality traits roughly in the order length, strength and micronaire. The textile spinner, on the other hand has no interest in yield, but considers traits that affect the efficiency of their yarn production in addition to all the spinning variables such as yarn type, breakages, imperfections, etc. The SCI is an attempt to condense the needs of the spinner into an index value that could be of use by the breeder. Historical and more modern attempts, such as Qscore are poorly documented or not empirically derived. The SCI index is a documented attempt to combine the important fiber traits into a single index, which enables us to select the multiple fiber traits even it is imprecisely presented. Assuming SCI is a suitable proxy for actual spinning performance or at least yarn strength, whether one uses the HVI or AFIS instruments, our results indicate the relative importance of easily measured individual traits and suggests how they can be combined into a useful index. Although there were some discrepancies in the ranking of the determined fiber traits, all the developed statistical models agreed that they can be used to predict the spinning consistency index, which is also positively associated with yarn strength. In short, this study revealed that spinning consistency index (SCI) can be used as an alternative and efficient selection index for combining the multiple fiber traits to enhance yarn spinning.

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CHAPTER 5: CONCLUSIONS

Reniform nematode is one of the major plant pathogens for cotton, which causes heavy economic loss in Southern United States. Due to lack of resistant/tolerant commercial cotton varieties, application of nematicides, and crop rotation with nonhost crop species have been implemented to manage the reniform nematode in cotton fields to some extent. Over last two decades, extensive screening was conducted to identify sources of reniform resistance within the cotton germplasm pool available in the National Cotton Germplasm Collection. To date, a number of cotton genotypes, which are resistant to reniform nematode have been identified, but little information is available about their response across reniform isolates collected from different geographical regions. This study showed that tolerant/resistant cotton genotypes had different responses across the reniform isolates collected from renifrom infested fields across Louisiana. Compared to other genotypes, both Lonren-2 and *G. arboreum* (A_2-190) exhibit a high level of resistance regardless of reniform isolates or itsgeographic origin. Within a cotton breeding program, both Lonren-1 and Lonren-2 (both tetraploids) are good sources of resistance and relatively amenable to use though they both, especially Lonren-1, have other agronomic performance deficiencies (poor yield). The diploid cotton *G. arboreum* (A_2-190) exhibited the highest level of resistance across the reniform isolates, but would be more problematic to use within a breeding program.

This study also showed that there is a significant variation in reproduction and pathogenicity among reniform isolates collected from reniform infested cotton fields across Louisiana. Across reniform isolates, the Evan isolate had the highest reproduction and pathogenicity compared to other reniform isolates, suggesting that the Evan isolate may build up a juvenile population in the field faster than other reniform isolates. Although there were limited

studies in site specific management for a reniform isolates, different management strategies are needed to reduced damage from specific reniform nematode isolates that are specific to geographical regions. This study provides the foundation for the nematologists and agronomists to identify reniform isolates or races within or across specific geographical regions and develop appropriate management strategies to suppress the reniform populations in reniform infested cotton fields. The presence of an interaction between cotton genotypes with different sources of reniform resistance and reniform isolates from different geographic regions suggests that distinct races may exist, potentially describable with a differential host series. This information will enable the cotton breeders and pathologists to select and deploy different resistant/tolerant cotton genotypes to effectively manage reniform populations existing in production fields.

Salt stress has become a serious problem worldwide, and one, which may already be limiting cotton production in the Macon Ridge and Red River regions of Louisiana. To date, there have been a limited number of studies seeking to identify salt tolerant cotton germplasm and, there is no comprehensive data available. The lack of information hinders researcher's efforts to both understand the mechanism of salt tolerance and to select appropriate salt tolerant cotton genotypes for use in the development or breeding of salt tolerant cotton varieties. This study showed that both hydroponics and pot culture are fast, efficient and effective in the screening of a large number of cotton germplasm accessions against elevated salt concentrations. Compared to pot culture, the effect of sodium chloride on plant parameters under the hydroponic technique was more rapidly expressed and able to discriminate the salt tolerant genotypes in a short period of time. In terms of time and space, the use of the hydroponic technique has an advantage over pot culture in that it can handle a large number of accessions. Logistically, it can be laborious to randomize the genotypes and collect the data. The hydroponic system was also

highly sensitive to abrupt change in water temperature, which might cause complete shut down the plant growth.

Although pot culture takes more time and space than hydroponic techniques, it is a way to identify the salt tolerant cotton genotypes, which might perform well under salt stress in the field due to its greater similarity. Although there were a few discrepancies between the two systems, a combination of both systems is an efficient way to identify salt tolerant genotypes. It is suggested to use the hydroponic technique to first discriminate salt tolerant genotypes from a larger germplasm pool and reconfirm the reaction of promising accessions by using pot culture. This study showed that salt stress affects the morphology and physiology of cotton genotypes. It also showed the presence of variation in degree of salt tolerance among cotton germplasm by using different salt concentrations. All measured plant parameters were affected to some extent due to elevated salt concentrations. The effect on plant parameters was slightly varied, which results in a discrepancy in the ranking of genotypes across salt treatments. Compared to other genotypes, MT1219, MT11, MT45, and MT245 consistently performed better in all the measured parameters.

In addition to biotic and abiotic stresses, cotton breeders are interested to develop the high yielding cotton cultivars with improved fiber quality to meet the standard yarn properties. Cotton breeders use HVI fiber trait to select the progenies/cultivars with high fiber quality. In addition to HVI, textile industries use AFIS fiber traits to some extent because they are interested in variability of individual fiber properties in the cotton bales. Although various fiber parameters are measured by HVI and AFIS, it is still challenging to give priority a fiber trait or group of traits to select best fiber for industrial uses. Historical and more modern attempts, such as Qscores are poorly documented or not empirically derived based on HVI fiber properties. The

SCI index is a documented attempt to combine the important fiber traits into a single index, which enables us to select the multiple fiber traits even it is imprecisely presented. Based on the developed statistical models, fiber length and strength are the two important parameters in determining the spinning consistency index of cotton fibers. This result also supports the selection criteria used by cotton breeders over 50 years to improve the fiber quality. The results also showed that the amount of neps, coarse fibers, immature and short fiber contents, and trash contents in the bales reduce the spinnability of cotton fibers. Assuming SCI is a suitable proxy for actual spinning performance or at least yarn strength, whether one uses the HVI or AFIS instruments, our results indicate the relative importance of easily measured individual traits and suggests how they can be combined into a useful index. Although there were some discrepancies in the ranking of the determined fiber traits, all the developed statistical models agreed that they can be used to predict the spinning consistency index, which is also positively associated with yarn strength. In short, this study revealed that spinning consistency index (SCI) can be used as an alternative and efficient selection index for combining the multiple fiber traits to enhance yarn spinning.

APPENDIX

TABLE A.1: LIST OF DAY NEUTRAL PRIMITIVE COTTON ACCESSIONS USED IN HYDROPONICS TECHNIQUE.

S. No	Genotypes	S. No	Genotypes	S. No	Genotypes	S. No	Genotypes
1	MT4	38	MT257	75	MT677	112	MT86
2	MT764	39	MT149	76	MT347	113	MT368
3	MT636	40	MT634	77	MT242	114	MT60
4	MT620	41	MT460	78	MT96	115	MT171
5	MT763	42	MT224	79	MT725	116	MT640
6	MT610	43	MT73	80	MT178	117	M-238
7	MT477	44	MT633	81	MT250	118	MT62
8	MT115	45	MT245	82	MT215	119	MT320
9	MT281	46	MT29	83	MT668	120	MT76
10	MT52	47	MT241	84	MT33	121	MT27
11	MT53	48	MT239	85	MT72	122	MT188
12	MT11	49	MT338	86	MT104	123	MT100
13	MT1291	50	MT249	87	MT293	124	MT106
14	MT212	51	MT786	88	MT81	125	MT466
15	MT1219	52	MT235	89	MT32	126	MT36
16	MT701	53	MT68	90	MT101	127	MT180
17	MT244	54	MT89	91	MT1117	128	MT117
18	MT71	55	MT74	92	MT246	129	MT206
19	MT278	56	MT43	93	MT91	130	MT1175
20	MT209	57	MT237	94	MT41	131	MT17
21	MT650	58	MT223	95	MT754	132	MT31
22	MT478	59	MT50	96	MT198	133	MT612
23	MT70	60	MT255	97	MT113	134	MT228
24	MT493	61	MT240	98	MT55	135	MT175
25	MT202	62	MT219	99	MT1004	136	MT63
26	MT216	63	MT93	100	MT24	137	MT30
27	MT220	64	MT173	101	MT1000	138	MT99
28	MT6	65	MT199	102	MT201	139	MT154
29	MT195	66	MT243	103	MT1063	140	MT121
30	MT1195	67	MT221	104	MT1046	141	MT140
31	MT790	68	MT664	105	MT77	142	MT155
32	MT48	69	MT226	106	MT7	143	MT182
33	MT119	70	MT139	107	MT88	144	MT156
34	MT57	71	MT804	108	MT326	145	MT122
35	MT18	72	MT64	109	MT116	146	MT2
36	MT67	73	MT247	110	MT641	147	MT164
37	MT120	74	MT720	111	MT61	148	MT45
						149	MT90
						150	MT570

VITA

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