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Morpho-Physiological Characterization of Cotton Chromosome Substitution Lines for Abiotic Stresses

Akansha Awasthi

Morpho-physiological characterization of cotton chromosome substitution lines for abiotic stresses

By

Akanksha Awasthi

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By

Akanksha Awasthi

Approved:

_____

K. Raja Reddy
(Major Professor)

_____

Teddy P. Wallace
(Committee Member)

_____

Sukumar Saha
(Committee Member)

_____

Mark W. Shankle
(Committee Member)

_____

Michael S. Cox
(Graduate Coordinator)

_____

J. Mike Phillips
(Department Head

_____

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Akanksha Awasthi

Date of Degree: December 9, 2016

Institution: Mississippi State University

Major Field: Agronomy

Major Professor: K. Raja Reddy

Title of Study: Morpho-physiological characterization of cotton chromosome substitution lines for abiotic stresses

Pages in Study 55

Candidate for Degree of Master of Science

The chromosome substitution (CS) lines constitute an important genetic and breeding resources, increasing the genetic diversity of Upland cotton from other alien tetraploid species. Two experiments were conducted to quantify low temperature and drought stress effects during early growth stages in 21 cotton CS-lines with parent, Texas Marker (TM)-1. In Experiment I, plants were grown at optimum (30/22°C) and low (22/14°C) temperatures under optimum water and nutrient conditions. In Experiment II, plants were grown at optimum water and drought conditions. Above- and below-ground growth parameters including several root traits were assessed at 25 days after seeding. CS-lines varied significantly varied for many traits measured. Combined low temperature and drought response indices, derived from all measured parameters, showed CS-T04 and CSB08sh showed significantly higher and lower tolerance to low temperature, respectively, while CS-T04 and CS-B22sh showed significantly higher and lower tolerance to drought condition compared to TM-1.

DEDICATION

I dedicate this dissertation to my mother Mrs. Malti Awasthi, my father Mr. Ashok Awasthi, and my beloved husband Vivek Dixit. Without their love, affection, teaching, inspiration, encouragement and sacrifices this step of my life was never possible.

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DAS	Days after sowing
PH	Plant height
LN	Number of leaves
LDW	Leaves dry weight
SDW	Stems dry weight
RDW	Root dry weight
TDW	Total dry weight
LA	Leaf area
RL	Root length
SA	Root surface area
RAD	Average root diameter
RLVL	Root length per volume
RV	Root volume
RN	Number of roots
RNT	Number of tips
RNF	Number of forks
RNC	Number of crossings
ILTRI	Individual low temperature response index
IDSRI	Individual drought stress response index

CLTRI	Combined low temperature response index
CDSRI	Combined drought stress response index

CHAPTER I

GENERAL INTRODUCTION

Cotton, as the world's leading textile fiber plant, forms a vital part of global agriculture and is a mainstay of the economy of many developed and developing countries. It produces the basic raw materials, such as cellulose, protein, and oil, in quantity and quality surpassed by few plant species. Due to the immense importance of cotton crop, the cotton breeders have made great strides for improving cotton plant utilizing available genetic resources which resulted in numerous high yielding cultivars with better fiber quality traits. But due to the increasing consumption of fiber and greater competition from synthetic fibers, there is need to further speed up efforts for continued genetic improvement in cotton plant for yield and fiber quality traits. There is a great need to develop the capacity to increase the quantity and quality of food and fiber to meet the demands of the rising population.

Cotton Origin, History and Distribution

The history of the cotton starts with the evolution of the genus possibly 10-20 million years ago (Seelanan et al., 1997). This original entity released into several geographic centers of diversity including Africa, Australia, Arabia, and Mesoamerica. Gossypium is the cotton genus. It comprises of 50 species (Brubaker et al. 1999). The two old world cotton species, G. arboretum and G. herbaceum arose from the African-Arabian gene pool, whereas, *G. barbadense* and *G. hirsutum*, evolved in the New World

(Smith and Cothren, 1999). Commercial species of cotton plant are *G. hirsutum* (>90% of world production), *G. barbadense* (3-4%), *G. arboreum* and *G. herbaceum* (together, 2%). Within their different, non-overlapping geographical areas, each species independently attracted the attention of 4 different groups of early domesticators.

Cotton is grown in a wide geographic area and exhibits flexibility in growth to environmental stresses because of its indeterminate growth habit, perennial nature, and sympodial fruiting pattern (Lee, 1984; Reddy et al., 2007). The almost 40 species of *Gossypium* occur in many parts of the world and are adapted to a variety of habitats, many of them arid Cotton is currently the leading plant fiber crop worldwide and is grown commercially in the temperate and tropical regions of more than 50 countries. The leading producing countries in the world are China, USA, India, Pakistan, Uzbekistan, and Turkey. World cotton commerce is about US$20 billion annually (Saranga *et. al.,* 2001). *Gossypium hirsutum*, also known as upland cotton or Mexican cotton, is native to Mexico, the West Indies, northern South America, Central America and possibly tropical Florida (Wendel et al., 1992). *G. hirsutum*, is an indeterminate perennial plant that produces dehiscent fruit that is harvested for lint which serves numerous purposes (Turner et al., 1986). It is an important dual-use crop which provides not only natural fiber to the textile industry but also seed nutrition components for both humans and livestock. Upland cotton is being grown globally across both tropical and temperate latitudes.

Only *G. hirsutum* and *G. barbadense* are important textile crops, with *G. hirsutum* dominating the world cultivation due to its superior yield, but it has lower fiber quality than *G. barbadense*. In the United States, Pima cotton (*G. barbadense* L.) accounts for

less than 5% of US cotton production and is grown primarily in the arid southwest with an air temperature of >40°C (Radin, 1992). *G. barbadense* L. line 3–79 is lower in yield, has smaller bolls and known as extra –long staple cotton with fiber properties of length, micronaire, and strength than upland cotton. *G. mustelinum* is the only cotton species native to Brazil; it is endemic to the semi-arid region of the northeast. The populations are found near perennial and semi-perennial sources of water, such as ponds or pools in intermittent streams. *G. tomentosum*, is native to Hawaiian Islands due to which it is known as Hawaiian cotton. It inhabits low shrub lands. It is not cultivated for fiber, but is occasionally found as an ornamental. Its lint are short and reddish brown, unsuitable for spinning or twisting into thread.

Cotton Production in United States

US is the third largest producer of cotton in the world. The Cotton Belt spans the southern half of the United States, from Virginia to California. Cotton is grown in 17 states and is a major crop in 14 dominated by Texas, California, Arizona, Mississippi, Arkansas, and Louisiana. Cotton continues to be the basic resource for thousands of useful products manufactured in the U.S. and overseas. Cotton production is a $25 billion-per-year industry in the United States and is the world's top exporter of cotton, shipping some 12 million bales per annum. Cotton Incorporated estimates that around 60% of US cotton area is grown without irrigation. Planting begins in February in south Texas and as late as June in northern areas of the Cotton Belt. Most of the U.S. cotton acreage is grown only on rain moisture. Cotton is machine harvested in the U.S., beginning in July in south Texas and in October in more northern areas of the Belt. Stripper harvesters, used chiefly in Texas and Oklahoma, have rollers or mechanical

brushes that remove the entire boll from the plant. In the rest of the Belt, spindle pickers are used. *G. hirsutum* is the most widely planted species of cotton in the United States, constituting some 95% of all cotton production. American Pima accounts for less than 5% of U.S. cotton production. It is grown chiefly in California, with small acreages in West Texas, New Mexico and Arizona.

Cotton Growth and Development

The growth stages for cotton can be divided into five main stages: (1) germination and emergence (2) seedling establishment (3) leaf area and canopy development (4) flowering and boll development and (5) maturation. For cotton, the threshold temperature is 60˚F; based on growing degree days (DD) concept where little or no development occurs below that temperature.

Table 1.1 The average number of days and heat units

Growth Stage	**Days**	**Heat Units – DD60s**
Planting to Emergence	4 to 9	50 to 60
Emergence to First Square	27 to 38	425 to 475
Square to Flower	20 to 25	300 to 350
Planting to First Flower	60 to 70	775 to 850
Flower to Open Boll	45 to 65	850 to 950
Planting to Harvest	130 to 160	2200 to 2600

(Oosterhuis, 1996)

Vegetative Phase

The cotton plant has a noticeable main stem, which is an outcome of the elongation and development of the terminal bud or apical meristem. The main stem has an indeterminate growth habit and it is made up of a series of nodes and internodes. The length of the internodes and the number of nodes are influenced by environmental factors

such as climate, nutrients, soil moisture, disease and insects as well as by the genetics. At the emergence stage, the fully expanded cotyledons reach 1 to 2 inches above the soil surface and are arranged directly opposite the main stem. Main stem leaves and branches are spirally arranged on the stem in a three-eighths phyllotaxy above the cotyledonary node. Two types of branches which are known as monopodial; the vegetative branches, and sympodial; the fruiting branches are produced in the development stage. Monopodial branches are structurally similar to the main stem. Sympodial branches are produced by the main stem and monopodial branches grow at an acute angle to the main stem. Every sympodial branch has a main stem leaf associated with the branch. Each new fruiting node has an extending leaf and a fruiting structure or square at each node, as the branch extends from the main stem. The development of this branch ends in a square, but a second leaf and square develop in the axil of the first leaf and similarly extend away from the first leaf and square by internode elongation.

Reproductive Phase

About 4 to 5 weeks after planting, the reproductive growth begins to appear with the formation of the floral buds or squares in the terminal of the plant. The fruiting branches tend to be produced at each successive main-stem node once fruiting is started. Due to its indeterminate growth habit, the cotton plant will continue adding vegetative growth at the same time as the reproductive development throughout the remainder of the season. The developing bolls become the major sink for photosynthetic products. The first position white flower moves closer to the terminal of the plant. Thus, the new node or square development further happens slowly. Within 3 weeks after the fertilization, the boll develops rapidly and reaches its full size. Seeds do not reach maturity until shortly

before the boll opens, but reach their full size. The maximum growth rate occurred during the first 10 to 15 days of the period. At about 25 days after fertilization, fibers attain their full length. Thickening of the fiber begins at about 16 days after fertilization and continues until the boll is mature. Cotton quality is defined by the length, maturity, strength and micronaire of the fiber. The climatic conditions experienced by the crop, the genetic makeup of specific plant varieties, and the management of the crop through production and harvest determine the final fiber quality.

Root Growth in Cotton

Root growth dominates the growth of the cotton plant during germination and seedling establishment. As the cotton plant grows, the radicle that originally emerged from the seed becomes a taproot, from which lateral roots begin to form and grow. Lateral roots and the taproot collectively make up the basal root system. As the plant matures, the roots continue to spread and probe deeper in the soil profile for water and nutrients. Therefore, the distribution of roots tends to match the most fertile soil zones. The amount of roots generally peaks during the cotton flowering phase then declines as the plant partitions more carbohydrates to the developing bolls. The cotton plant's root system is very efficient at seeking moisture and nutrients from the soil. The optimum temperature for the growth of cotton roots has been reported to be 30 to 33C (Pearson et al., 1970), and 35C (McMichael and Burke, 1994).

Impact of Abiotic Stresses on Cotton

In an era of changing climate, diminishing natural resources, and global conflict, the increase in productivity can be achieved only with the help of technological

knowledge and improved agricultural practices. In production agriculture, every season is different in terms of amount and intensity of rain events, temperature and light energy received. Therefore, overall plant growth and development are all sensitive to variables or adverse environmental conditions (Lewis et al., 2000). In field, plants are often exposed to many environmental stresses which are responsible for the alteration in their reproductive growth and yield components. In order to understand the basis of stress tolerance on cotton, the diversity of the stress response and its utility for the survival of cotton plants should be investigated.

Abiotic stress, as a natural part of ecosystem, will affect organisms in a variety of ways. Their impact can be determined depending upon the location of the area and then we can say that these effects are beneficial or detrimental. Plant response to abiotic stresses is very complex and cause extensive losses to agricultural production worldwide. Plant adaptation to environmental stresses is dependent upon the activation of cascades of molecular networks involved in stress perception, signal transduction, and the expression of specific stress related genes and metabolites (Huang, 2012). The adaptation of plants to different abiotic stresses would require an appropriate response customized to each of the individual stress conditions involved, as well as suitable for the need to adjust for some of the averse aspects of stress combination. Cotton is highly sensitive to environmental stresses. Among the various environmental stresses, drought and temperature are the two most important stresses affecting crop production globally (Saini et al., 2000). Cotton, being a perennial with an indeterminate growth habit and a complex fruiting pattern, is considered to have the most complicated response to

environmental conditions and management practices of the major row crops grown in the United States (Oosterhuis, 1990).

Therefore, producers and crop managers have to manage the crop to maximize yield potential regardless of what uncontrollable circumstances may be present in the environment (Stewart et al., 2010). For future it seems that some environmental stresses will assuredly intensify, and this coupled with such practices as utilizing land marginally suited to agriculture and growing desirable crop plants in climates for which the plants are ill adapted to create an increasing need for new stress- tolerant varieties. Developing rapid and inexpensive screening tools for abiotic stress tolerance is therefore needed and will be beneficial to breeding programs and selection of cultivars for a niche environment.

Changes in the climate are always associated with changes in the other variables such as precipitation patterns (Giorgi et al., 2008). As a result, drought affected areas are expanding and the trend is accelerating over time (Delmer, 2005). Lobell et al., (2007) reported a negative correlation between worldwide crop yields and recent changes in temperature and precipitation patterns. Today, one third of the total world cultivated area suffers from inadequate supply of water (Massacci et al., 2008), and future world crop production will be substantially affected by any changes that causes water supply depletion. Therefore, it is important to understand crop growth and developmental responses to change in temperature and water. Water stress is a condition when plant water and turgor potential declines enough to the extent that inhibits normal plant functions and it has a significant effect on cotton's growth and development. The effects of water stress depend on the severity and duration of the stress, the growth stage at

which stress is imposed, and the genotype of the plant (Kramer, 1983). The cotton crop is sensitive to water shortage at all growth stages, but particularly reproductive development is the most sensitive period to drought stress following seed germination and seedling establishment. Cotton producers in the southern United States, are starting to rely more on the use of artificial irrigation due to often occurrence of severe drought and high humidity (Howell T. A., 2001). Irrigation can be a large expenditure for producers. Therefore, an adequate understanding of cotton's growth and development and its water use efficiency (WUE) is needed to maximize profitability. Cotton water requirement is about 5000 to 8000 cu.m during the season for obtaining a normal yield.

Water deficit stress adversely affects plant performance and yield development throughout the world (Boyer, 1982). Water- deficit stress reduces cell and leaf expansion, stem elongation, leaf area index (Jordan et al., 1970; Turner., 1986; Ball et al., 1994). Leaf, stem, and root growth rate are sensitive to water stress because they are dependent on cell expansion (Hsiao, 1976; Hearn, 1994). Pettigrew (2004) reported that water-deficit stress resulted in a decrease in leaf size, but noted that this decrease was accompanied by an increase in the specific leaf weight (SLW), a phenomenon also observed by Wilson et al. (1987). Krieg and Snug (1986) reported that water stress caused a reduction in the whole plant leaf area by decreasing the initiation of new leaves, with no significant changes in leaf size. Significantly fewer nodes and lower dry weights of stems and leaves of water stressed plants compared to those of the control were reported by Pace et al. (1999). Malik et al. (1979) reported that root growth appears to be less affected by drought than shoot growth while Michael et al. (1991) observed decrease shoot-to-root ratios of plants under drought stress. Drought will reduce the total dry

weight and yield concurrently. Typically, in most plants, reproductive growth is more sensitive to plant water stress than vegetative growth (Fischer et al., 1965), and cotton is similar as the highest water demand occurs during reproductive growth (Hearn, 1980). Cotton plants tend to compensate for lack of moisture by shedding fruit and, thereby, to some extent alleviate stress on the remaining fruiting forms (Ramey, 1986). Water stress also effect the fiber quality, Lokhande and Reddy (2014a) found that fiber length, strength and uniformity declined with decrease in leaf water potential (LWP) while fiber micronaire increased with decrease in LWP.

One of the most important factors in the production of a large crop of cotton is a full stand of plants early in the season. The period of greatest mortality of cotton plants due to adverse environmental conditions is from the time the seed is planted until the seedling stage is past. It is during this time that cold wet weather so often makes early planted cotton a failure. It follows, therefore, that if a variety of cotton, excellent in other respects, could be found with more resistance to cold in the seedling stage, and farmers would be able to plant earlier or would be assured of better stands from plantings at the usual time. In either case they would secure greater yields. Low temperature exposure during cottonseed germination, emergence, and early seedling growth reduces stand establishment in addition to the physiological and morphological changes that reduce lint yields (Muller 1968). Kittock et al. (1987) concluded that physiological and morphological effects of low temperatures early in the cotton planting season may often contribute as much to reduce yield as to reduced stands. Ludwig, (1932) reported in his study that Pima and two varieties of *Gossypium nanking* exhibit the most rapid and most complete germination at low temperature. Marani and Dag (1962b) noted a pronounced

difference in the ability of different cotton varieties to germinate at low temperature. Generally *G. barbadense* varieties germinated better than *G. hirsutum* at 12°C. Temperatures that lie outside the range of those typically experienced can have severe consequences for crops, significantly reducing yields. Low temperatures decrease the rate of dry matter production and, at extremes, can cause production to cease (Grace, 1988). Cool average temperatures and low night temperatures (below 22.0°C) encumber fiber elongation by decreasing the axial growth rate of fibers within the early stage of elongation (before 15 DPA) (Gipson and Joham, 1969 and Xie et al., 1993). Moreover, fiber qualities were significantly decreased under low temperature stress (Liakatas et al., 1998). Burke (1994) showed that root growth was enhanced when the root tissue temperature were within or below cotton's thermal kinetic window of 23.5 to 32°C (Burke et al., 1988). During the first 22d after emergence of pima cotton cv. S-6, about 8 d were required to produce a node on the main stem at 21°C, whereas only about 3.5 d were required to produce a node at the optimum day/night temperature (30°C /22°C; Reddy et al., 1995). Wanjura et al. (1967) showed that a minimum soil temperature between 15.6 and 20 °C was needed for supporting seedling emergence.

In the US cotton belt, temperature variation is quite large with seasonal variation exceeding 20ºC and with greater diurnal variation (Reddy *et al.,* 1995a, b). The detrimental effect of high and low temperature on various physiological processes impacting crop yields are complex.

Screening Root and Shoot Traits

Screening of root and shoot traits which describe the genotypic variations can be used to improve early plant development. Typically, root systems are difficult to study

due to their highly structured underground distribution, complexity of vigorous interactions with the environment, and the diversity of their functions. Different methodologies have been developed to study root growth under both field and controlled environment but root scanning based on winRHIZO optical scanner is one of the efficient methods which allow the image analysis and examining the root morphological traits like cumulative root length (RCL), root surface area (RSA), average root diameter (RAD), length per volume (RLPV), root volume (RV), number of roots (RN), number of roots having laterals (RNL), number of tips (RNT), number of forks (RNF), and number of crossings (RNC). The development of the root system of the cotton (G. hirsutum L.) plant is under genetic control but may be modified by environmental factors. Since cotton has tap root system, rooting depth and rooting density can be significantly influenced by the water stress which directly affect the root function of cotton plant (Klepper et al., 1973).

Chromosome Substitution Lines

Chromosome substitution has been an indispensable method useful for genetic analysis and breeding (Campbell et al., 2003, 2004; Shah et al., 1999). Chromosome substitution lines are the lines in which a single chromosome of a donor genotype is substituted into the genome of recipient genotype using appropriate aneuploidy stocks. Compared to conventional breeding techniques, chromosome substitution provides a unique opportunity to precisely detect genetic effects for important traits, including those associated with higher drought and heat tolerance. CS lines are useful from several perspectives: 1) to improve genetic diversity for important traits in Upland cotton, 2) to discover the untapped potential of cryptic alleles from the wild and unadapted tetraploid species, 3) to understand the ramifications of epistasis on complex agronomic and fiber

traits and 4) to identify chromosomal locations of important fiber and agronomic traits. Methods for development of interspecific chromosome substitution in *G. hirsutum* were outlined by Endrezzi (1963). CS lines can lead to enhanced resolution in linkage mapping and facilitate targeted exploitation of exotic germplasm to improve fiber quality and agronomic traits in cotton breeding program (Saha et al., 2004).

To increase genetic diversity with elite upland cotton, introgression populations with wild species of cotton, *G. mustelinum* and *G. tomentosum*, were created. Backcrossed chromosome substitution lines (CS-B) have been developed with a homologous pair of chromosome arms of *Gossypium barbadense* germplasm substituted for the homologous *Gossypium hirsutum* (TM-1) chromosome. Several CS-B lines had significant homozygous and heterozygous dominance effects for different agronomic and fiber traits showing that specific CS-B lines may be useful for improving agronomic and fiber traits in hybrid cottons (Saha et al., 2008). Since the upland cotton has very narrow gene pool because of its unique evolutionary history, domestication, modern improvement practices, and on crossing a few elite lines of closely related genotypes (Small et al., 1999), cotton cultivars with enhanced fiber quality and productivity will allow US growers to compete with synthetic fibers.

Despite some farming management efforts made to alleviate stresses to a certain extent, the major objective of screening cotton breeding lines and germplasm for low temperature and drought stress tolerance is imperative to understand the early season responses in growth and development of these CS-lines. Similarly identification of traits associated with reproductive performance in genetically stable lines containing individual chromosome are crucial to improve cotton yield in the US to satisfy escalating world

demand for cotton. The methods employed and indices developed in this study will be of great use for many breeders to select best CS-lines ready to withstand low temperature and drought stress. Hence the objective of these study was to investigate early season responses with respect to low temperature and drought stress of chromosome substitution (CS) lines compared with an Upland cotton cultivar, Texas Marker (TM)-1.

CHAPTER II

MORPHO-PHYSIOLOGICAL RESPONSES OF THE COTTON CHROMOSOME SUBSTITUTION LINES FOR LOW TEMPERATURE AND DROUGHT STRESS

Abstract

The chromosome substitution (CS) lines constitute an important genetic and breeding resources, increasing the genetic diversity of Upland cotton from other alien tetraploid species. Two experiments were conducted to quantify low temperature and drought stress effects during seedling emergence and growth stages in 21 cotton CS-lines with parent, Texas Marker (TM)-1. In Experiment I, plants were grown at optimum (30/22°C) and low (22/14°C) temperatures under optimum water and nutrient conditions. In Experiment II, plants were grown at optimum water and drought conditions for plants grown at optimum temperature conditions. Above- and below-ground growth parameters of the CS lines were assessed with TM-1 at 25 days after seeding in both the experiments. Also, root morphological traits were assessed using WinRHIZO root image analysis system. CS-lines varied significantly for many traits measured, particularly, plant height, total plant dry weight, and root morphological parameters. Combined low temperature and drought response indices, derived from root, shoot and physiological parameters, showed CS-T04 and CSB08sh showed significantly higher and lower tolerance to low temperature, respectively compared to TM-1, while CS-T04 and CS-B22sh showed

significantly higher and lower tolerance to drought condition compared to TM-1 among 21 CS-lines tested. The identified low temperature and drought-tolerant CS-lines might be useful in cotton breeding programs for Upland cotton improvement.

Introduction

The vitality of future cotton industry depends on high yielding germplasm with fiber that meets or preferably exceeds the international standards of fiber quality. Since the Upland cotton, the dominant species that is grown over 95% of cotton growing area across world's Cotton Belt, has very narrow gene pool because it's unique evolutionary history, domestication, modern improvement practices, and origin from crossing of a few elite lines of closely related genotypes (Small et al., 1999). Cotton cultivars with enhanced fiber quality and productivity will allow growers to compete with synthetic fibers. New cultivars are especially important in the light of extreme and unpredictable year to year variations in weather along with projected changes in climatic conditions and associated environmental stresses, particularly temperature and drought (Reddy et al., 2000; IPCC, 2010).

Breeders have long aspired to breed cotton plants that are tolerant to drought and thermally stressful environments with high productivity and superior fiber quality. Their efforts, however, have been hindered by the limited knowledge of genetic and morpho-physiological traits that underscores the genetic potential under field conditions for improved productivity and quality. The lack of effective selection tools for low and high temperature and drought tolerance were another major constrain in developing stress tolerant cotton lines (Singh et al., 2007) because the only indicator available is the yield as the end product.

A previous study shows that high temperature during flowering affects more severely cotton pollen than ovule (Kakani et al., 2005). Most of the cotton squares and flowers are aborted when day and night temperatures were > 30/20°C (Reddy et al., 1992a, 1992b, 1997). Previous studies in corn (Schoper et al., 1987) and tomato (Sato et al., 2000) revealed that lower fruit and seed set in high temperature were due to non-viable pollen, unsuccessful anther dehiscence and pollen shed. Such activities reduced pollen tube penetration into the stigma and thereby damaged the female performance (Gross and Kigel, 1994). Lower fruit-set at high temperatures was related to decreased pollen production, poor pollen viability and decreased pollen germination in several other species including cotton (Reddy et al., 2005). In a recent review, Singh et al. (2007) concluded that there is an urgent need to develop heat and low temperature tolerant cultivars for todays' high, but variable temperature conditions across several cotton growing areas.

In addition to high temperature effects on cotton reproductive potential, low and variable temperatures during seed germination and seedling stages affects growth and development (Ashraf, 2002) as temperature and other weather conditions vary spatially and temporally across the cotton growing areas and seasons. In the U.S., cotton is exposed to diverse temperatures, above and below optimum temperatures during the growing season. The planting window for cotton in Mississippi Delta is listed as from April 27th to May 23rd. In recent years, cotton producers have shown an increased interest in early planting of cotton in hopes to capitalize on better yields and better fiber quality by being able to harvest earlier when weather and filed conditions are more favorable. Planting earlier can have advantages of earlier harvest and less risk of late

season rainfall hindering harvest. Cotton seedlings exposed to low temperatures early in the season take longer to develop, have a slower biomass accumulation, and could halt seedling emergence (Christiansen and Thomas, 1969). In addition, in spite of the importance of the root architecture that determines efficient acquisition of soil nitrogen and water, understanding of root growth and development is minimal. Therefore, understanding cotton root system during the stand establishment will be useful in addressing the challenges of seedling establishment and cultivar survival under adverse soil conditions such as low temperature (Lynch, 2005). Root system architecture has been referred to as an integrative result of lateral root initiation, morphogenesis, emergence, and growth (Dubrovsky and Forde, 2012) and thus provides key traits that could be used to screen cultivars for survival under low temperature conditions. Thus, an understanding of the internal and external cues that determine root architecture will lead to the development of tools and management practices that optimize root development with changing weather and climate conditions.

Drought stress, on the other hand, is a syndrome that affects several physiological processes affecting both sources and sinks (Reddy et al., 1997; Lokhande and Reddy, 20). In a preliminary study, we found that pollen produced under drought stressed cotton is not the causative factor in lowering the boll-load, but processes such gas exchange will ultimately leads lower boll set and resulting smaller bolls and poor lint quality. Many studies addressed several aspects of cotton growth, development and reproductive potential as affected by water stress (Gerik et al., 1996; Grimes et al., 1969). Drought stress effects on several growth and physiological processes resulting have been reported that caused stunted plant growth (Gerik et al., 1996), reduced leaf area, and decreased

CO_2 assimilation rate, increased boll-shedding from carbon starvation, fewer numbers of bolls and smaller boll weights, and ultimately reductions in seedcotton yield (Gerik et al., 1996; Pettigrew, 2004a). Also, changes in soil and plant water status modifies the growth and fruiting patterns in cotton and limits the productivity by affecting boll retention (Onder et al., 2010), lint yield (Pettigrew, 2004a) and fiber quality (Basal et al., 2009). However, information on how the cotton CS-lines will respond under stress conditions is lacking.

Historically, introduction of genes from exotic un-adapted species have contributed beneficial alleles of agronomic value and yield quality improvements in other crops (Tranksley and McCough, 1997), utilization of those techniques in sister species of *G. hirsutum* have been under-utilized. Recently, scientists at USDA/ARS developed (Stelly et al. 2005) a very valuable germplasm resources of backcrossed chromosome substitution (CS) lines of *G.* barbadense (CS-B lines), *G. mustelinum* (CS-M lines), and *G. tomentosum* (CS-T lines) backcrossed into, TM-1, a line considered genetic standard of Upland *(G. hirsutum)* cotton. . These backcross CS lines resulting from this cytogenetic manipulation are genetically identical to TM-1 genetic background except that each differ by the replacement of a specific homologous pair of chromosome or chromosome arm from those sister species of *G. barbadense*, *G. mustelinum* or *G. tomentosum*. Observation and measurement of different chromosome substitution lines in such a uniform genetic background will detect the effect of the group of genes that a specific substituted chromosome carries in upland chromosome background (Saha et al., 2006, 2010). Previous studies demonstrated that comparative analysis of CS lines with TM-1 provide a unique opportunity to detect and quantify genetic effects on a

chromosome-by-chromosome basis for important traits (Saha et al., 2013; Jenkins et al., 2007). The identification of lines that are phenotypically stable under field conditions could lead to crucial cultivar improvements. *Gossipium tomentosum* is one of the most heat-resistant species of the genus (Percival et al., 1999) and recently scientists have developed some heat tolerance cotton lines using genetic resources from Pima cotton (Lu et al., 1999, 1998). The Pima or Sea Island cottons (*G. barbadense)* are known for their superior fiber length, strength, and fineness, which offers price dividends because of its fiber traits at the mill. Therefore, breeders have attempted to combine the best fiber quality attributes of Pima cottons with yield and agronomic attributes of Upland cotton through conventional breeding methods (Lacape et al., 2005; He et al., 2008). However, conventional methods of interspecific introgression using sister species of cotton have not been fully successful in bringing the traits of importance for fiber quality or stress tolerance because of complex interactions among genes at the interspecific level(Renisch et al., 1994; Ulloa et al., 2005; Jiang et al., 2000). An alternative strategy to complement the conventional introgression method is to use interspecific chromosome substitution lines from the sister tetraploid species for improved stress tolerance.

The objectives of this study were to quantify early-season seedling vigor responses of 21 CS-lines from *G. barbadense, G. mustelinum, and G. tomentosum* backcrossed into Upland cotton genetic and molecular standard cultivar, TM-1. The comparative analysis of the parental line with that of the true breeding homozygous CS-lines under similar uniform genetic background except the substituted chromosome or chromosome segment from the alien species will provide an ideal opportunity to associate many of these traits with individual substituted chromosome and chromosome

segments and detect their effects on morpho-physiological processes under low temperature and drought conditions.

Materials and Methods

Experimental Facility

The sunlit plant growth chambers know as Soil-Plant-Atmosphere-Research (SPAR) units located at the Rodney Foil Plant Science Research Center, Mississippi State (33°28′ N, 88°47′ W), Mississippi, USA were used to carry out these experiments. Each SPAR unit consists of a steel soil bin (1 m deep by 2 m long by 0.5 m wide) to accommodate the plants root system, a Plexiglas chamber (2.5 m tall by 2 m long by 1.5 m wide) to accommodate plant canopy, and a heating and cooling system connected to air ducts that pass conditioned air through the plant canopy to cause leaf flutter. The Plexiglas transmits 97% of the incoming photosynthetically active radiation (PAR) without spectral variability in absorption (wavelength 400–700 nm), however it blocks solar UV-B radiation [33]. During this experiment the incoming daily solar radiation (285–2800 nm) outside of the SPAR units was measured with a pyranometer (Model 4-8; The Eppley Laboratory Inc., Newport, RI) and ranged from 6.69 to 29.00 MJ m^{-2} d^{-1} with an average of 23.07 ± 1.32 MJ m^{-2} d^{-1}.

Variable density shade black cloths placed around the edges of the plant canopy to mimic solar radiation attenuation through the canopy and were adjusted regularly to match canopy height. The SPAR units have the capacity to precisely control air temperatures and chamber [CO_2] at determined set points and at near ambient levels of solar radiation. A heating and cooling system connected to air ducts that pass conditioned air through the plant canopy to cause leaf flutter. Chamber air temperature, [CO_2], and

soil watering in each SPAR unit, as well as continuous monitoring of environmental and plant gas exchange variables, were controlled by a dedicated computer system. More details of operation and control of SPAR chambers have been described by Reddy et al. (2001). Briefly, air ducts located on the northern side of each SPAR unit were connected to the heating and cooling devices. Conditioned air was passed through the plant canopy with sufficient velocity to cause leaf flutter (4.7 km h^{-1}) and was returned to the air-handling unit just above the soil level. Chilled ethylene glycol was supplied to the cooling system via several parallel solenoid valves that opened or closed depending on the cooling requirement. To fine-tune the air temperature, two electrical resistance heaters provided short pulses of heat, as needed. Chamber air temperature, carbon dioxide concentration [CO_2], and soil watering in each SPAR unit, as well as continuous monitoring of environmental and plant gas exchange variables, were controlled by a dedicated computer system (Reddy et al., 2001) (Table 1). The chamber [CO_2] was maintained either at 400 µmol mol^{-1} by a dedicated infrared model LI-6252 (LI-COR Biosciences, Lincoln, NE, USA) gas analyzer. Pure CO_2 was supplied from a compressed gas cylinder through a system that included a pressure regulator, solenoid and needle valves, and a calibrated flow meter (Reddy et al., 2001). The relative humidity (RH) of each chamber was monitored with a humidity and temperature sensor (HMV 70Y, Vaisala, Inc., San Jose, CA) installed in the returning path of airline ducts. The vapor pressure deficits (VPD) in the units were estimated from these measurements as per Murray (1967).

Materials Used

Twenty one chromosome substitution (CS) lines, containing different pairs and short segments of chromosomes from *G. barbadence* (CS-B lines), *G. mustelinum* (CS-M lines), and *G. tomentosum* (CS-T lines) (Saha et al., 2004; Stelly et al., 2005) and 1 Upland parent (*G. hirsutum*), Texas Marker (TM)-1 were used in the experiments (Table 2.1). CS-B02, CS-M02, CS-T02, CS-B04, CS-M04, CS-T04, CS-B06, CS-M06, CS-T06, and CS-B08sh, CS-M08sh, CS-T08sh, CS-B11sh, CS-M11sh, CS-T11sh, CS-B15sh, CS-M15sh, CS-T15sh, CS-B22sh, CS-M22sh, CS-T22sh lines contained a pair of short arm of chromosome 8, 11, 15, and chromosome 22 of *G. barbadense, G. mustelinum, and G. tomentosum* in the background of TM-1 (*G. hirsutum*) as a recurrent parent, respectively.

Treated seed of all CS lines and TM-1 with fungicide were sown in PVC pots (15.2 cm diameter and 30.5 cm height) filled with the soil medium consisting of 3:1 sand: top soil classified as sandy loam (87% sand, 2% clay, and 11% silt) with a 500 g of gravel at the bottom of each pot. Initially, four seeds were sown in each pot and 4 days after emergence, the plants were thinned to one pot^{-1}. Pots were arranged as a randomized complete block in 11 rows with 2 pots row^{-1} in each SPAR chamber. Three temperature and drought stress treatments were randomly arranged in 9 SPAR units. Except for the treatments, the other growth conditions were same during the experiment for all the units. For each treatment, three replications were maintained by using one SPAR unit as one replication.

Table 2.1 CS-lines (twenty one) and one parent TM-1 used in the experiment.

S. no.	CS- line
1	CS-M02
2	CS-T02
3	CS-B02
4	CS-M04
5	CS-T04
6	CS-B04
7	CS-M08sh
8	CS-T08sh
9	CS-B08sh
10	CS-M06
11	CS-T06
12	CS-B06
13	CS-M11sh
14	CS-T11sh
15	CS-B11sh
16	CS-M15sh
17	CS-B15sh
18	CS-T15sh
19	CS-M22sh
20	CS-B22sh
21	CS-T22sh
22	TM-1

Treatments

Nine SPAR units, three for each treatment, were used in this experiment. Each CS line was placed randomly within each of three replications in each treatment of control, 30/22°C with well-watered, low temperature 22/13°C well-watered and drought stress (DS), 30/22°C and 50% evapo-transpiration (ET) of the control treatment. The temperature treatments were imposed at seeding, while the DS treatment was imposed 6 days after seeding and 2 days after seedling emergence. The daytime temperatures were initiated at sunrise and returned to the nighttime temperature 1 h after sunset. The

environmental data for mean temperature, vapor pressure deficit, carbon dioxide concentration measured soil moisture content are presented in the Table. 1.

Plants were irrigated three times a day through an automated and computer-controlled drip system with full-strength Hoagland's nutrient solution (Hewitt, 1952), delivered at 0700, 1200 and 1700 h, based on treatment-based evapotranspiration values. Evapotranspiration rates expressed on a ground area basis (L d^{-1}) throughout the treatment period were measured in each SPAR unit as the rate at which the condensate was removed by the cooling coils at 900-s intervals (McKinion and Hodges, 1985; Reddy et al., 2001; Timlin et al., 2007). They were obtained by measuring the mass of water in collecting devices connected to a calibrated pressure transducer.

Measurements

Shoot Parameters

The seedling emergence was recorded as the number of days from sowing to 50% emergence in each pot. Plant heights were measured and nodes were counted on all plants at final harvest, 25 DAP. Leaf area was measured using the LI-3100 leaf area meter (LI-COR, Biosciences). Plant total dry weights (TD) including leaves, stems, and roots were recorded after oven drying for 5 days at 75°C.

Root Morphology

After separating the stem from individual root systems of each plant, roots were washed by placing the pot on sieves and gently spraying with water. The cleaned individual root systems were floated in 5 mm of water in a 0.3- by 0.2-m Plexiglas tray and gray-scale root images were acquired according to the procedure described by

Wijewardana et al. (2015). Briefly, roots were untangled and separated with a plastic paintbrush to minimize root overlap. The tray was placed on top of a specialized dual-scan optical scanner (Regent Instruments, Inc.), linked to a computer. Gray-scale root images were acquired by setting the parameters to high accuracy (resolution 800 by 800 dpi). Acquired images were analyzed for root length, root surface area, average root diameter, root volume, and number of tips, forks, and crossings using WinRHIZO Pro software (Regent Instruments, 2009).

Photosynthetic Pigments

Leaf photosynthetic pigment contents (chlorophyll a, chlorophyll b, and carotenoids) were measured from the cotyledonary leaves for each CS line and treatments at the final harvest, 25 days after sowing. Five leaf discs, each with 2.0 cm^2 area, from each treatment were collected randomly and placed in vials containing 5 mL of dimethyl sulphoxide for chlorophyll (Chl) extraction. Absorbance of the supernatant was measured using a Bio-Rad ultraviolet/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA) at 470, 648, and 663 nm. The total chlorophyll and carotenoids were estimated by using the equation of Lichtenthaler (1987) as described by Chappelle et al. (1992) and expressed on leaf area basis (µg cm^{-2}).

Data Analysis

To test the significance of low temperature and drought on growth and biomass components of cotton CS-lines, analysis of variance was performed by using general linear model PROC GLM (SAA Institute Inc., 2003). Fisher protected LSD tests at P= 0.05 was used to determine significance of treatment effects. Sigma plot 13 was used to

plot the graphs. Additionally, regression analysis was used to determine correlation between roots and shoot vigor indices.

Calculation of Stress Response Indices

The CS-lines were classified into low temperature and drought stress tolerance by combined low temperature response index (CLTRI) and combined drought stress response index (CDSRI). Initially, individual low temperature response index (ILTRI) was calculated by dividing the value of a parameter (PL) at low temperature by the value of the same parameter (Po) at control (optimum temperature) of each cultivar (Eq. 1.1).Then, combined low temperature response index (CLTRI) was calculated with sum of 18 ILTRI of each cultivar that includes plant height (PH), leaf area (LA), leaf number (LN), stem dry weight (SDW), leaf dry weight (LDW), root dry weight (RDW), root length (RL), root surface area (RSA), average root diameter (RAD), length per volume (RLPV), root volume (RV), number of roots (RN), number of roots having laterals (RNL), number of tips (RNT), number of forks (RNF), and number of crossings (RNC) , root-shoot ratio (RSR), total pigment content (TPC) [Equation (Eq). 1.3]. Similarly, individual drought response index (IDSRI) was calculated by dividing the value of a parameter (PD) at drought (50% ET) by the value of the same parameter (Po) at control (100% ET) of each cultivar (Eq. 1.2). Then the combined drought stress response index was calculated by adding all the 19 IDSRI of shoot and root parameters.

$$ILTRI = [PL/Po] \quad (2.1)$$

$$IDSRI = [PD/Po] \quad (2.2)$$

$$CLTRI = [PHl / Pho] + [LAl / LAo] + [LN1/LNo]+ [SDWl / SDWo] + [LDWl / LDWo] + [RDWl / RDWo] + [RLl /RLo] + [RSAl / RSAo] + [RADl / RADo] + [RLPVl / RLPVo] + [RVl / RVo] + [RNl / RNo] + [RNLl / RNLo] + [RNTl / RNTo] + [RNFl / RNFo] + [RNCl / RNCo] + [RSRl / RSRo] + [TPCl / TPCo] \quad (2.3)$$

Results and Discussions

Shoot Parameters

The protocol developed in this experiment to achieve the study objectives on CS lines using sunlit plant growth chambers to study early-season vigor identified stress tolerant lines for low temperature and drought stress. Except for the treatments variables, there were no differences among the treatments. The measured average temperatures were significantly different ($P = 0.05$) among the temperature treatments, 18.7 ± 0.58 °C and 25.8 ± 0.15°C for the low (22/13°C) and optimum (30/22°C) temperature treatments, respectively. Accordingly, the measured vapor pressure deficits were significantly different ($P = 0.05$) among the temperature treatments, 0.95 ± 0.05 and 2.06 ± 0.07 kPa the low and optimum temperature treatments, respectively. The measured carbon dioxide concentrations were not significantly different among the treatments, 422 ± 3.5 µmol mol^{-1}. The measured average soil moisture levels, however, were significantly ($P = 0.05$) different among the two water stress treatments, 0.167 ± 0.016 m^3 m^{-3} for the well-watered and 0.105 ± 0.014 m^3 m^{-3}. This is the first study to identify early season growth, developmental and physiological traits including several root traits of several CS lines for low temperature and drought stress. Knowledge of these CS-lines performance in low

temperature and water stress conditions will be valuable for manipulating breeding techniques for yield improvement and for developing or selecting CS-line best suited for early season planting in many cotton growing areas.

There were no differences ($P = 0.05$) in plant height measured at 25 days after sowing among all the CS lines when plants were grown at optimum temperature and water conditions, averaged 14.9 cm plant^{-1}, and they were not different from the TM-1 cultivar (Table 1). Low temperature and drought stress significantly impacted plant stem elongation and thus plant height in all CS lines including TM-1, stem lengths were 71 and 97% shorter than the control, when averaged across the CS lines, for low temperature and drought conditions, respectively. Similar to the control, CS lines grown in low temperature conditions were not different for plant height except for the CS-B02, which showed a 155% increase suggesting the potential effects of the genes associated with the substituted chromosome two from *G. barbadense*. On the other hand, all CS lines, irrespective of substitution of chromosome arms or segments from three different species of cotton showed shorter plants than TM-1 by 30%. The CS lines, CS-T02, CS-M04, CS-B06, CS-M11sh, and CST15sh were significantly shorter than TM-1 (Table 2.2) suggesting the effects of the substituted chromosome or chromosome segment from the alien species causing this trait differences.

Mainstem pre-fruiting node numbers were drastically affected by low temperature than the drought stress treatments in this experiment; about 5 nodes plant^{-1} were produced during the 25-day period, compared to one and 3.7 nodes plant^{-1} under the low temperature and drought stress treatments, respectively. Similar low temperature effects were recorded in both Upland and Pima cotton cultivars on pre-fruiting and post-fruiting

periods in other studies (Reddy et al., 1992a, 1992b, 1993, 1995). CS-B02 in low temperature treatment produced significantly higher number of pre-fruiting leaves compared to TM-1 suggesting the effect could be the effect of the substituted chromosome. Contrary to this, CS lines CS-T02, CS-M04, CS-B06, CSM15sh, and CS B22sh produced significantly fewer pre-fruiting leaves compared to TM-1 under drought stress conditions (Table 2.2). Reddy et al. (1997a) and Lokhande and Reddy (2014) found linear decline in stem elongation and node addition rates during linear phase of cotton growth and flowering periods of Upland cotton implying that drought stress impacts cotton by affecting cell elongation and division under drought stress conditions. Even though plant height extension and leaf addition rates are recognized as basic phenomena of shoot morphogenesis and growth, the CS-lines are not bringing any positive changes under well-watered or drought stressed conditions. A positive change or increases in plant height and mainstem nodes would have improved cotton growth and developmental processes as the size these organs could affect overall canopy development and finally lint yield (Reddy et al., 1997a; Gerik et al., 1996, 1998). Similarly Roussopoulos et al., (1998) have found reduction in plant height and node number under low temperature stress.

Similar to plant height response to drought and low temperature treatments, whole plant and individual (2nd leaf) leaf areas also declined in all CS lines (Table 2.3). Plants grown in low temperature treatment had no second leaf that is fully mature in any of the treatments. When averaged across all CS lines, the whole plant leaf area in the control treatment was 386 cm plant-1, and drought and Low temperature treatment caused about 65 and 91% reduction in leaf area, respectively, compared to the plants grown under

optimum conditions. The reduction in whole plant leaf area were partly due to reductions in individual leaf sizes and fewer number of leaves produced under low temperature and drought stress treatments (Table 2.2 and 2.3). Reductions in leaf areas have been reported in other studies for plants grown under drought during early season (Ball et al., 1994; Gerik et al., 199; Reddy at al., 1997a) and during early-flowering stages (Lokhande et al., 2014) and low temperature treatments during early-season (Reddy et al, 1992a, 1992b, 1997a6). The 2nd leaf in the CS lines, CS-T06 and CS-B22sh, was 25% larger than TM-1 under optimum conditions and CS-line, CS-B22sh showed 44% smaller leaf area (Table 2.3). Individual and whole plant leaf area was not different among the CS lines for plants grown under drought stress conditions. Whereas, CS lines, CS-B08 and CS-M22sh, had significantly lower whole plant leaf area compared to TM-1 under low temperature conditions showing the effects could be due to the substituted chromosome or chromosome segment from the alien species (Table 2.3).

Table 2.2 Shoot morphological traits of cotton CS-lines.

CS-Lines	Plant height, cm			Leaves, no. plant^{-1}		
	C	LT	DS	C	LT	DS
TM1	15.87	0.47^{A}	6.9^{B}	5.00	1.0^{A}	4.00
CS-B02	17.43	1.2^{Aa}	5.9^{B}	5.67	2.0^{Aa}	3.7
CS-M02	18.50	0.6^{A}	6.63^{B}	5.33	1.3^{A}	3.3
CS-T02	11.37	0.4^{A}	3.27^{Bb}	4.67	0.7^{A}	3.0^{b}
CS-B04	12.87	0.27^{A}	4.27^{B}	4.67	1.0^{A}	3.7
CS-M04	12.37	0.33^{A}	3.77^{Bb}	4.33	1.0^{A}	3.0^{b}
CS-T04	11.97	0.37^{A}	5.47^{B}	4.33	1.3^{A}	4.0
CS-B06	15.80	0.27^{A}	3.63^{Bb}	5.33	1.0^{A}	3.0^{b}
CS-M06	15.10	0.6^{A}	5.33^{B}	5.33	1.3^{A}	4.0
CS-T06	16.30	0.33^{A}	5.1^{B}	5.00	1.0^{A}	4.3
CS-B08sh	15.97	0.2^{A}	5.27^{B}	4.67	0.0	3.3
CS-M08sh	16.47	0.73^{A}	6.47^{B}	5.33	1.3^{A}	4.0
CS-T08sh	11.90	0.37^{A}	4.93^{B}	4.33	1.0	3.3
CS-B11sh	15.50	0.37^{A}	4.6^{B}	5.00	1.0	3.3
CS-M11sh	11.90	0.33^{A}	4.13^{Bb}	4.33	1.0	3.3
CS-T11sh	14.20	0.4^{A}	5.2^{B}	4.67	1.0	3.3
CS-B15sh	16.17	0.4^{A}	4.93^{B}	5.00	0.8	3.0^{b}
CS-M15sh	16.70	0.77^{A}	5.3^{B}	5.00	1.3	3.7
CS-T15sh	16.83	0.4^{A}	4.13^{Bb}	5.33	1.0	4.0
CS-B22sh	16.20	0.43^{A}	3.9^{Bb}	5.00	1.0	3.0^{b}
CS-M22sh	13.33	0.23^{A}	4.93^{B}	4.67	1.0	3.7
CS-T22sh	15.00	0.27^{A}	4.30	5.00	0.7	3.7
Mean	14.90	0.40	4.30	4.91	1.0	3.7

'A' and 'B' represents the significant differences at P = 0.05 level for low temperature and drought stress treatments, respectively, from the control.
'a' and 'b' represents the significant difference at 0.05 level from TM-1 under each treatment condition.
Measurements were taken under control (C), low temperature (LT) and drought stress (DS) 25 days after sowing.

Table 2.3 Shoot morphological traits of cotton CS-lines.

	Second leaf area, cm^2			Total leaf area, cm^2 $plant^{-1}$		
	C	LT*	DS	C	LT	DS
TM1	89.31	-	35.94^{B}	388.48	40.23^{A}	167.00^{B}
CS-B02	63.26	-	31.93^{B}	403.22	65.27^{Aa}	143.65^{B}
CS-M02	83.84	-	38.22^{B}	426.57	50.80^{A}	140.11^{B}
CS-T02	65.50	-	23.55^{B}	272.59	31.96^{A}	84.87b^{B}
CS-B04	67.06	-	34.86^{B}	363.78	35.19^{A}	134.67^{B}
CS-M04	72.27	-	29.74^{B}	321.90	35.42^{A}	103.06^{B}
CS-T04	78.43	-	43.21^{B}	317.87	33.47^{A}	153.85^{B}
CS-B06	90.86	-	34.78^{B}	471.30	26.63^{A}	144.91^{B}
CS-M06	84.43	-	34.20^{B}	462.26	44.21^{A}	145.22^{B}
CS-T06	112.00^{a}	-	35.37^{B}	464.27	32.42^{A}	152.00^{B}
CS-B08sh	98.30	-	36.26^{B}	360.05	23.14^{Aa}	130.88^{B}
CS-M08sh	50.70^{a}	-	27.11^{B}	356.47	37.15^{A}	134.04^{B}
CS-T08sh	72.86	-	30.74^{B}	285.18	35.14^{A}	127.39^{B}
CS-B11sh	101.42	-	36.25^{B}	479.92	38.07^{A}	150.16^{B}
CS-M11sh	91.21	-	41.00^{B}	363.54	26.27^{A}	134.13^{B}
CS-T11sh	89.32	-	37.34^{B}	371.44	35.15^{A}	129.25^{B}
CS-B15sh	98.90	-	30.71^{B}	416.50	31.03^{A}	113.98^{B}
CS-M15sh	67.52	-	25.62^{B}	399.68	39.26^{A}	128.40^{B}
CS-T15sh	76.36	-	34.07^{B}	454.63	35.59^{A}	135.14^{B}
CS-B22sh	111.00^{a}	-	27.55^{B}	487.57	33.52^{A}	102.70^{B}
CS-M22sh	58.24	-	24.04^{B}	278.56	23.01^{Aa}	105.37^{B}
CS-T22sh	68.86	-	30.90	345.22	28.13^{A}	125.19^{B}
Mean	81.44	-	32.88	385.95	35.24	133.38

'A' and 'B' represents the significant differences at P = 0.05 level for low temperature and drought stress treatments, respectively, from the control.
'a' and 'b' represents the significant difference at 0.05 level from TM-1 under each treatment condition.
*The second leaf in low temperature treatment was not fully matured when the experiment was terminated at 25 days after sowing.
Measurements were taken under control (C), low temperature (LT) and drought stress (DS) 25 days after sowing.

Root Parameters

Root growth and developmental dynamics are integral parts of plant stand establishment during the early-season and improving root traits will have a positive impact on for soil-plant-atmosphere water dynamics and crop yield. Therefore, several

attempts have been used to address and to quantify environmental factors on cotton growth and development using other non-destructive methods such mini-rhizotron systems (Bland, 1993; Reddy et al. 1997c, 1997d; McMichael et al., 2010). In this experiment, low temperature and drought stress effects on early-season total root length, root surface area, root diameter, which are indicators of the root size and functions (Costa et al., 2002), and also are useful parameters in determining nutrient uptake efficiency and performance under varied stress conditions (McMichael et al., 1996; Hammer et al., 2009; Rosolem et al., 1994; Wijewardana et al., 2015) were quantified using winRhizo optical root image system and analysis among CS-lines (Table 2.4). When averaged across all CS-lines, plants grown under low temperature and drought stressed conditions produced 85 and 44% less total root length and 78 and 44% less root surface area, respectively, compared to plants grown under optimum conditions (Table 2.4). The average root diameters, on the other hand, was greater (35%) under low temperature and unchanged under drought stress treatments when compared to plants grown under optimum conditions (Table 2.4).

Total root length, was significantly lower by 18-24% in CS-M08sh and CS-T22sh and by 47-59% in CS-M22sh and CS-B08sh when compared to TM-1(Table 3). Among the drought stressed treatment, CS-T04 was the only line that showed significantly lower total root length (38%) when compared TM-1 under the same conditions suggesting the genes located on the substituted chromosome from *G. tomentosum* causing this difference. The CS-M22sh under low temperature and CS-T02 and CS-B08sh under drought stressed treatments showed 40-47% reduction in total root surface area, when compared to TM-1 (Table 2.4). The Cs-M08sh was the only line that showed 14%

increase in average root diameter under low temperature treatment when compared to TM-1 grown under the same condition suggesting the effects of the alien species substituted chromosome with this phenotype (Table 2.4).

Table 2.4 Root growth traits of cotton chromosome substitution (CS) lines.

CS-Lines	Root length, cm plant^{-1}			Root surface area, cm^2 plant^{-1}			Average root diameter, cm		
	C	LT	DS	C	LT	DS	C	LT	DS
TM1	4348.1	952.0^{A}	2980.9^{B}	628.34	185.58^{A}	430.22^{B}	0.46	0.62^{A}	0.46
CS-B02	5065.3	1027.8^{A}	3156.7^{B}	707.47	186.37^{A}	474.16^{B}	0.45	0.60^{A}	0.48
CS-M02	5563.8	819.1^{A}	2527.8^{B}	800.33	161.63^{A}	378.79^{B}	0.45	0.63^{A}	0.47
CS-T02	3961.3	694.2^{A}	1836.7^{Bb}	554.85	129.00^{A}	252.41^{Bb}	0.45	0.59^{A}	0.44
CS-B04	5169.7	924.2^{A}	3015.4^{B}	758.37	163.67^{A}	432.85^{B}	0.46	0.56^{A}	0.46
CS-M04	4176.9	758.2^{A}	2147.2^{B}	623.63	146.93^{A}	325.68^{B}	0.47	0.62^{A}	0.48
CS-T04	4532.0	1131.8^{A}	3388.5^{B}	657.13	207.17^{A}	480.15^{B}	0.47	0.60^{A}	0.45
CS-B06	5837.5^{a}	748.6^{A}	2737.1^{B}	900.60	144.34^{A}	384.36^{B}	0.49	0.61^{A}	0.45
CS-M06	5818.0^{a}	731.0^{A}	3022.5^{B}	835.90	135.40^{A}	442.10^{B}	0.46	0.58^{A}	0.46
CS-T06	4831.4	711.8^{A}	2863.7^{B}	736.52	142.86^{A}	444.22^{B}	0.49	0.64^{A}	0.49
CS-B08sh	4357.9	386.0^{Aa}	1967.6^{B}	570.15	73.84^{Aa}	254.19^{Bb}	0.41	0.62^{A}	0.42
CS-M08sh	3530.0	421.0^{Aa}	2159.5^{B}	537.20	94.07^{Aa}	302.48^{B}	0.48	0.71^{Aa}	0.45
CS-T08sh	3718.6	746.7^{A}	2835.0^{B}	519.12	145.30^{A}	394.54^{B}	0.44	0.62^{A}	0.45
CS-B11sh	5397.9	800.0^{A}	2957.4^{B}	880.56	149.34^{A}	430.38^{B}	0.52^{a}	0.61	0.47
CS-M11sh	4698.7	668.7^{A}	2518.7^{B}	755.68	134.47^{A}	389.21^{B}	0.51	0.64^{A}	0.49
CS-T11sh	3652.6	773.0^{A}	2275.9^{B}	540.96	149.42^{A}	355.93^{B}	0.47	0.62^{A}	0.49
CS-B15sh	4337.0	675.4^{A}	2286.8^{B}	618.28	136.47^{A}	342.43^{B}	0.45	0.64^{A}	0.47
CS-M15sh	5387.6	1093.3^{A}	2252.1^{B}	794.44	185.23^{A}	312.58	0.47	0.55	0.44
CS-T15sh	4995.9	556.9^{A}	2793.8^{B}	743.34	107.96^{A}	402.79^{B}	0.47	0.62^{A}	0.46
CS-B22sh	5828.8^{a}	727.3^{A}	1969.2^{B}	813.13	147.70^{A}	290.35^{B}	0.45	0.66^{A}	0.48
CS-M22sh	3167.3	500.9^{Aa}	2151.5^{B}	463.05	97.42^{Aa}	314.54^{B}	0.46	0.63^{A}	0.47
CS-T22sh	4106.9	721.4^{A}	2582.2^{B}	601.03	146.21^{A}	373.94^{B}	0.46	0.65^{A}	0.46
Mean	4658.3	803.23	2599.51	683.64	152.90	385.08	0.46	0.62	0.46

'A' and 'B' represents the significant differences at P = 0.05 level for low temperature and drought stress treatments, respectively, from the control.
'a' and 'b' represents the significant difference at 0.05 level from TM-1 under each treatment condition.
Measurements were taken under control (C), low temperature (LT) and drought stress (DS) 25 days after sowing.

Physiological Parameters

Plants grown under low temperature treatment produced significantly lower (about 40%) total chlorophyll content as well individual chlorophyll a and chlorophyll b

contents when averaged across all CS-lines (Table 2.5). Plants grown under drought stressed conditions, on the other hand, did not show any difference in pigments components and total pigments, when expressed on a leaf area basis. The CS-lines grown under drought stressed conditions also didn't show any differences when compared to TM-1 for total or individual pigments components (chlorophyll a, and chlorophyll b) (Table 2.5). The chlorophyll a content in CS-lines (CS-M06, CS-T08sh and CS-M15sh) and chlorophyll b content in CS-M06 and total chlorophyll in CS-M06 and CS-M15sh, for plants grown under low temperature conditions produced significantly lower amounts (34-47%) when compared to the respective treatment TM-1 plants suggesting the effects due to the substituted chromosome or chromosome segment from the alien species (Table 2.5). This could be due to lowered synthesis of pigments and its components under low temperature conditions as have been reported (Nie and Baker, 1991; Haldiman, 1997). Kornerova el al., (1999) have found a strong decrease in the contents of chlorophylls when the plants were subjected to low temperature.

Table 2.5 Physiological traits of cotton chromosome substitution (CS) lines

CS-Lines	Chlorophyll a, µg cm^2			Chlorophyll b, µg cm^2			Total chlorophyll , µg cm^2		
	C	LT	DS	C	LT	DS	C	LT	DS
TM1	18.97	14.93^A	20.06	9.04	6.22^A	9.71	28.01	21.14^A	29.77
CS-B02	18.26	18.33	19.73	8.59	8.72	9.42	26.85	27.05	29.15
CS-M02	18.21	12.67^A	18.88	8.33	5.79^A	8.62	26.54	18.47^A	27.50
CS-T02	21.07	11.27^A	20.76	10.00	5.08^A	9.90	31.07	16.35^A	30.66
CS-B04	18.87	15.23	19.54	9.21	7.08	9.39	28.08	22.31	28.93
CS-M04	18.53	10.49^A	18.27	9.13	4.82^A	8.85	27.67	15.30^A	27.13
CS-T04	19.94	10.28^A	21.28	9.56	4.53^A	10.30	29.51	14.81^A	31.59
CS-B06	19.53	14.82^A	20.83	9.03	6.44^A	10.03	28.56	21.25^A	30.86
CS-M06	19.04	8.64^{Aa}	20.12	9.05	3.83^{Aa}	9.61	28.09	12.46^{Aa}	29.74
CS-T06	20.39	10.42^A	19.26	9.68	4.69^A	9.22	30.07	15.11^A	28.49
CS-B08sh	21.74	10.18^A	20.63	10.50	4.40^A	9.62	32.24	14.58^A	30.25
CS-M08sh	20.87	10.08^A	20.03	9.99	4.59^A	9.88	30.87	14.68^A	29.91
CS-T08sh	20.39	7.91^{Aa}	19.45	9.66	3.56^{Aa}	9.13	30.05	11.47^{Aa}	28.58
CS-B11sh	19.57	10.37^A	20.19	9.50	4.78^A	9.85	29.08	15.15^A	30.04
CS-M11sh	21.60	11.20^A	20.00	10.33	6.08^A	9.36	31.93	17.28^A	29.36
CS-T11sh	19.46	13.61^A	19.70	9.32	6.33^A	9.38	28.78	19.94^A	29.08
CS-B15sh	20.24	9.99^A	19.97	9.69	4.91^A	9.53	29.93	14.91^A	29.50
CS-M15sh	20.48	9.41^{Aa}	20.56	9.84	4.55^A	10.05	30.32	13.96^{Aa}	30.61
CS-T15sh	21.16	14.61^A	22.57	10.14	6.98^A	11.05	31.30	21.59^A	33.62
CS-B22sh	20.42	12.45^A	20.56	9.86	5.69^A	9.75	30.28	18.14^A	30.31
CS-M22sh	19.32	12.34^A	20.57	9.11	5.47^A	9.53	28.43	17.81^A	30.11
CS-T22sh	19.72	18.69	19.90	9.37	9.37	9.60	29.09	28.06	29.50
Mean	19.90	12.18	20.13	9.50	5.63	9.63	29.40	17.81	29.76

'A' and 'B' represents the significant differences at P = 0.05 level for low temperature and drought stress treatments, respectively, from the control.
'a' and 'b' represents the significant difference at 0.05 level from TM-1 under each treatment condition.
Measurements were taken under control (C), low temperature (LT) and drought stress (DS) 25 days after sowing.

Plant-component and Total Weight

Above-ground, root and total dry weights of plants grown under low temperature and drought stress conditions varied for certain CS-lines (Table 5). When averaged across the CS-lines, plants grown under low temperature produced 77-78 and 86% lower above-

ground and root and total dry weights, respectively when compared to plants grown at optimum conditions. Similarly, plants grown under drought stress treatments also produced lower biomass by 54, 33, and 50% for above-ground, root and total dry weights, respectively, when compared to plants grown under optimum conditions (Table 2.6). Low temperature condition in this experiment caused drastic reduction in plant-component and total dry weights than drought stress conditions. The reduction under low temperature and drought stress treatments are similar many previous studies during other stages of cotton (Bradow, 1990; Reddy et al., 1992a, 1992b; Lokhande and Reddy, 2014a, 2014b).

There were no differences (P = 0.05) in above-ground, root and total plant dry weights measured at 25 days after sowing among all the CS lines when plants were grown at optimum temperature and water conditions, except CS-B06, which showed 44% more above-ground dry weight than TM-1 suggesting the association of the substituted chromosome causing this difference in dry weight (Table 2.6). The CS-B02 (43%) and CS-M04 (-43%) produced significantly higher and lower above-ground plant dry weight under low temperature and drought stress treatments, respectively, compared to TM-1 at the respective treatments. Similarly, root biomass production was lower by 50% in CS-B08sh and CST22sh for plants grown under low temperature treatment, and CST02 and CS-B-08sh by 58% when compared to respective treatment-dependent TM-1 plants. Similar to root dry weight responses, CS-B08sh and CS-CS22sh produced significantly lower total biomass compared to TM-1 for plants grown under low temperature treatment. The CS-T02 and CS-B22sh and CS-M22sh produced 40 and 66% less total

biomass, respectively, when compared to TM-1, when plants were grown under drought stressed condition (Table 2.6).

In general, when averaged across all CS-lines, 91 and 54% more biomass was portioned to root (root /shoot ratio) for plants grown at low temperature and drought stressed treatments, respectively, compared to biomass partitioning to roots for plants grown under optimum conditions (Table 2.7). Similar biomass partitioning responses were recorded in earlier at other growth stages under low temperature (Hodges et al., 1993; Reddy et al., 1992a, 1992b) and drought stress conditions (Lokhande and Reddy, 2014b). The root/shoot ratio was significantly lower in CS-lines, CS-T08sh (about 50%) and CS-B08sh (39%) when compared to respective treatment-dependent TM-1 plants under low temperature and drought stressed condition, respectively suggesting the potential role of the substituted chromosome segment causing this differences (Table 2.7).

Table 2.6 Plant dry weight of cotton chromosome substitution (CS) lines

CS-Lines	Above- ground weight, g plant^{-1}			Root dry weight, g plant^{-1}			Total dry weight, g plant^{-1}		
	C	LT	DS	C	LT	DS	C	LT	DS
TM1	2.45	0.30^{A}	1.33^{B}	0.49	0.13^{A}	0.41	2.94	0.44^{A}	1.74^{B}
CS-B02	2.78	0.43^{Aa}	1.17^{B}	0.54	0.16^{A}	0.33^{B}	3.32	0.59^{A}	1.49^{B}
CS-M02	3.00	0.37^{A}	1.06^{B}	0.62	0.14^{A}	0.32^{B}	3.62	0.51^{A}	1.38^{B}
CS-T02	1.90	0.25^{A}	0.8^{Bb}	0.48	0.11^{A}	0.24^{Bb}	2.37	0.35^{A}	1.04^{Bb}
CS-B04	2.30	0.30^{A}	1.09^{B}	0.58	0.13^{A}	0.38^{B}	2.88	0.43^{A}	1.47^{B}
CS-M04	1.89	0.27A	0.76^{Bb}	0.49	0.12^{A}	0.29^{B}	2.38	0.39^{A}	1.05^{Bb}
CS-T04	1.92	0.31^{A}	1.25^{B}	0.49	0.16^{A}	0.45	2.41	0.47^{A}	1.70^{B}
CS-B06	3.53^{a}	0.24^{A}	1.31^{B}	0.73	0.13^{A}	0.37^{B}	4.27^{a}	0.37^{A}	1.68^{B}
CS-M06	3.08	0.31^{A}	1.19^{B}	0.74	0.12^{A}	0.44^{B}	3.82	0.43^{A}	1.63^{B}
CS-T06	2.88	0.29^{A}	1.41^{B}	0.63	0.11^{A}	0.46^{B}	3.51	0.40^{A}	1.87^{B}
CS-B08sh	2.45	0.23^{A}	1.25^{B}	0.45	0.06^{Aa}	0.24^{Bb}	2.90	0.29^{Aa}	1.49^{B}
CS-M08sh	2.46	0.34^{A}	1.07^{B}	0.47	0.09^{A}	0.39	2.93	0.43^{A}	1.46^{B}
CS-T08sh	1.76	0.33^{A}	0.98^{B}	0.41	0.12^{A}	0.36^{B}	2.16	0.45^{A}	1.34^{B}
CS-B11sh	3.16	0.30^{A}	1.28^{B}	0.69	0.11^{A}	0.39^{B}	3.84	0.41^{A}	1.67^{B}
CS-M11sh	2.49	0.28^{A}	1.15^{B}	0.55	0.12^{A}	0.50	3.04	0.40^{A}	1.66^{B}
CS-T11sh	2.20	0.26^{A}	1.05^{B}	0.47	0.12^{A}	0.34^{B}	2.67	0.39^{A}	1.39^{B}
CS-B15sh	2.86	0.24^{A}	0.99^{B}	0.51	0.11^{A}	0.34^{B}	3.37	0.34^{A}	1.33^{B}
CS-M15sh	2.63	0.35^{A}	1.01^{B}	0.61	0.14^{A}	0.28^{B}	3.24	0.49^{A}	1.29^{B}
CS-T15sh	3.32	0.27^{A}	1.18^{B}	0.64	0.10^{A}	0.37^{B}	3.96	0.37^{A}	1.55^{B}
CS-B22sh	3.33	0.32^{A}	0.87^{B}	0.64	0.13^{A}	0.27^{B}	3.96	0.45^{A}	1.14^{Bb}
CS-M22sh	1.85	0.23^{A}	0.90^{B}	0.38	0.07^{Aa}	0.26^{B}	2.23	0.29^{Aa}	1.16^{Bb}
CS-T22sh	2.40	0.26^{A}	1.21^{B}	0.46	0.12^{A}	0.36^{B}	2.85	0.38^{A}	1.56^{B}
Mean	2.53	0.29	1.14	0.55	0.12	0.37	3.07	0.42	1.54

‘‘A’ and ‘B’ represents the significant differences at P = 0.05 level for low temperature and drought stress treatments, respectively, from the control.
‘a’ and ‘b’ represents the significant difference at 0.05 level from TM-1 under each treatment condition.
Measurements were taken under control (C), low temperature (LT) and drought stress (DS) 25 days after sowing.

Table 2.7 Root / Shoot ratio of cotton chromosome substitution (CS) lines.

CS-Lines	Root/Shoot ratio		
	C	LT	DS
TM1	0.20	0.46^{A}	0.31
CS-B02	0.19	0.40^{A}	0.35^{B}
CS-M02	0.21	0.39^{A}	0.31
CS-T02	0.26	0.43^{A}	0.30
CS-B04	0.26	0.43^{A}	0.35^{B}
CS-M04	0.26	0.43^{A}	0.37^{B}
CS-T04	0.31	0.52^{A}	0.36
CS-B06	0.21	0.56^{A}	0.29
CS-M06	0.24	0.41^{A}	0.39^{B}
CS-T06	0.22	0.38^{A}	0.33
CS-B08sh	0.18	0.21^{a}	0.19^{b}
CS-M08sh	0.18	0.25^{a}	0.36^{B}
CS-T08sh	0.23	0.37^{A}	0.37^{B}
CS-B11sh	0.21	0.38^{A}	0.30
CS-M11sh	0.22	0.44^{A}	0.46^{B}
CS-T11sh	0.21	0.45^{A}	0.32^{B}
CS-B15sh	0.18	0.44^{A}	0.34^{B}
CS-M15sh	0.23	0.40^{A}	0.28
CS-T15sh	0.19	0.37^{A}	0.32^{B}
CS-B22sh	0.20	0.39^{A}	0.32^{B}
CS-M22sh	0.20	0.30	0.29
CS-T22sh	0.19	0.46^{A}	0.30^{B}
Mean	0.22	0.42^{A}	0.34^{B}

'A' and 'B' represents the significant differences at P = 0.05 level for low temperature and drought stress treatments, respectively, from the control.
'a' and 'b' represents the significant difference at 0.05 level from TM-1 under each treatment condition.
Measurements were taken under control (C), low temperature (LT) and drought stress (DS) 25 days after sowing.

Combined Low Temperature Response Index (CLTRI) and Combined Drought Stress Response Index (CDSRI)

The CS-lines were classified into low temperature and drought stress tolerance by combined low temperature response index (CLTRI) and combined drought stress

response index (CDSRI) using all the 19 parameters measured. This method integrates all the parameters and their responses to drought/low temperature stress conditions.

Among the 21 CS-lines tested for CLTRI, all except CS-T04 showed lower values compared to TM-1 and CS-B08sh was significantly lower (48%) CLTRI when compared to TM-1. The CS-line, CS-T04, exhibited higher (36%) CLTRI showing a potential for further studies for low temperature tolerance (Fig. 2.1). Similarly, the CS-line, CS-T04, was the only line among all lines tested exhibited higher (31) drought tolerance. Even though, all CS-lines except CS-T04 showed lower CDSRI values compared TM-1, the CS-B22sh showed significantly lower (50%) CDSRI value showings its susceptibility under drought conditions (Fig. 2.2). Our results suggested that CS-T04 would have good potential under drought stress condition and CS-B22sh has lowest potential under drought stress condition. Further investigation with CS-T04 will be helpful in improving Upland cotton germplasm under drought stress condition.

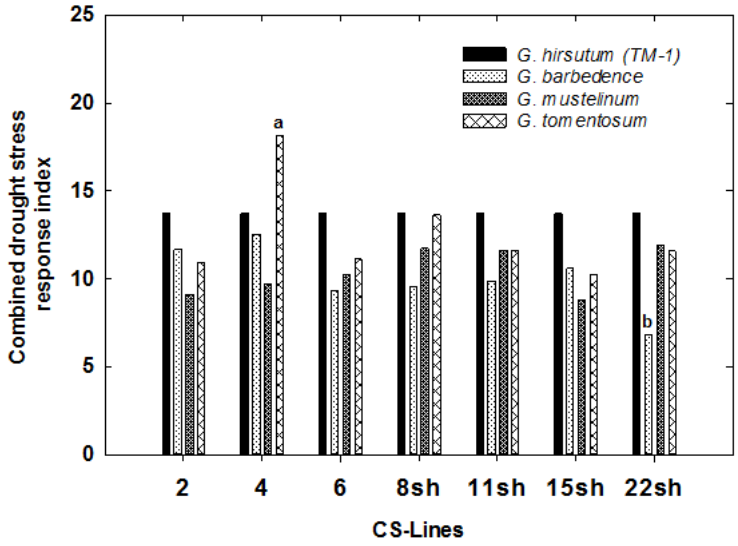

Figure 2.1 Combined low temperature stress response index of cotton CS-lines.

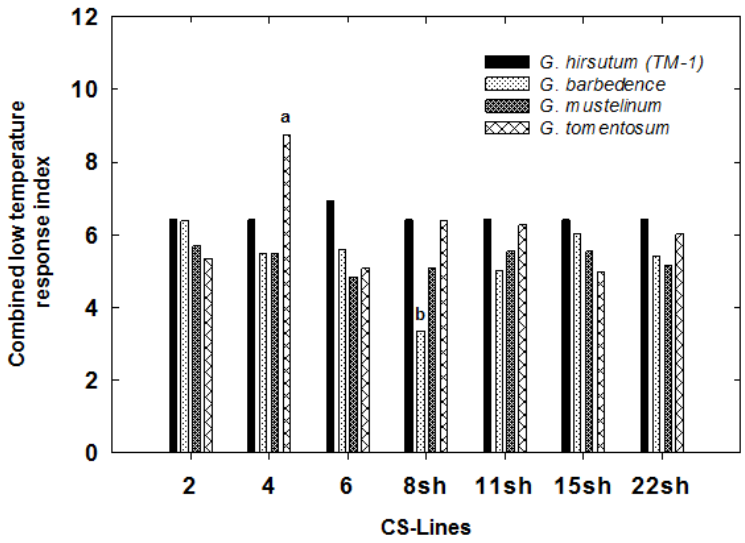

Figure 2.2 Combined drought stress response index of cotton CS-lines.

Inter-relationships between Shoot and Root Vigor Indices and Shoot and Root Vigor Indices and Combined Stress Response Indices

Figure 2.1 and 2.2 illustrate the relationships between total shoot, root and combined stress response vigor indices utilizing the CS-lines and treatments. We found a linear relationship between shoot and root combined vigor indices (R2 = 0.64) indicating either indices could be used for screening CS-lines for various abiotic stresses (Fig. 2.3). However, a very strong linear relationships were obtained between combined vigor index and shoot (R2 = 0.92) and root traits (R2 = 0.86) indicating that indices developed based on both root and shoot traits will be more helpful in developing indices and in screening CS-lines for abiotic stress tolerance (Fig. 2.4)

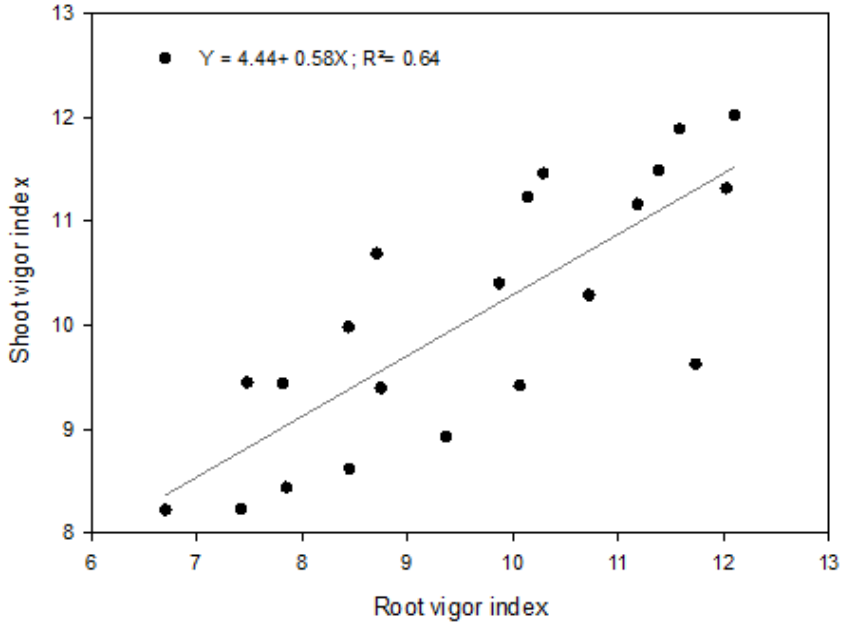

Figure 2.3 The relationship between root and shoot vigor index.

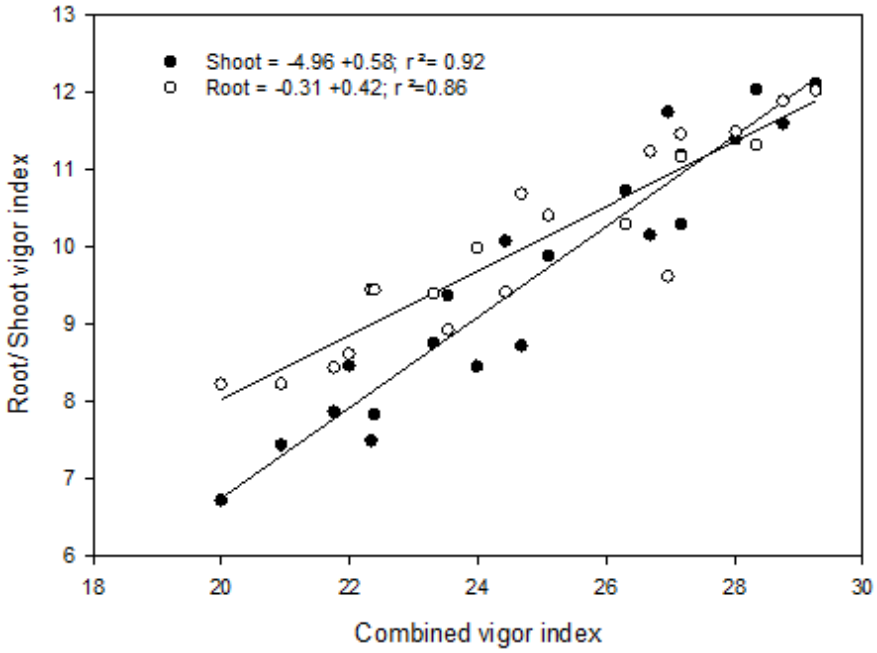

Figure 2.4 The relationship between root/shoot and combined vigor index.

In these studies, we have investigated 21 CS-lines for low temperature and drought stress tolerance when compared to the parental cotton line, TM-1. The results show that low temperature stress impacted more severely on all plant traits measured during seedling growth stage. All CS-lines showed shorter plants and fewer number of leaves under low temperature conditions. Similarly, plant growth in terms of plant components dry weights and total dry weight and whole plant leaf area were also lower for plants grown under low temperature conditions compared to plants grown under optimum conditions. Among 21-CS lines tested, CS-T04 more tolerant while CS-B08sh was less tolerant to low temperature. These CS-lines warrants further investigations to identify stress tolerance mechanisms. Similarly, CS-T04 was also identified as more tolerant to drought stress among the 21 CS-lines tested in this study. However, CS-B22sh was found less tolerant to drought stressed condition among the CS lines tested in this study. Since CS-T04 was identified as more tolerant to both low temperature and drought stressed conditions, further investigations are needed to unravel mechanisms of tolerance in this line when compared to the TM-1.

CHAPTER III

SUMMARY AND CONCLUSIONS

To meet the demands for fiber quality with increasing population and to face the competition from synthetic fibers, Upland cotton yield and fiber quality should be enhanced. The narrow genetic base of Upland cotton, however, makes it a challenge to optimize management to increase productivity and fiber quality. The chromosome (CS)-lines from sister species with superior fiber quality and abiotic stress tolerance will provide a platform to increase genetic diversity available in Upland cotton. To assess cold tolerance and root morphology in a set of CS lines in which G. hirsutum chromosome or chromosome segment substituted with the respective chromosome or chromosome segments of *G. barbadense* (CS-B), *G. mustelinum* (CS-M) or *G. tomentosum* (CS-T) germplasm. Twenty-one CS-lines were evaluated for their level of tolerance to low temperature and drought conditions by comparing with TM-1. In Experiment I, plants were grown at low (20/12 °C) and optimum (30/22°C) temperatures under optimum water and nutrient conditions. In the Experiment II, plants were grown under drought (50% evapo-transpiration, ET) and well-water 100% ET)-based irrigation under optimum temperature condition. Above- and below-ground growth parameters including several root traits using WinRHIZO root image analysis system were assessed at 25 d after seeding in both the experiments. The CS-lines varied significantly for many traits measured, particularly, days to 50% seedling emergence, plant height, dry matter

weights, and root morphological parameters. CS-B08sh showed early emergence in both the experiments, but other CS-lines were not different from TM-1. Plant height at low temperature ranged from 0.2 cm (CS-B08sh) to 1.2 cm (CS-B02) with an average of 0.44 cm, and under drought stress it varied from 3.2 (CS-T02) to 6.6 cm (CS-M02) with an average of 4.8 cm, whereas at optimum temperature, it varied from 11.3 (CS-T02) to 17.4 cm (CS-B02) with the average of 14.8 cm. The average root diameter was greater at low temperature treatment (0.64 mm) than at optimum temperature (0.47 mm), while under drought stress it was about 0.46 mm. Combined low temperature- and drought response index, a measure of all response indices combined at low to optimum temperature and drought to optimum and well-watered, was used to categorize CS-lines early-season tolerance to low temperature and drought stress. Among the 21 CS-lines tested, CS-T04 and CSB08sh showed significantly higher and lower tolerance to low temperature, respectively compared to isogenic TM-1, while CS-T04 and CS-B22sh showed significantly higher and lower tolerance to drought condition compared to isogenic TM-1. Strong linear and positive correlation between shoot and root vigor indices and linear correlation between combined vigor indices indicate that shoot or root traits could be used to test stress tolerance among the CS-lines. The identified low temperature and drought-tolerant CS-lines might be useful in cotton breeding programs for Upland cotton improvement.

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