PHOTOSYNTHETIC THERMOTOLERANCE AND PHYSIOLOGICAL CONTRIBUTORS TO YIELD IN DIVERSE COTTON GENOTYPES

By

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(Under the Direction of John L. Snider)

ABSTRACT

Past cotton breeding programs have primarily focused on enhancing yield through increasing lint percent and selecting a limited set of desirable agronomic traits. However, without targeted efforts to select for other functional yield drivers or tolerance to abiotic stress, cotton crops are at a greater risk of experiencing yield losses caused by environmental extremes. To further improve cotton cultivars, breeding programs should incorporate diverse germplasm and prioritize the selection of traits that contribute to yield under varying environmental conditions. This research included two field experiments and one controlled-environment experiment. The objectives of the field experiments were to assess genotypic variation in thermotolerance of thylakoid component processes for diverse cotton genotypes and quantify differences in physiological (∑IPAR, RUE, and HI) and yield component contributors to yield in a diverse set of field-grown cotton genotypes. The first experiment highlighted genotypic variations in thermotolerance of photosystem II, intersystem electron transport, and photosystem I. Intersystem electron transport exhibited greater heat sensitivity than photosystem II or I. In the second experiment, lint yield, biomass production, light interception, and harvest index were affected by genotype, with harvest index being a better predictor of lint yield than other traits. Boll production and intra-boll yield components were also genotype-dependent. The objective of the controlled-environment experiment was to assess the effects of growth temperature and genotype on early plant growth, single-leaf physiology, and thermotolerance of thylakoid processes for cotton exposed to optimal and supra-optimal temperature conditions. Significant interactions between genotype and growth temperature were

observed for all growth metrics, and some of the physiological processes, and for thermotolerance of photosystem II. Genotypes with higher growth-specific thermotolerance showed greater leaf area and lower nighttime respiration and stomatal conductance under high temperatures relative to the optimum. Net photosynthesis was not predictive of growth response to high temperature. Photosystem II acclimated more readily to high temperature in some genotypes than in others, but high temperature thresholds for PSII were not consistently predictive of growth under high temperature extremes. It is concluded that genotypic differences in thermotolerance will depend on acclimation of multiple processes.

INDEX WORDS: *Gossypium hirsutum*; *Gossypium barbadense*; Thermotolerance; Photosynthesis; Thylakoid reactions; Physiological contributors; Intra-boll components; Lint yield; Heat acclimation

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CHAPTER 1

INRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Cotton is the most important fiber crop in the world, and *Gossypium hirsutum*, also known as Upland cotton, accounts for the majority of global production. Growth, development, and productivity of a cotton plant can be determined by its genotype and environment. Having a diverse set of genetic material to mine through selective breeding is important for 1) continued ability to respond to future threats such as climate change and 2) continued agronomic improvement of cotton cultivars. Daily mean temperatures in many cotton producing regions are already near the upper limit of the optimum temperature range for growth and development or this range has already been surpassed. Furthermore, climate change is expected to increase the duration, severity, and intensity of heat wave events, which will likely have negative implications for crop production. This has made the selection of heat tolerant genotypes essential, and identifying some of the most heat sensitive processes will help define the weakest links in plant performance under high temperature extremes.

Cotton breeding efforts have produced consistent yield improvement through selection for increases in lint percent. However, yield is ultimately a product of solar radiation absorbed by the canopy, the efficiency with which radiation is converted to dry matter, and the fraction of total biomass accounted for by lint. The importance of each of these functional traits in driving genetic differences in yield has received only limited attention in cotton, and should be explored further. To this end, the currently proposed research focuses on genotypic variation in thermotolerance and the physiological contributors to yield variation in diverse cotton genotypes.

1.2. Literature Review

Cotton production and crop development:

Cotton refers to any plant within the genus *Gossypium* from the family Malvaceae that is grown for spinnable fibers, which are hair-like cellulosic extensions of seed coat cells (Jabran et al., 2020). Cotton is the most widely-grown fiber crop in the world. The genus *Gossypium* includes approximately 50 species, but not all the species are used for cultivation nor do they produce spinnable fibers (Fryxell 1992). The most widely cultivated species of cotton is *Gossypium hirsutum* L. which is commonly known as Upland cotton. It is a high-yielding cotton species and accounts for more than 90% of cotton production around the world (Jabran et al., 2020). The other cultivated species of cotton are *G. barbadense* L. (Egyptian cotton), *G. herbaceum* L. (Arabian/Levant cotton) and *G. arboreum* L. (tree cotton) (Lee and Fang 2010). The United States is one of the top cotton producing countries in the world, following only India and China in total production. During the year 2019/2020, the US produced 922 kg ha^{-1} of cotton on 4.7 million hectares (USDA 2021). Cotton is grown across the southern half of the US in a region referred to as the cotton belt, and the state of Georgia is second only to Texas in total cotton production.

The cotton plant is an indeterminate, perennial shrub that is most commonly grown as an annual crop (Mauney 1984). The growth and development of a cotton plant is usually divided into four stages: germination and seedling emergence, leaf area and canopy development, flowering and boll development, and maturation. Cotton exhibits epigeal germination where the hypocotyl rapidly elongates and forms a hook that pulls the cotyledons upwards and out of the soil (Rahman and Farooq 2019). The emergence of the radicle from the seed coat usually takes place within 2-3 days and the seedlings emerge out of soil in about 5-10 days after planting. Root growth occurs at a faster rate than the shoot, with the primary root reaching up to 25 cm before unfolding of the

cotyledons (Taylor and Ratcliff 1969). Cotyledons expand and become green after exposure to light and start photosynthesizing, signaling a transition from dependence on seed storage reserves to autotrophy.

Canopy development follows a typical sigmoidal growth pattern, with leaf area production exhibiting a lengthy lag phase (Snider and Oosterhuis, 2015; Snider et al., 2021). For example, the only leaves on the plant are mainstem leaves initially, and the addition of first 4-5 true leaves occurs every few days in an alternate pattern up the mainstem. However, once the cotton plant begins producing branches and fruiting sites, along with a subtending leaf at each fruiting site, leaf area development will enter an exponential growth phase near first flower (~60 days after planting). The cotton plant has two types of branches: vegetative (monopodial) and reproductive or fruit bearing (sympodial) branches. The sympodial branches have a zig-zag pattern with multiple meristems where the monopodial branches have only a single meristem (Mauney 1984).

Reproductive growth starts about 4-5 weeks after planting. Flowers start as squares which are small green structures and pyramidal in shape. It takes about 25 days for a pin-head square to develop into a white flower (Tharp 1965). Pollination occurs shortly after opening of the flower, and the time period between pollination and fertilization is affected by temperature (Oosterhuis and Jernstedt 1999). After fertilization, the ovary begins to expand, and will eventually develop into a type of fruit called a capsule. The capsule in cotton is referred to as a boll. The boll reaches its maximum size at about 25 days after anthesis (Mauney 2015). The mature bolls start drying and split open 40-45 days after pollination (Balls 1915).

Maturity of the cotton crop is usually described as physiological maturity, harvest maturity or agronomic maturity (Bruns, 2009). Physiological maturity is attained when the number of mainstem nodes above the uppermost, first position white flower (NAWF) are no more than 5

because fruit produced after this point in the season do not often mature to a harvestable boll that will contribute appreciably to yield (Bourland et al., 1992). This point is also referred to as cutout. The cotton crop is considered mature for harvesting if it has produced enough open bolls to achieve maximum economic production (Gwarthmey et al., 2016). Agronomic maturity is the developmental stage of the cotton crop when it is ready for the application of harvest-aid chemicals (Brecke et al., 2001). The cotton crop is generally considered to have reached agronomic maturity at 60% open boll.

Genetic diversity:

Globally, cotton has wide genetic diversity, where wild relatives of cotton are substantially different from the current, improved cultivars in many ways. In their native habitats, wild cotton plants are indeterminate perennials resembling shrubs or small trees. In contrast, cotton is widely grown as an annual crop. *G. hirsutum* has been found to be more genetically diverse within the species than the other three major cultivated species of cotton (Abdurakhmonov et al., 2008). G. *hirsutum* originated in Mesoamerica (Mexico and Guatemala), but it spread throughout Central America and the Caribbean (Wendel 1995). As documented by Mauer (1954), G. *hirsutum* can be divided into four groups of sub-species: 1) G. *hirsutum ssp mexicanum*; 2) G. *hirsutum ssp paniculatum*; 3) G. *hirsutum ssp punctatum* and 4) G. *hirsutum ssp euhirsutum*. These four groups encompass all wild and domesticated races of *G. hirsutum* from 80 different countries (Lacape et al., 2007). The sub species *mexicanum* includes the wild races with bushy, perennial habit with medium sized leaves and short fruiting branches and low seed germination percentage (Abdurakhmonov et al., 2004). The sub species *paniculatum* includes cultivated-tropical races with semi-sympodium and sympodium bushy habit having big leaves and long branches and low seed germination (Abdurakhmonov et al., 2004). *Punctatum* sub-species includes the half-wild

and primitive cultivated races having shrubs with slender stems, three-lobed leaves, small to medium seeds and short fibers and comparatively higher seed germination rate (Abdurakhmonov et al., 2004). The sub-species group *euhirsutum* includes semi-herbal bushes with sympodial stems and mainly sympodial branches, varying leaf sizes and shapes, fiber quality and high seed germination rates (Abdurakhmonov et al., 2004).

Selective breeding has utilized a number of valuable traits from wild cotton accessions, but continued selection for agronomically desirable traits has led to a reduction in genetic diversity for modern cotton genotypes. For instance, a wilt resistant variety named 'Tashkent' was developed using G. *hirsutum ssp mexicanum var nervosum* germplasm in Uzbekistan (Abdullaev et al., 2009). Day-neutral genes were introduced into primitive *G. hirsutum* accessions, and the resulting progeny served as the genetic base for future breeding efforts (McCarty et al., 1979, Liu *et al* 2000). Robinson et al. 2007 documented introgression of nematode resistance into G. *hirsutum* from G. *longicalyx*. After the domestication of cotton, breeding efforts were made within smaller sets of genetic materials through crossing and re-selection, which led to losses in genetic diversity for modern cotton (Wendel et al., 1992, Brubaker et al., 1999). May et al. (1995) documented that in the late 1980s, reselection and repeated crossing within genetically related material was done to develop proprietary cultivars. Bowman et al. (1996) also reported low genetic variation among the Upland cotton cultivars released between 1970 and 1990. Conaty and Constable (2020) reported in Australian breeding programs that selection for improved cultivars has led to increased total dry matter production and carbon assimilation rates, but increased harvest index (through increases in lint percent) has been the dominant driver of yield improvement.

In addition to reductions in genetic diversity among cultivars released by modern breeding programs, there is an even higher level of genetic homogeneity among the most widely-grown

cotton cultivars in the US (Mehboob-ur-Rahman et al., 2012, Van Esbroeck et al., 1998). Specifically, Van Esbroeck et al. (1998) concluded that cultivars widely grown by producers had a higher coefficient of parentage (common genotypes in their pedigree) than was typical of most breeding programs. Thus, producers were typically only utilizing a portion of the genetic diversity available in public breeding programs. Because breeding efforts have largely focused on traits that consistently generate yield improvement, other potentially valuable traits such as tolerance to environmental stresses, have not been intentionally selected for in most breeding programs. Even more concerning is the possibility that declines in genetic diversity through selective breeding may have made cotton more genetically vulnerable to environmental stresses (Paterson et al., 2004) at a time when climate change is expected to increase the frequency and intensity of extreme weather events (heat waves, drought periods, rainfall events; Meehl et al., 2004; Tebaldi et al., 2006).

In the US, there are well-established breeding programs focused on improving crop performance in their respective production environments. The MAR (Multi-adversity resistance) program focused on traits that contributed to improved resistance to pests as well as other stresses (Bird, 1982; El-Zik 1995). In addition, improved germplasm from this program showed higher lint yields, better fiber quality, earlier maturation, and improved stability over multiple environments (El-Zik and Thaxton 1998).

Acala germplasm and cultivars have high lint yield and are known for exceptional quality fiber when cultivated in areas of the western US such as Mexico, California, and Arizona (Ulloa et al., 2009). The New Mexico cotton breeding program was established in 1926 for the development and improvement of Acala cotton and has released dozens of cultivars or germplasm with high fiber quality and Verticillium wilt tolerance (Zhang et al., 2005). Recently, efforts have been made for the improvement of heat tolerance in Acala cotton as well as other Upland cotton germplasm,

but the development of rapid and reliable screening methods for heat tolerance phenotyping represents a major constraint to these efforts (Percy et al., 2006; Ulloa et al., 2009).

The PeeDee germplasm program is based in South Carolina, was established in 1935, and has released over 80 improved cultivars and germplasm lines since that time (Campbell et al., 2011). The program's original goals at its inception were to improve yield and boll weevil tolerance of Sea Island cotton (G. *barbadense* L.) and to produce Upland cotton (G. *hirsutum* L.) with fiber properties comparable to Sea Island cotton (Culp and Harrel 1973). By the mid-1940s the focus of the Pee Dee program had shifted away from Sea Island cotton to selection of Upland cotton germplasm with improved agronomic performance and quality (Campbell et al., 2011).

Future efforts to select for traditionally-neglected traits, such as stress tolerance, will likely utilize genetic material from the major germplasm programs and material from exotic lines as well. The genotypes used in the proposed research were developed under different programs. 'DES 56' was developed by crossing PD 2164 and 'Stoneville 213' (Bridge and Chism 1978). DES 56 was an early maturing and high yielding cultivar in the Mississippi Delta (Bridge and Chism 1978; Bridge and Meredith 1983). This genotype is present in the pedigrees of a large proportion of commercially-grown cotton cultivars (Van Esbroeck et al., 1998). 'Acala Maxxa' was developed in 1975 by USDA Cotton Research Station, Shafter, California. It was developed by crossing T7538 and S4959. The plants of this cultivar show improved yield characteristics as well as fiber quality (CPCSD 1990). 'Tamcot Sphinx' was released in 1995 by the Texas Agricultural Experiment Station. It was developed under the Texas Multi-Adversity Resistance (MAR) Genetic Improvement Program (El-Zik and Thaxton 1996). This cultivar was developed from a cross between the strain MAR-CDP37HPIH-1-1-86 and a selection from 'Paymaster 145' (El-Zik and Thaxton 1996). Tamcot Sphinx is highly resistant to reniform nematode, has a cylindrical growth

habit and storm-resistant bolls (El-Zik and Thaxton 1996). UA 48 is a conventional cultivar of cotton released in November 2010 by the Arkansas Agricultural Experiment Station (Bouland and Jones 2012). UA 48 was developed by crossing Arkot 8712 and FM 966 (Bouland and Jones 2012). UA 48 is early maturing, is resistant to bacterial blight, has exceptional fiber quality and high yield (Bourland and Jones 2012). T0018MDN, T0246BC3MDN, and MDN0101 (GH191) are exotic genotypes of cotton that tend to be late flowering and have extensive vegetative growth at the expense of reproductive growth (Jiang et al., 2018).

The elite, industry checks included in the current study were as follows. DP 1646 B2XF (Bayer Crop Science) was selected because it was the most widely grown cultivar in the US at the start of our project. DG 3615 B3XF (Nutrien Ag Solutions) was selected for its superior yields in the University of Georgia on-farm variety trials (UGA on-farm cotton variety trials). ST 5020 GLT (BASF-Stoneville cotton) was selected because it exhibited unique root anatomical traits and greater seedling vigor than most commercial cultivars tested in previous experiments (Snider et al., 2022).

Heat stress effects on physiology and yield

The cotton canopy is often not at the same temperature as the air, where leaf temperatures are usually less than the air temperature at mid-day during a typical summer day. In contrast with plant shoots, root temperature is quite similar to soil temperature (Burke and Wanjura 2010). Furthermore, high soil temperatures can negatively affect seedling germination, emergence, and early growth of roots and shoots, especially given the close proximity of shoot tissues to the soil surface (Arndt, 1945; Ashraf et al., 1994; Nabi and Mullins, 2008). Nabi and Mullins (2008) determined that roots and shoots of cotton seedlings grown at 38°C were 50% and 61% shorter, respectively, than for seedlings grown at 32°C. Arndt (1945) subjected cotton seedlings to

temperature regimes ranging from 18 to 39 °C in 3 °C increments and determined that germination percent was lowest at 39 °C. Roots of seedlings grown under 39 °C showed signs of heat injury and hypocotyl length was also reduced. Reddy et al. (1992) reported that mainstem growth of cotton plants decreased at temperatures above 35 °C and stem elongation rates were lower at 40 °C. Leaf area was decreased by 50% in plants grown at 40 °C than the plants grown at 30 °C. The number of mainstem nodes were similar to optimum temperature during initial stages but it was reduced at later stages. Cotton plants grown at high temperature have been shown to abort squares and bolls during the reproductive stages (Reddy et al., 1991, Reddy et al., 1992).

In addition to limiting root and shoot growth, high temperature also reduces photosynthetic rates in cotton (Cottee et al., 2010). There have been multiple studies aimed at addressing the mechanistic basis for heat-induced photosynthetic inhibition (Crafts-Brandner and Salvucci, 2000; Hu et al., 2018; Snider et al., 2010; Salvucci and Crafts-Brandner, 2004; Wise et al., 2004). High temperature has been shown to cause structural alterations in the plastids, decrease chlorophyll content, limit PSII efficiency, inhibit electron transport, and inactivate Rubisco activase (Matos et al., 2002; Semenova, 2004; Snider et al., 2009; Oosterhuis and Snider 2011, Carmo-Silva et al., 2012; Hejnak et al., 2015).

Photosystem II (PSII) is associated with the oxygen evolving complex which catalyses the photolysis of water and production of molecular oxygen (Taiz and Zeiger 2010). High temperature can alter the oxidation-reduction properties of PS II acceptors, affecting overall electron transport (Mathur et al., 2014). Moderate heat stress induces photoinhibition of PS II (Berry and Bjorkman 1980) and inhibits the repair of PS II by inhibiting *de novo* synthesis of the D1 protein as well as other proteins associated with PS II (Akhverdiev et al., 2008), whereas severe heat stress induces the inactivation of oxygen evolving complex (Murata et al., 2006). Other authors have reported

that the maximum quantum yield of PS II (F_v/F_m) in cotton was significantly decreased at 40°C relative to optimal temperature conditions (30°C) (Hejnak et al., 2015; Westhuizen et al., 2020). However, Agarwal and Jajoo (2021) reported that PS II recovered within 10 minutes after being subjected to 40 °C in spinach leaves, and recent publications have documented the exceptional ability of PSII to acclimate to prevailing temperature conditions in cotton (Snider et al., 2013; Hu et al., 2018). Thus, in experiments where chlorophyll fluorescence and net photosynthesis have been compared side by side, F_v/F_m generally does not decline until leaf temperatures far exceed values necessary to substantially limit photosynthetic CO2 assimilation (Law and Crafts-Brandner, 1999; Snider et al., 2010, 2013, 2015a,b). Furthermore, a number of studies have indicated that either limitations to electron transport, at sites other than PSII (Schrader et al., 2004; Wise et al., 2004) or inactivation of rubisco activase (Feller et al., 1998; Law and Crafts-Brandner, 1999; Salvucci and Crafts Brandner, 2004) are likely the functional limitation to photosynthesis in cotton. High temperature stress can also cause overproduction of reactive oxygen species (ROS), resulting in oxidative stress. For example, ROS are mainly produced in the chloroplasts (PSI and PSII), mitochondria and peroxisomes (Soliman et al., 2011), and a certain amount of ROS production is a normal response to environmental stimuli or developmental triggers (Taiz and Zeiger 2010). Furthermore, ROS production is an important step in the initiation of signaling cascades that upregulate protective processes in the plant, but overproduction could potentially damage photosynthetic tissues.

The reproductive stages of cotton are reportedly more heat sensitive than vegetative stages (Hodges et al., 1993; Reddy et al., 1995; Reddy et al., 1999). Heat stressed plants have poorly developed flowers, abnormal pollen development, reduced fertilization of available ovules, shedding of squares and flowers, low boll retention due to shedding of young fruit, reduced boll

size, fewer seeds per boll, and less total fiber production per plant (Reddy et al., 1999, Loka and Oosterhuis 2010, Snider et al., 2009; Ton 2011). Not surprisingly, cotton yield can be negatively affected under high temperature stress (Lewis et al., 2000; Oosterhuis, 2000). While yield reductions under heat stress are predominantly associated with reductions in boll retention, declines in the number of seed per boll can also contribute to heat-induced yield loss (Reddy et al., 1995, 1992; Zhao et al., 2005; Pettigrew, 2008; Cottee et al., 2010).

High temperatures have been implicated as a contributor to yield variability in the US (Oosterhuis 2000), and South Asian countries like India and Pakistan already experience yield-limiting high temperatures during typical growing seasons (up to 48°C) (Gur et al., 2010). With the changing climate and increasing daily mean temperatures, the duration, intensity, and frequency of heat wave events is also expected to increase (Meehl and Tebaldi, 2004). Singh et al. (2007) reported that with every 1°C increase in daily temperature, cotton production decreased by 110 kg ha^{-1}. Therefore, screening and identification of heat tolerant genotypes and development of heat tolerant cultivars will become more important in the future (Azhar et al., 2009).

A genotype is considered heat tolerant when it performs better under high temperature conditions than another genotype; however, the response variables used in the determination of heat tolerance can vary substantially from one study to the next. Cottee et al. (2010) evaluated multiple measures of plant performance under high temperature and concluded that electron transport rate (as determined via chlorophyll fluorescence) and membrane integrity measurements were the most rapid and reliable estimates of heat tolerance. Similarly, Bibi et al. (2008) and Wu et al. (2013) have suggested that chlorophyll fluorescence measurements could be used to select for heat tolerant upland cotton germplasm. Liu et al. (2006) used pollen germination, pollen tube growth, and boll retention as methods for screening of 14 different cotton genotypes for heat

tolerance. While reproductive tissues would likely be the most relevant indicators of heat tolerance in cotton, research conducted by Snider et al. (2010, 2011) indicated that a cultivar with greater thermostability of thylakoid processes also exhibited greater reproductive heat tolerance. Thus, individual leaf measurements would be more logistically feasible at a large scale.

Some of the previously-mentioned studies require controlled environment facilities or specially built structures for evaluation of heat tolerance in the field, limiting the logistical feasibility of heat tolerance screening for field-grown plants. Another approach to heat tolerance screening involves collecting leaf samples from the field, incubating each leaf sample under a range of temperature conditions and utilizing chlorophyll fluorescence measurements to quantify photosynthetic performance at each temperature. Once temperature response curves have been developed for each sample, the temperature causing a 15% decline in photosynthetic efficiency (T_{15}) can be used as a standardized measure of heat tolerance as has been described elsewhere (Snider et al., 2010, 2013, 2015a,b; Hu et al., 2018). Traditional fluorescence methods quantify the maximum (F_v/F_m) and actual (Φ_{PSII}) quantum efficiency of photosystem II (Maxwell and Johnson, 2000). However, a relatively novel method termed OJIP fluorescence (the letters indicate steps in the fluorescence transient of an illuminated leaf sample) can estimate the quantum yield of PSII (ϕ_{P0}), of inter-photosystem electron transport (ϕ_{E0}), and of PSI end electron acceptor reduction (ϕ_{R0}) (Strasser et al., 2010). Combining this technique with the T_{15} approach above will allow us to identify cultivar variation in heat tolerance of photosynthetic components in much the same way that yield components are often used as selection criteria for yield improvement rather than yield alone (Groves et al., 2016).

Physiological contributors to yield

According to Monteith (1977), crop growth is the product of cumulative light energy intercepted by the canopy during the growing season and the efficiency of its conversion to biomass. Specifically, dry matter production (DM) is equal to cumulative intercepted photosynthetically active radiation (∑IPAR) × radiation use efficiency (RUE). Radiation use efficiency (RUE) is the amount of dry matter produced per unit solar radiation intercepted (g MJ^{-1}) (Gonias et al., 2012) Photosynthetically active radiation (PAR) refers to radiation between the 400 and 700 nm wavelengths (Taiz and Zeiger 2010). Cumulative IPAR directly influences above ground biomass production by the canopy during the whole season (Monteith 1977, Liu et al., 2012, Pradhan et al., 2014). Light interception is most commonly assessed using a ceptometer, that simultaneously measures above and below canopy light intensity (Rosenthal and Gerik 1991, Kiniry et al., 2005). The efficiency with which the crop intercepts incoming solar radiation is defined by the light extinction coefficient (*k*), which provides a measure of the amount of light absorbed by the canopy per unit leaf area index (LAI). The value for *k* is cultivar and growth stage specific, where interception of light by the crop canopy is regulated by leaf angle, shape, and spatial orientation of leaves on the plant (Vargas et al., 2002, Boote and Loomis 1991). Previous research in Australia has indicated that genetic yield improvement has been associated negatively with an increase in light interception by the canopy (Conaty and Constable 2020), but similar efforts to evaluate the contribution of IPAR to yield in diverse genotypes are limited. Regarding RUE, Rosenthal and Gerik (1991) demonstrated a significant cultivar effect on radiation use efficiency (during the reproductive phase) in three different cotton cultivars (Acala SJ-2, Deltapine 50, and Tamcot CD3H), mainly due to differences in boll number and photosynthetic efficiency among the selected cultivars. Similarly, Conaty and Constable (2020) documented that yield

improvement in Australian breeding programs was positively associated with total biomass production along higher single-leaf photosynthetic rates. Thus, it will be important for the research proposed here to evaluate both IPAR and RUE as both of these parameters drive total biomass production.

Crop yield can be calculated as ∑IPAR × RUE × HI (Earl and Davis 2003), where ∑IPAR × RUE is equal to total biomass production, and HI is harvest index. For cotton, harvest index is the ratio of final lint yield to total biomass (Constable and Bange, 2015). Harvest index can be increased in cotton by increasing the ratio of reproductive dry weight to total dry weight (Wells and Meredith 1984), or by altering within-boll components without a co-occurring change of reproductive to vegetative dry matter partitioning (Conaty and Constable 2020).

Harvest index and total dry matter are both considered as primary yield determinants and are reported to contribute to genotypic variability in lint yield (Conaty and Constable 2020). Genotypes with early flowering and fruiting will typically produce less vegetative growth and a higher fraction of total biomass can be partitioned toward reproductive organs (Wells and Meredith 1984). Meredith and Wells (1989) showed that obsolete cotton cultivars had 24% lower lint yields than modern cultivars and the increases in lint yields was due to more dry matter distribution to reproductive structures than vegetative growth. Improvement in HI and lint yield over the years is mainly attributed to increase in lint percent (Campbell et al., 2011, Bridge and Meredith 1971, Bridge et al., 1971, Culp and Green 1992). Campbell et al. (2011) reported that there was a 9% increase in lint percent of PeeDee germplasm cultivars over the span of 70 years. Bridge and Meredith (1971) evaluated obsolete and modern cultivars of cotton and observed an increase in lint percent. They suggested that lint yield is closely associated with lint percent and it might be a major contributor in the improvement of lint yield.

1.3. Rationale

As mentioned previously, changes in climate and an increase in average global temperatures will require the development of more heat tolerant and high yielding cultivars. However, breeding programs have mainly focused on yield improvement by placing selection pressure on only a limited number of agronomically valuable traits. Future improvement programs should utilize diverse cotton germplasm for the selection of valuable traits such as heat tolerance or functional components of yield. Both of these efforts should have positive, long-term effects on yield improvement and stability under challenging production conditions. This proposed research will 1) define the variability in thermotolerance that exists among a diverse set of cotton genotypes, 2) establish the relative heat sensitivity and acclimation potential of photosynthetic components of the thylakoid reactions, and 3) assess genotypic variation in physiological contributors to yield (∑IPAR, RUE, and HI).

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CHAPTER 2

VARIATION IN THERMOTOLERANCE OF PHOTOSYSTEM II ENERGY TRAPPING, INTERSYSTEM ELECTRON TRANSPORT, AND PHOTOSYSTEM I ELECTRON ACCEPTOR REDUCTION FOR DIVERSE COTTON GENOTYPES

Kaur, N., J.L. Snider, A.H. Paterson, T.L. Grey, C. Li, G. Virk, and V. Parkash. 2023. *Plant Physiology and Biochemistry*, 201: 107868.

ABSTRACT

Cotton breeding programs have focused on agronomically-desirable traits. Without targeted selection for tolerance to high temperature extremes, cotton will likely be more vulnerable to environment-induced yield loss. Recently-developed methods that couple chlorophyll fluorescence induction measurements with temperature response experiments could be used to identify genotypic variation in photosynthetic thermotolerance of specific photosynthetic processes for field-grown plants. It was hypothesized that diverse cotton genotypes would differ significantly in photosynthetic thermotolerance, specific thylakoid processes would exhibit differential sensitivities to high temperature, and that the most heat tolerant process would exhibit substantial genotypic variation in thermotolerance plasticity. A two-year field experiment was conducted at Tifton and Athens, Georgia, USA. Experiments included 10 genotypes in 2020 and 11 in 2021. Photosynthetic thermotolerance for field-collected leaf samples was assessed by determining the high temperature threshold resulting in a 15% decline in photosynthetic efficiency (T_{15}) for energy trapping by photosystem II (Φ_{Po}), intersystem electron transport (Φ_{Eo}), and photosystem I end electron acceptor reduction (Φ_{Ro}). Significant genotypic variation in photosynthetic thermotolerance was observed, but the response was dependent on location and photosynthetic parameter assessed. Φ_{Eo} was substantially more heat sensitive than Φ_{Po} or Φ_{Ro}. Significant genotypic variation in thermotolerance plasticity of Φ_{Eo} was also observed. Identifying the weakest link in photosynthetic tolerance to high temperature will facilitate future selection efforts by focusing on the most heat-susceptible processes. Given the genotypic differences in environmental plasticity observed here, future research should evaluate genotypic variation in acclimation potential in controlled environments.

Keywords: Gossypium hirsutum; *Gossypium barbadense*; Thermotolerance; Photosynthesis; Thylakoid reactions

2.1. INTRODUCTION

Cotton is the most important fiber crop in the world, and *Gossypium hirsutum*, also known as Upland cotton, accounts for the majority of global cotton production. As with all plants, growth, development, and productivity of a cotton plant can be determined by its genotype and environment. Having a diverse set of genetic material to mine through selective breeding is important for 1) continued ability to respond to future threats such as climate change and 2) continued agronomic improvement of cotton cultivars (Paterson et al., 2004). Daily mean temperatures in many cotton producing regions are already near the upper limit of the optimum temperature range for growth and development or this range has already been surpassed. Climate change is expected to increase the duration, severity, and intensity of heat wave events, which will likely have negative implications for crop production. This has made the selection of heat tolerant genotypes essential along with identification of the weakest links in plant performance under high temperature extremes (Constable et al., 2001; Bita and Gerats, 2013).

Excessively high temperatures negatively influence a number of physiological processes during vegetative and reproductive development. For example, Nabi and Mullins (2008) determined that roots and shoots of cotton seedlings grown at 38 °C were 50% and 61% shorter, respectively, than for seedlings grown at 32 °C. Reddy et al. (1992) reported that mainstem growth of cotton plants decreased at temperatures above 35 °C, and leaf area was decreased by 50% in plants grown at 40 °C, relative to plants grown at 30 °C. In addition to limiting root and shoot growth, high temperature also reduces photosynthetic rates in cotton (Cottee et al., 2010). There have been multiple studies aimed at addressing the mechanistic basis for heat-induced

photosynthetic inhibition (Crafts-Brandner and Salvucci, 2000; Hu et al., 2018; Snider et al., 2010; Salvucci and Crafts-Brandner, 2004; Wise et al., 2004). High temperature can alter the oxidation-reduction properties of photosystem II (PS II) electron acceptors, affecting overall electron transport (Mathur et al. 2014). Moderate heat stress induces photoinhibition of PS II (Berry and Bjorkman 1980) and inhibits the repair of PS II by inhibiting de novo synthesis of the D1 protein as well as other proteins associated with PS II (Akhverdiev et al. 2008). Severe heat stress induces the inactivation of oxygen evolving complex (Murata et al., 2006). Other authors have reported that the maximum quantum yield of photosystem II (F_v/F_m) in cotton was significantly decreased at 40 °C relative to optimal temperature conditions (30 °C) (Hejnak et al., 2015; Westhuizen et al., 2020). However, Agarwal and Jajoo (2021) reported that PS II recovered within 10 minutes after being subjected to 40 °C in spinach leaves. Others have also documented the exceptional ability of PSII to acclimate to prevailing temperature conditions in cotton (Snider et al., 2013; Hu et al., 2018). Thus, in experiments where chlorophyll fluorescence and net photosynthesis have been compared, F_v/F_m generally does not decline until leaf temperatures far exceed values necessary to substantially limit photosynthetic CO_2 assimilation (Law and Crafts-Brandner, 1999; Snider et al., 2010, 2013, 2015a,b). A number of studies have indicated that either limitations to electron transport, at sites other than PSII (Schrader et al., 2004; Wise et al., 2004) or inactivation of rubisco activase (Feller et al., 1998; Law and Crafts-Brandner, 1999; Salvucci and Crafts Brandner, 2004) are likely the functional limitation to photosynthesis in cotton plants exposed to heat stress.

The reproductive stages of cotton are more heat sensitive than vegetative stages (Hodges et al., 1993; Reddy et al., 1995; Reddy et al., 1999). Heat stressed plants have poorly developed flowers, abnormal pollen development, reduced fertilization of available ovules, shedding of squares and flowers, low boll retention due to shedding of young fruit, reduced boll size, fewer

seeds per boll, and less total fiber production per plant (Reddy et al., 1999, Loka and Oosterhuis 2010, Snider et al., 2009; Ton 2011). Not surprisingly, cotton yield can be negatively affected under high temperature stress (Lewis et al., 2000; Oosterhuis, 2002). While yield reductions under heat stress are predominantly associated with reductions in boll retention, declines in the number of seeds per boll can also contribute to heat-induced yield loss (Reddy et al., 1995, 1992; Zhao et al., 2005; Pettigrew, 2008; Cottee et al., 2010).

High temperatures have been implicated as a contributor to yield variability in the US (Oosterhuis 2002), and South Asian countries like India and Pakistan already experience yield-limiting high temperatures during typical growing seasons (up to 48°C) (Gur et al., 2010). With the changing climate and increasing daily mean temperatures, the duration, intensity, and frequency of heat wave events is also expected to increase (Meehl and Tebaldi, 2004). Singh et al. (2007) reported that with every 1°C increase in daily mean temperature, cotton production decreased by 110 kg ha^{-1} in terms of lint yield. Therefore, screening and identification of heat tolerant genotypes and development of heat tolerant cultivars will become more important in the future (Azhar et al., 2009).

A genotype is considered heat tolerant when it performs more efficiently under high temperature conditions than another genotype; however, the response variables used in the determination of heat tolerance can vary substantially from one study to the next. Cottee et al. (2010) evaluated multiple measures of plant performance under high temperature and concluded that electron transport rate (as determined via chlorophyll fluorescence) and membrane integrity measurements were the most rapid and reliable estimates of heat tolerance. Similarly, Bibi et al. (2008) and Wu et al. (2014) have suggested that chlorophyll fluorescence measurements could be used to select for heat tolerant Upland cotton germplasm. Liu et al. (2006) used pollen germination,

pollen tube growth, and boll retention as methods for screening of 14 different cotton genotypes for heat tolerance. While reproductive tissues would likely be the most relevant indicators of heat tolerance in cotton (Snider et al., 2009; Snider and Oosterhuis, 2011), research conducted by Snider et al. (2010, 2011) indicated that a cultivar with greater thermostability of thylakoid processes also exhibited greater reproductive heat tolerance. Thus, individual leaf measurements would be more logistically feasible at a large scale.

Some of the previously-mentioned studies require controlled environment facilities or specially built structures for evaluation of heat tolerance in the field, limiting the widespread adoption of heat tolerance screening for field-grown plants. Another approach to heat tolerance screening involves collecting leaf samples from the field, incubating each leaf sample under a range of temperature conditions and utilizing chlorophyll fluorescence measurements to quantify photosynthetic performance at each temperature. Traditional fluorescence methods primarily quantify the maximum (F_v/F_m) and actual (Φ_{PSII}) quantum efficiency of photosystem II (Maxwell and Johnson, 2000). However, another method termed OJIP fluorescence (the letters indicate steps in the fluorescence transient of an illuminated leaf sample) can estimate the quantum yield of PSII (Φ_{Po}), of inter-photosystem electron transport (Φ_{Eo}), of PSI end electron acceptor reduction (Φ_{Ro}), and a number of PSII-specific structural indicators or reaction center-specific fluxes (Strasser et al., 2010). Using OJIP fluorescence, authors have documented the effects of heat stress, chilling injury, drought, and other abiotic stresses on the structure and functionality of the photosynthetic apparatus in multiple species (Brestic et al., 2012; Oukarroum et al., 2009; Strasser et al., 2010; Strauss et al., 2006; Zushi et al., 2012). Brestic et al. (2012) utilized OJIP parameters to document genotypic differences in thermotolerance plasticity in wheat (*Triticum aestivum* L.). Chen et al. (2016) documented differences in thermotolerance plasticity among croftonweed (*Ageratina*

adenophora) populations using an OJIP-based heat sensitivity index. Controlled environment studies in wheat and creeping bentgrass have utilized OJIP methods to document cultivar-specific differences in heat tolerance using multiple OJIP-derived parameters (Fan and Jespersen, 2023; Oukarroum et al., 2009). For cotton, previous studies have developed fluorescence-temperature response curves and used the temperature causing a 15% decline in photosynthetic efficiency (T_{15}) as a standardized measure of heat tolerance (Snider et al., 2010a, 2013, 2015a,b; Hu et al., 2018). Previous research conducted in our laboratory has coupled the T_{15} approach with OJIP measurements to evaluate the effect of low growth temperature on thermotolerance acclimation for multiple thylakoid specific components in cotton under controlled environment conditions (Hu et al., 2018). Snider et al. (2015) also documented seasonal variation in thermotolerance of photosystem II, intersystem electron transport, and PSI end electron acceptor reduction. However, there are no studies to date that have utilized OJIP fluorescence to document genotypic differences in thermotolerance or thermotolerance plasticity for the aforementioned processes in field grown cotton. We hypothesized that 1) diverse cotton genotypes would exhibit significant differences in thermotolerance for specific thylakoid processes, 2) specific component processes of the thylakoid reactions would differ significantly in thermotolerance under field conditions, and 3) diverse upland cotton genotypes will exhibit differences in their thermotolerance plasticity for the most heat-sensitive thylakoid specific process. Thus, the objectives of this study were to 1) assess genotypic variation in thermotolerance of thylakoid component processes for diverse cotton genotypes, 2) assess differences in heat tolerance of thylakoid component processes, and 3) quantify differences in thermotolerance plasticity of the most heat sensitive thylakoid component process in upland cotton genotypes.

2.2. MATERIALS AND METHODS

2.1.1 Plant material

The current study was conducted at two University of Georgia research farms: Lang-Rigdon Research Farm, Tifton, Georgia and Iron Horse Farm, Athens, Georgia, USA. Experiments were planted on June 2 in 2020 at both locations and May 10, 2021 at Tifton and June 18, 2021 at Athens. The soil type at the Tifton location is classified as a Tifton sandy loam, and the soil at the Athens site is characterized as a Pacolet sandy loam. The study included 10 cotton genotypes in 2020 and 11 genotypes in 2021. The experiment was arranged in a randomized complete block design with 8 replications and 3.05 m long single-row plots with a 1.83 m inter-row spacing and 3 m bare soil alleys separating each range of research plots. Soil fertility, irrigation, and pest management practices followed University of Georgia Cooperative Extension Service recommendations for the production of high-yielding cotton (1681 kg ha^{-1} lint yield goal) (Whitaker et al., 2019). The Upland cotton (*Gossypium hirsutum* L.) genotypes used in this study were selected from different breeding programs across the US. DES 56 was developed by crossing PD 2164 and Stoneville 213 (Bridge and Chism 1978). DES 56 is an early-maturing and high-yielding cultivar developed in the Mississippi Delta (Bridge and Chism 1978; Bridge and Meredith 1983). This genotype is present in the pedigrees of a large proportion of commercially-grown cotton cultivars (Van Esbroeck et al., 1998). Acala Maxxa was developed in 1975 by USDA Cotton Research Station, Shafter, California. It was developed by crossing T7538 and S4959. Plants of this cultivar show improved yield characteristics as well as fiber quality (CPCSD 1990). Tamcot Sphinx was released in 1995 by the Texas Agricultural Experiment Station. It was developed under the Texas Multi-Adversity Resistance (MAR) Genetic Improvement Program (El-Zik and Thaxton 1996). This cultivar was developed from a cross between the strain MAR-CDP37HPIH-1-1-86

and a selection from Paymaster 145 (El-Zik and Thaxton 1996). Tamcot Sphinx is highly resistant to reniform nematode, has a cylindrical growth habit and storm-resistant bolls (El-Zik and Thaxton 1996). UA 48 is a conventional cultivar of cotton released in November 2010 by the Arkansas Agricultural Experiment Station (Bourland and Jones 2012). UA 48 was developed by crossing Arkot 8712 and FM 966 (Bourland and Jones 2012). UA 48 is early maturing, is resistant to bacterial blight, has exceptional fiber quality and high yield (Bourland and Jones 2012). T0018MDN, T0246BC3MDN and MDN0101 (GH191) are exotic genotypes of Upland cotton that tend to be late flowering and have extensive vegetative growth at the expense of reproductive growth (Jiang et al., 2018). The elite, industry checks included in the current study were as follows. DP 1646 B2XF (Bayer Crop Science) was selected because it was the most widely grown cultivar in the US at the start of our project. DG 3615 B3XF (Nutrien Ag Solutions) was selected for its superior yields in the University of Georgia on-farm variety trials (www.ugacotton.com). ST 5020 GLT (BASF-Stoneville cotton) was selected because it exhibited unique root anatomical traits and greater seedling vigor than most commercial cultivars tested in previous experiments (Snider et al., 2022). In 2021, a commercially-available Pima cotton (*Gossypium barbadense* L.) cultivar (DP 341 RF) was included in the experiment at both field sites. Pima cotton is commonly grown in the Southwestern United States, where it is not unusual for daytime temperatures to exceed 42 °C during the summer months.

2.1.2 Sample collection and temperature incubation

Because cotton is especially sensitive to high temperatures during flowering (Snider and Oosterhuis, 2011), leaf samples were collected between the first flower and peak bloom (Oosterhuis 1990) at both locations and in both growing seasons. Sample dates were August 11 for Tifton and August 13 for Athens in 2020, and July 24 and August 24 in 2021 for Tifton and

Athens, respectively. The average daily maximum temperature, average daily minimum temperature, and the highest temperature observed in the two weeks preceding each sample date for each location obtained from Georgia Weather Network (www.georgiaweather.net) is provided in Table 2.1.

Table 2.1.: The average daily maximum temperature (Max. temp.), minimum temperature (Min. temp.) and highest temperature observed during the two-week period prior to sample collection (Highest temp.) for each location.

Location	Growing season	Max. temp. (°C)	Min. temp. (°C)	Highest temp. (°C)
Tifton	2020	34.25	22.60	36.61
Athens	2020	32.92	21.07	34.34
Tifton	2021	32.12	21.88	33.48
Athens	2021	31.95	21.49	34.47

Although fruiting branch leaves represent important sources of carbohydrate for boll development (Ashley, 1972), the diverse collection of cotton genotypes utilized here varied substantially in phenology. Therefore, measurement of fruiting branch leaves from a common position on the plant and point in the growing season would not have been possible. As a result, uppermost, fully expanded mainstem leaves were utilized for all assessments as leaves from these positions would had similar leaf ages and peak photosynthetic activity (Constable and Rawson, 1980). Specifically, uppermost, fully-expanded leaves from the fourth mainstem node below the terminal were collected, placed in plastic bags containing moist paper towels to prevent desiccation

and then placed in an insulated container and kept at room temperature (~21 °C). Complete dark adaptation of leaves which causes all the photosynthetic reaction centers to be open is a requirement for OJIP assessments of thylakoid-dependent processes (Strasser et al., 2010). Leaves are often dark adapted for 20 minutes to 1 hour, likely because this represents the minimum amount of time required for all reaction centers to be in the open state (Jedmowski and Bruggemann, 2015; Mishra et al., 2016, Rodriguez et al., 2017; Bussotti et al., 2020). However, to ensure full dark adaptation, leaves were kept under dark conditions overnight. Overnight dark adaptation has been implemented extensively in ecophysiology studies using OJIP fluorescence as it represents the longest period of time a leaf could possibly be exposed to dark conditions in the natural environment (Strauss et al., 2006; Kalaji et al., 2014; Snider et al., 2015b; Hu et al., 2018; Koller et al., 2020; Khan et al., 2021; Virk et al., 2021; Fan and Jespersen 2023). Following dark adaptation, leaf discs of ~1cm diameter were excised from each leaf sample and placed on moist filter paper in direct contact with a large thermal gradient table described extensively elsewhere (Chastain et al., 2016). Fluorescence-based temperature response experiments are commonly conducted on detached leaves and excised leaf discs due to the logistical constraints to conducting comparable measurements in situ (Burke, 1990; Lazar and Ilik, 1997; Froux et al., 2004; Burke, 2007; Gimeno et al., 2009). Leaf segments were first incubated at 30 °C for six minutes prior to the first chlorophyll fluorescence measurement (measurements described in more detail below). This temperature is widely considered optimal for photosynthesis in cotton (Burke and Wanjura, 2010). Thereafter, samples were progressively incubated at 35, 40, 45, and 50 °C for six minutes at each temperature prior to fluorescence measurements. The incubation times used here were chosen because preliminary research conducted in *Rhus glabra* and *Gossypium hirsutum* evaluated leaf segments incubated for 2 to 30 minutes and assessed F_v/F_m every two minutes. It was observed

that incubation times longer than 4 minutes did not produce appreciably different temperature response curves. These personal observations formed the basis of subsequently published research (Snider et al., 2010a,b). In many studies, even shorter incubation times (five minutes or less at each temperature) are commonly used for T_{15} and critical temperature determination (Epron 1997; Ladjal et al., 2000; Froux et al., 2004; Bordignon et al., 2019). Furthermore, all incubation times in the current paper were the same for all site-years and genotypes, so variation in T_{15} accurately reflects relative differences in heat tolerance among processes, genotypes, or environments.

2.1.3 OJIP Fluorescence measurements

An Opti-Sciences OS5p fluorometer (Opti-Sciences Inc., Hudson, NH, USA) was used to do fluorescence induction measurements at each temperature. During each measurement, fluorescence intensity prior to exposure to a saturating flash of light (F_0) was first determined. The leaf sample was then exposed to a saturating flash of light (3500 μmol m^{-2} s^{-1}), and the fluorescence intensity at the J (F_j; 2 ms), I (F_i; 30 ms), and P (maximum fluorescence intensity reached, F_m, irrespective of time) steps were quantified along with the initial slope of the fluorescence transient (M_0). In addition to identifying genotypic differences in heat tolerance, it was also important in the current study to identify the relative sensitivities of thylakoid component processes. As a result, the parameters calculated from our OJIP fluorescence readings included quantum yield of energy trapping by photosystem II ($\mathbf{\Phi_{Po}}$), quantum yield of electron transport between photosystem II and photosystem I ($\mathbf{\Phi_{Eo}}$), and quantum yield of photosystem I end electron acceptor reduction ($\mathbf{\Phi_{Ro}}$). The three quantum efficiencies evaluated here are interdependent in the sense that the quantum efficiency of each thylakoid-specific process cannot be higher than the quantum efficiency of the preceding step. However, differences in the response of these parameters to environmental stresses have been reported in numerous other studies (Zushi et al., 2012; Hu et al., 2018; Snider et al.,

2018; Gupta 2019; Virk et al., 2021), and the inclusion of Φ_{Po}, Φ_{Eo} and Φ_{Ro} provides a common method to asses thermotolerance at PSII and locations beyond PSII. These parameters were calculated as described in Strasser et al. (2010). Each quantum efficiency was plotted versus temperature for each sample, and a third order polynomial function was fit to the resulting data in order to estimate high temperature thresholds for each process (described in the statistical analysis section).

Table 2.2: List of OJIP-derived parameters, their definition and the calculation of each parameter (Strasser et al., 2010).

Parameter	Definition	Calculation
Φ_{Po}	Quantum yield of energy trapping by photosystem II (Pheophytin and Q_A reduction)	$\frac{F_m - F_0}{F_m}$
Φ_{Eo}	Quantum yield of electron transport between photosystem II and photosystem I (intersystem electron transport from reduced Q_A to intersystem electron acceptors)	$\frac{F_m - F_J}{F_m}$
Φ_{Ro}	Quantum yield of photosystem I end electron acceptor (Ferredoxin and NADP) reduction	$\frac{F_m - F_i}{F_m}$

2.1.4 Statistical analysis

First, the effect of genotype on each photosynthetic parameter of interest was evaluated using a mixed effects analysis of variance. Specifically, genotype was a fixed effect, replication was a random effect, and the quantum efficiency of interest (Φ_{Po}, Φ_{Eo} and Φ_{Ro}) was the response variable. Post hoc analysis for genotypic means separation was conducted using Fisher's protected LSD test ($p < 0.05$). The analysis was performed within each site-year and incubation temperature separately. The analysis was performed within each site-year because of the different number of genotypes in each growing season and the environmental differences between Tifton and Athens. For the second approach, the photosynthetic component of interest was plotted versus incubation temperature for each sample, and polynomial regression (third order regression) was utilized to interpolate the temperature causing a 15% decline in photosynthetic efficiency relative to 30 °C (T_{15}; Figure 2.1). The T_{15} approach to assess heat tolerance is a widely utilized method in plant ecophysiology (Froux et al., 2003; Gimeno et al., 2009; Snider et al., 2010; Snider et al., 2015). Thereafter, a mixed-effects analysis of variance was utilized, where T_{15} was the dependent variable of interest, block was considered a random effect, and genotype was the fixed effect of interest. The relative heat tolerance of photosynthetic components was evaluated by performing a mixed effects analysis of variance with T_{15} as the dependent variable of interest, block as a random effect and photosynthetic component as a fixed effect. To asses genotypic variation in thermotolerance plasticity, the environment T_{15} value for the most heat sensitive component was calculated for each site year (average of all genotypes in a given site year). Then, the mean T_{15} values for each genotype were plotted against the environment mean T_{15} values. The linear regression lines were fitted for each genotype and the slopes were calculated. Multiple pairwise homogeneity of slopes tests were conducted using analysis of covariance. Specifically, when $P < 0.05$ for the interaction

term [environment mean x genotype], two cultivars differed significantly in their slopes. Genotypes with higher slopes are considered more environmentally plastic, whereas genotypes with lower slopes are considered less responsive to environment. All statistical analyses were conducted using JMP Pro 15 software. Figures were created using Sigmaplot 14.0 software.

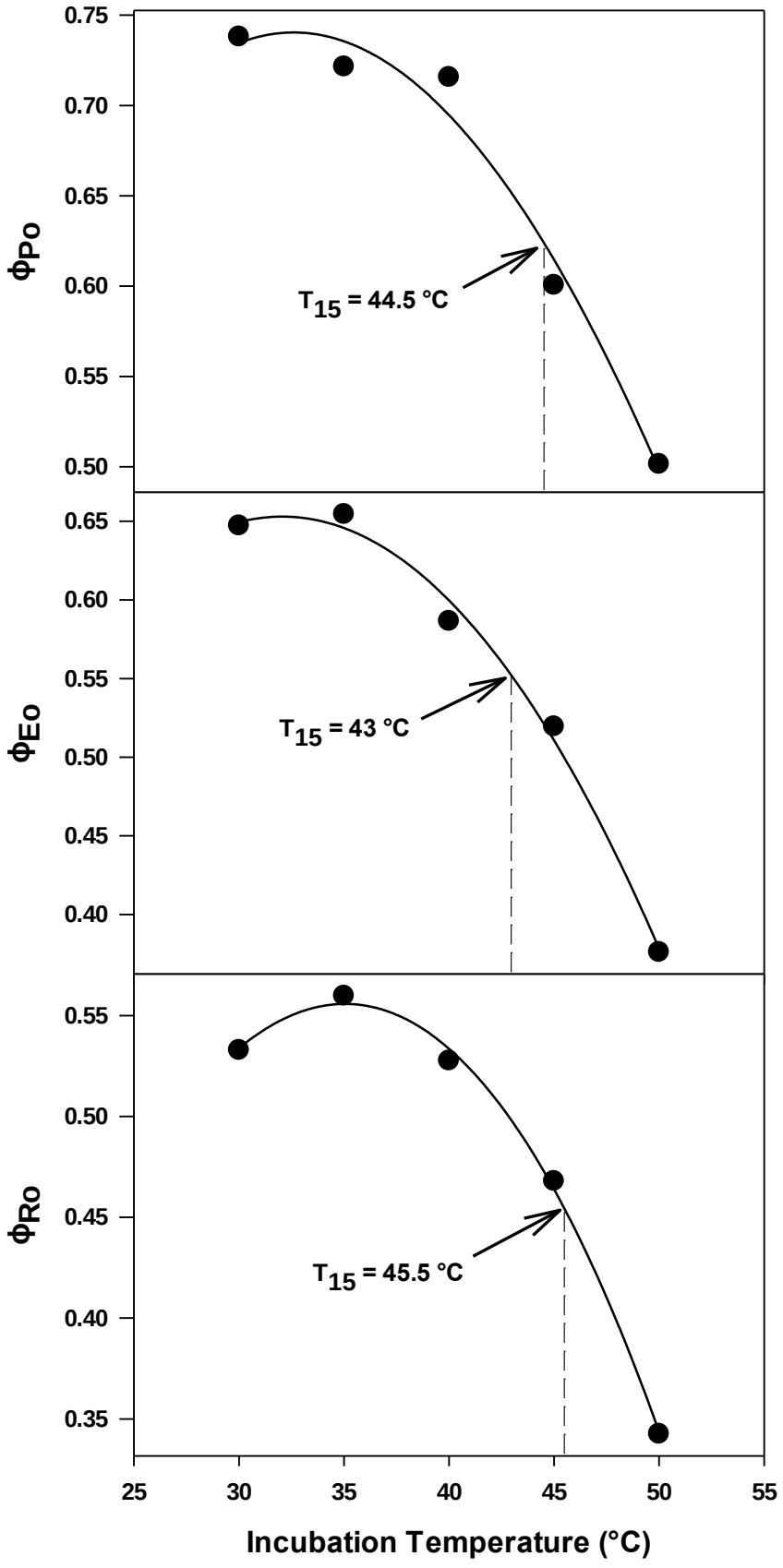
0.75
0.70
0.65
0.60
0.55
0.50
ΦPo
T15 = 44.5 °C
0.65
0.60
0.55
0.50
0.45
0.40
ΦEo
T15 = 43 °C
0.55
0.50
0.45
0.40
0.35
ΦRo
T15 = 45.5 °C
25
30
35
40
45
50
55
Incubation Temperature (°C)

Figure 2.1. An example graph illustrating how T_{15} was calculated for a single leaf and three different quantum efficiencies (defined in Table 2). A third-order polynomial function was fit to the quantum efficiency x incubation temperature data and the temperature causing a 15% decline in efficiency relative to 30 °C was interpolated from the resulting function. T_{15} is indicated with a vertical dashed line.

2.3. RESULTS

2.3.1. Effect of genotype on photosynthetic efficiencies within incubation temperature

Significant genotype effects in the quantum yield of energy trapping by Photosystem II (Φ_{Po}), the quantum yield of inter-system electron transport (Φ_{Eo}) and the quantum yield of photosystem I end electron acceptor reduction (Φ_{Ro}) were observed but the trends were dependent on site-year and incubation temperature. The responses of these photosynthetic components are provided in Figures 2.2-2.4 for both years and locations. Regarding the Φ_{Po}, a significant genotype effect was observed at 30, 35 and 40 °C at Athens in 2020 (Figure 2.2A). However, at the highest incubation temperatures, no genotype effect was observed for Φ_{Po}. For the Tifton location in 2020, genotypes significantly affected Φ_{Po} at 35, 40, 45 and 50 °C (Figure 2.2B), but we will focus our observations on effects at the high temperature extremes, 45 and 50 °C. At these two incubation temperatures, MDN0101 (GH191), T0246BC3MDN, and DG 3615 were the genotypes that exhibited consistently the highest Φ_{Po} values (0.678 to 0.452 for 45 and 50 °C). By comparison DP 1646, UA 48, DES 56, Acala Maxxa and Tamcot Sphinx were the genotypes that had the lowest Φ_{Po} values at 45 and 50 °C, where average photosynthetic efficiency of energy trapping ranged from 0.641 to 0.324. In 2021, significant differences were observed at 30, 35 and 45 °C in Athens (Figure 2.2C) and at 30, 35, 40 and 50 °C in Tifton (Figure 2.2D). A Pima cotton cultivar (DP 341) was added to the study in 2021, and this genotype, along with the Upland genotypes

T0246BC3MDN, T0018MDN and Acala Maxxa, showed the highest Φ_{Po} values at the 45 °C incubation temperature in Athens. Tamcot Sphinx, DP 1646 and Acala Maxxa had the lowest Φ_{Po} values at 45 °C for the Athens-2021 site-year. At 50 °C for the Tifton site in 2021, DP 341, Tamcot Sphinx and DES 56 produced the highest Φ_{Po} values (0.450), whereas UA 48, DG 3615, Acala Maxxa, T0018MDN, DP 1646 and T0246BC3MDN had the lowest (0.348).

A significant genotype effect was observed for Φ_{Eo} only at 30 °C at Athens in 2020 and no effect was observed at higher temperatures (Figure 2.3A). For the Tifton location in 2020, the genotypes were significantly different in their Φ_{Eo} at all the incubation temperatures (Figure 2.3B). MDN0101 (GH191), T0246BC3MDN, and DG 3615 were the genotypes that exhibited consistently the highest Φ_{Eo} values at 45 and 50 °C (0.678 to 0.452). The lowest Φ_{Eo} values were exhibited by DP 1646, UA 48, DES 56, Tamcot Sphinx and Acala Maxxa at 45 and 50 °C (0.374 to 0.128) in 2020 at Tifton location. In 2021, significant differences were observed at 30 and 35 °C in Athens (Figure 2.3C) and only at 50 °C in Tifton (Figure 2.3D). At the Tifton site, DP 341 and Tamcot Sphinx had the highest Φ_{Eo} values (0.334) whereas Acala Maxxa, DG 3615, T0246BC3MDN, T0018MDN, UA 48, DP 1646, MDN0101 (GH191), ST 5020 and DES 56 had the lowest Φ_{Eo} values (0.235) at 50 °C.

As for Φ_{Ro}, a significant genotype effect was observed only at 40 °C at Athens in 2020 (Figure 2.4A), whereas the genotypes were significantly different in their Φ_{Ro} at 40, 45 and 50 °C at the Tifton location in 2020 (Figure 2.4B). At 45 and 50 °C, MDN0101 (GH191), T0246BC3MDN, and DG 3615 were the genotypes that exhibited consistently the highest Φ_{Ro} values (0.505 to 0.297 for 45 and 50 °C). By comparison, DP 1646, UA 48, DES 56, Acala Maxxa and Tamcot Sphinx had the lowest Φ_{Ro} values at 45 and 50 °C, where average photosynthetic efficiency of photosystem I end electron acceptor reduction ranged from 0.442 to 0.239. In 2021, significant differences were

observed at 30 and 35 °C in Athens (Figure 2.4C) and only at 50 °C in Tifton (Figure 2.4D). At the Tifton site in 2021, DP 341, Tamcot Sphinx and DES 56 had the highest Φ_{Ro} values (0.294) whereas UA 48, DG 3615, Acala Maxxa, DP 1646, T0018MDN, T0246BC3MDN, ST 5020 and MDN0101 (GH191) had the lowest Φ_{Ro} values (0.220) at 50 °C.

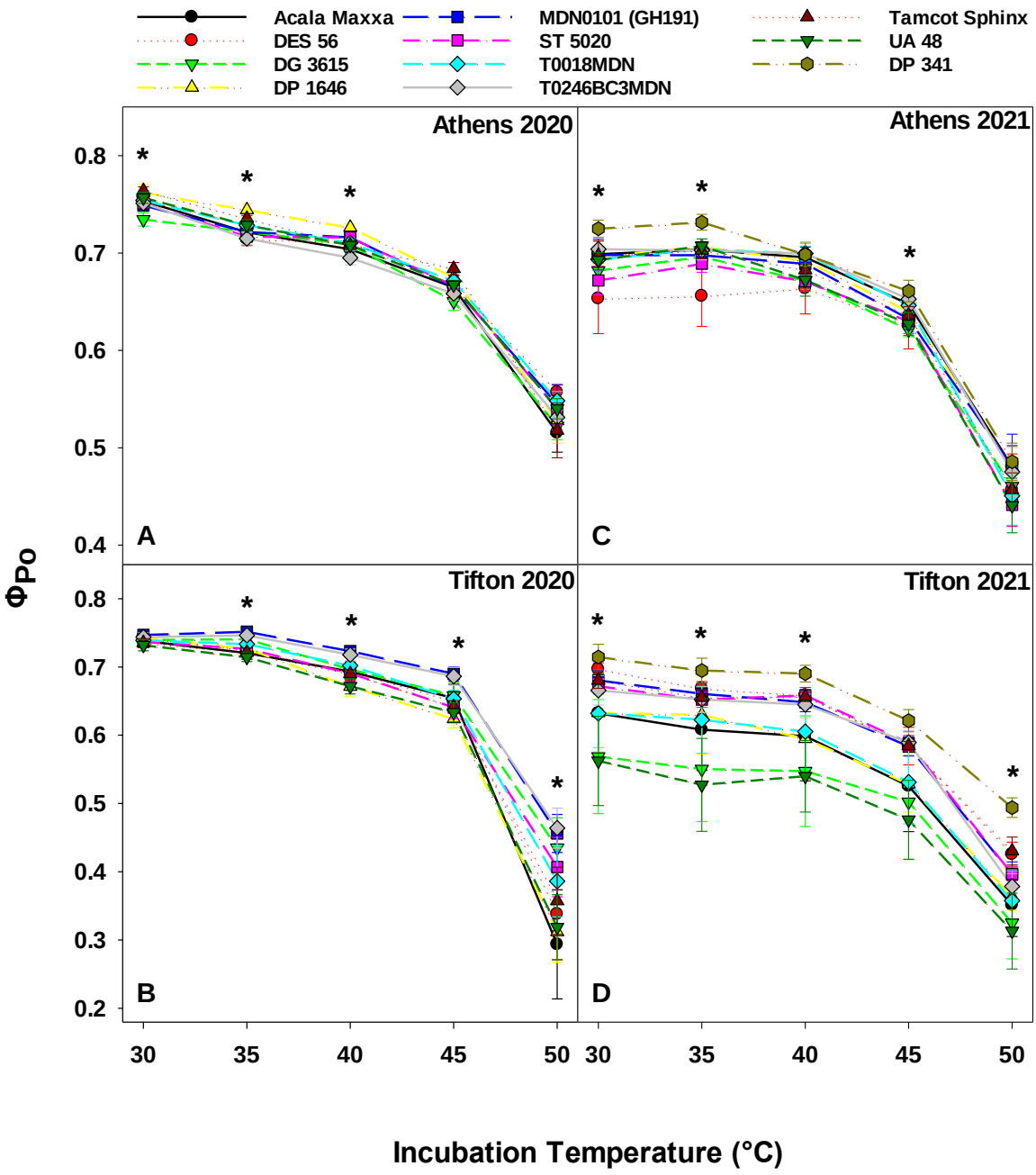

Figure 2.2. The response of maximum quantum yield of energy trapping by photosystem II (Φ_{Po}) to increasing incubation temperatures for 10 cotton genotypes in 2020 (A and B) and 11 genotypes in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Each data point represents the means ± standard error of eight replications, and asterisks indicate a significant genotype effect at a given incubation temperature ($p < 0.05$).

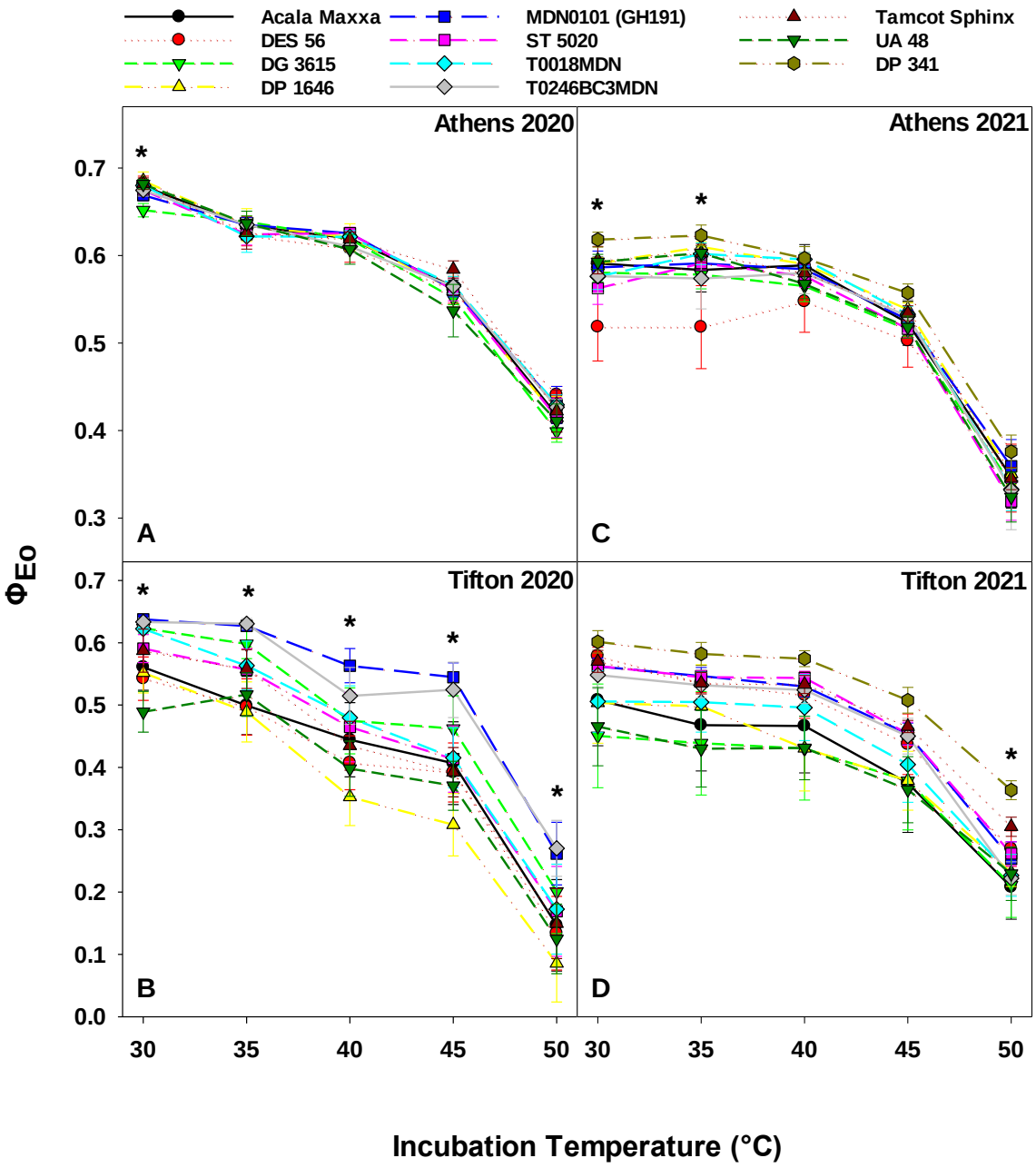

Figure 2.3. The response of maximum quantum yield of electron transport between photosystem II and photosystem I (Φ_{Eo}) to increasing incubation temperatures for 10 cotton genotypes in 2020 (A and B) and 11 genotypes in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Each data point represents the means ± standard error of eight replications, and asterisks indicate a significant genotype effect at a given incubation temperature ($p < 0.05$).

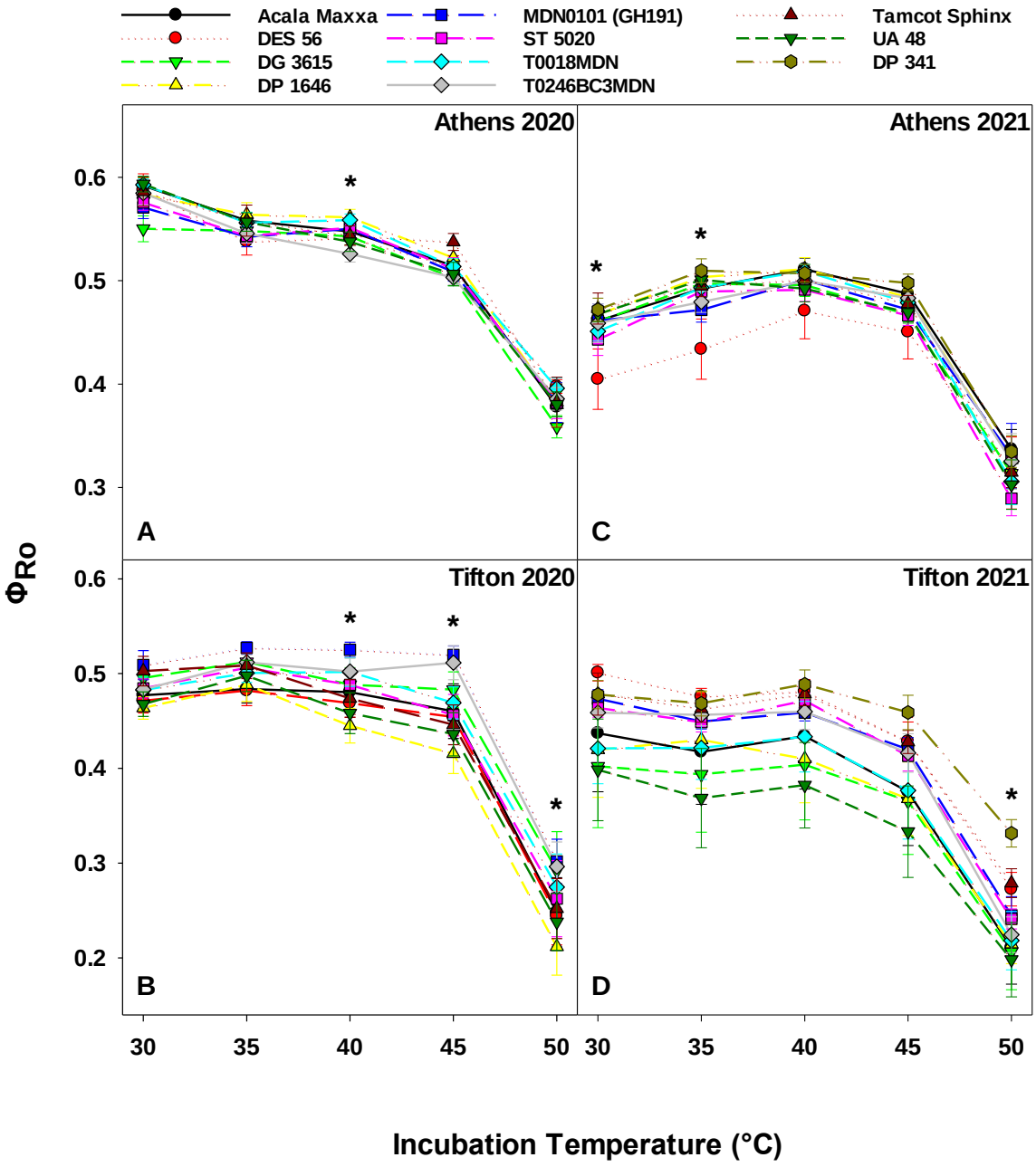

Figure 2.4. The response of maximum quantum yield of photosystem I end electron acceptor reduction (Φ_{Ro}) to increasing incubation temperatures for 10 cotton genotypes in 2020 (A and B) and 11 genotypes in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Each data point represents the means ± standard error of eight replications, and asterisks indicate a significant genotype effect at a given incubation temperature ($p < 0.05$).

2.3.2. High Temperature Thresholds (T_{15}) for Photosynthetic Processes

The high temperature thresholds ($T_{15;}$ the temperature causing a 15% decline in photosynthetic efficiency) for the efficiency of the three photosynthetic parameters of interest are provided in Figures 2.5-2.7 for both years and locations. In the 2020 season, the cotton genotypes did not significantly differ in their T_{15} values for Φ_{Po} at Athens or Tifton (Figure 2.5A and 2.5B). T_{15} averaged 46.4 °C for all genotypes in Athens and 46.7 °C for all genotypes in Tifton during 2020. Similarly, at Athens in 2021, T_{15} for Φ_{Po} was unaffected by genotype and averaged 45.1 °C (Figure 2.5C). However, thermotolerance of Φ_{Po} was significantly affected by genotype in 2021 at Tifton (Figure 2.5D). T0246BC3MDN, DP341, Acala Maxxa and T0018MDN were the genotypes that exhibited the greatest heat tolerance of photosystem II (average T_{15} = 46 °C), whereas DP 1646 and MDN0101 (GH191) were the least tolerant in 2021 at Tifton (average T_{15} = 43.8 °C).

Similarly, there were no significant differences among the genotypes for thermotolerance of intersystem electron transport [T_{15} (Φ_{Eo})] in either year at Athens (Figure 2.6A and 2.6C) or in 2020 at Tifton (Figure 2.6B). In Athens, T_{15} averaged 43.9 °C for all genotypes in 2020 and 43.8 °C in 2021, and for Tifton, T_{15} averaged 44.8 °C for all genotypes during 2020. However, there were significant genotypic differences in heat tolerance observed in 2021 at Tifton (Figure 2.6D). DP 341, MDN0101 (GH191), T0246BC3MDN and Acala Maxxa (average T_{15} = 43.1 °C) exhibited the most heat tolerant intersystem electron transport, whereas DP 1646, Tamcot Sphinx, T0018MDN, DG 3615 and DES 56 (average T_{15} = 37.6 °C) were the least heat tolerant.

For T_{15} of Φ_{Ro}, there were no significant differences among the genotypes in either year for the Athens location (Figure 2.7A and 2.7C) or for the 2021 season at Tifton (Figure 2.7D). For all genotypes at the Athens location, T_{15} averaged 45.6 °C in 2020 and 45.6 °C in 2021, and T_{15} was 45.6 °C for all genotypes in Tifton during 2021. However, significant differences in heat tolerance

were observed in 2020 at the Tifton location (Figure 2.7C). DP 1646, T0018MDN, DG 3615, T0246BC3MDN, Acala Maxxa, DES 56, ST 5020 and UA 48 (average T_{15} = 47.7 °C) showed the greatest thermotolerance for end electron acceptor reduction by PSI, whereas MDN0101 (GH191) and Tamcot Sphinx (Average T_{15} = 46.3 °C) were the least heat tolerant for this process.

Thylakoid component processes also showed significant differences in heat tolerance in all of the four site-years evaluated (Figure 2.8). Quantum yield of energy trapping by photosystem II and end electron acceptor reduction by PSI were the most heat tolerant processes, whereas intersystem electron transport was the most heat sensitive process. For example, T_{15} values ranged from 46.7 °C at the Tifton 2020 site-year to 45.1 °C at the Athens 2021 site year for Φ_{Po} and from 47.4 °C at the Tifton 2020 site-year to 45.5 °C at the Tifton 2021 site year for Φ_{Ro}. By comparison, T_{15} values for Φ_{Eo} ranged from 44.8 °C at the Tifton 2020 site-year to 40.4 °C at the Tifton 2021 site year.

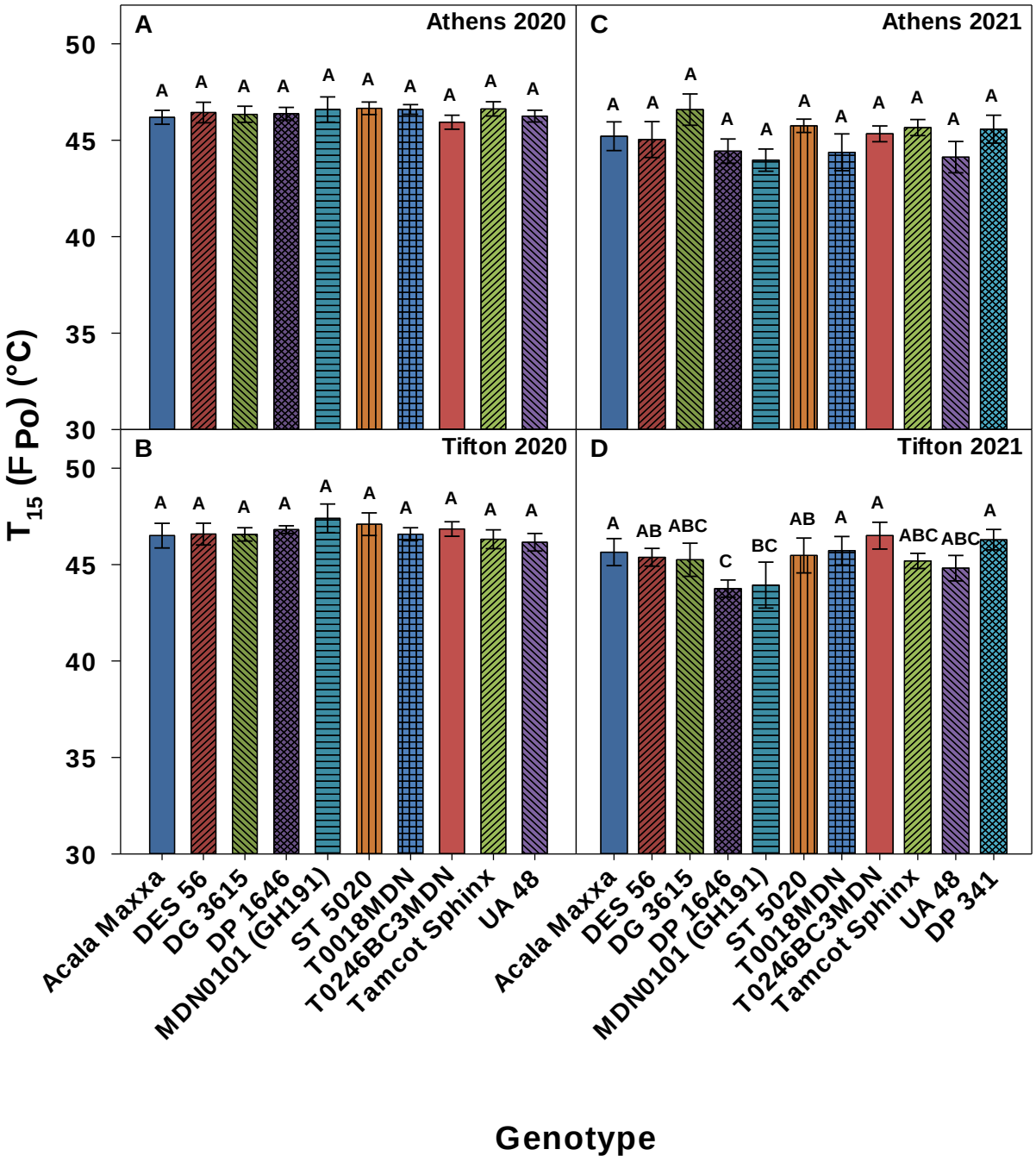

Figure 2.5. High temperature thresholds (T_{15}) for the efficiency of energy trapping by Photosystem II [T_{15} (Φ_{Po})] for 10 diverse cotton genotypes in 2020 (A and B) and 11 in 2021 (C

and D) at field sites in Athens and Tifton, Georgia. Data are means ± standard error (n = 8) and bars not sharing a common letter within a given site-year are significantly different ($p < 0.05$).

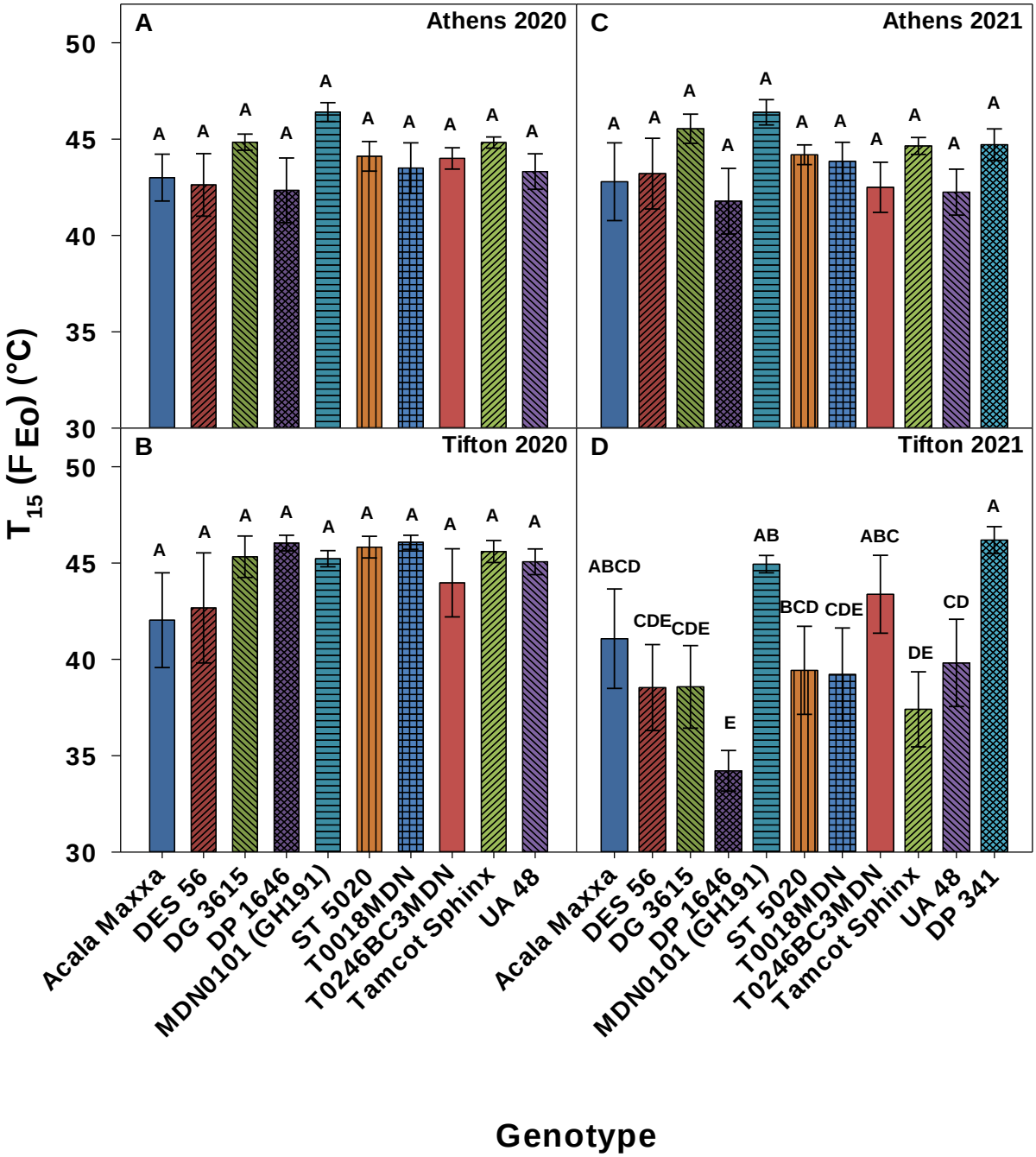

Figure 2.6. High temperature thresholds (T_{15}) for the efficiency of intersystem electron transport [T_{15} (Φ_{Eo})] for 10 diverse cotton genotypes in 2020 (A and B) and 11 in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Data are means ± standard error (n = 8) and bars not sharing a common letter within a given site-year are significantly different ($p < 0.05$).

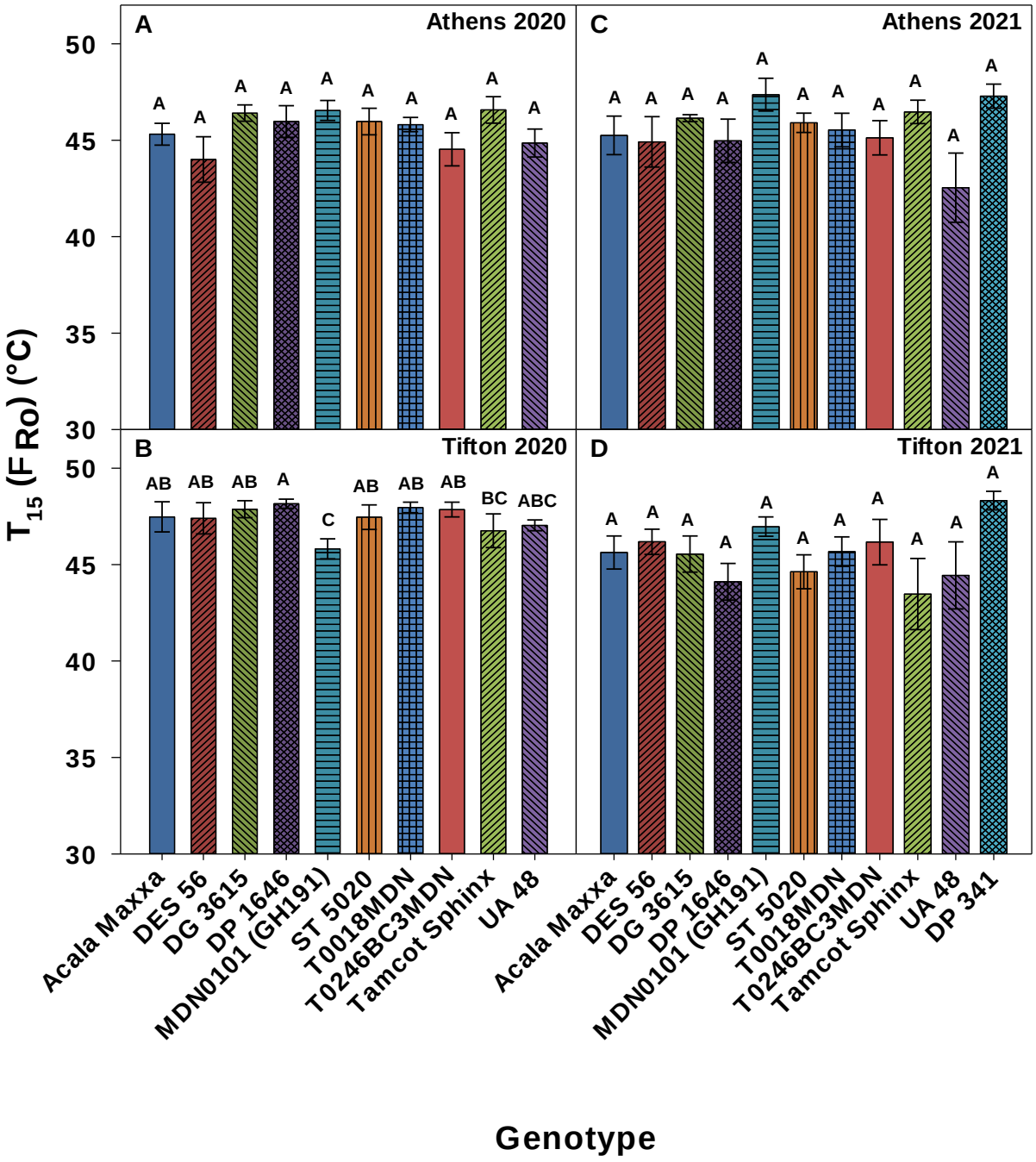

Figure 2.7. High temperature thresholds (T_{15}) for the efficiency of photosystem I end electron acceptor reduction [T_{15} (Φ_{Ro})] for 10 diverse cotton genotypes in 2020 (A and B) and 11 in 2021

(C and D) at field sites in Athens and Tifton, Georgia. Data are means ± standard error (n = 8) and bars not sharing a common letter within a given site-year are significantly different ($p < 0.05$).

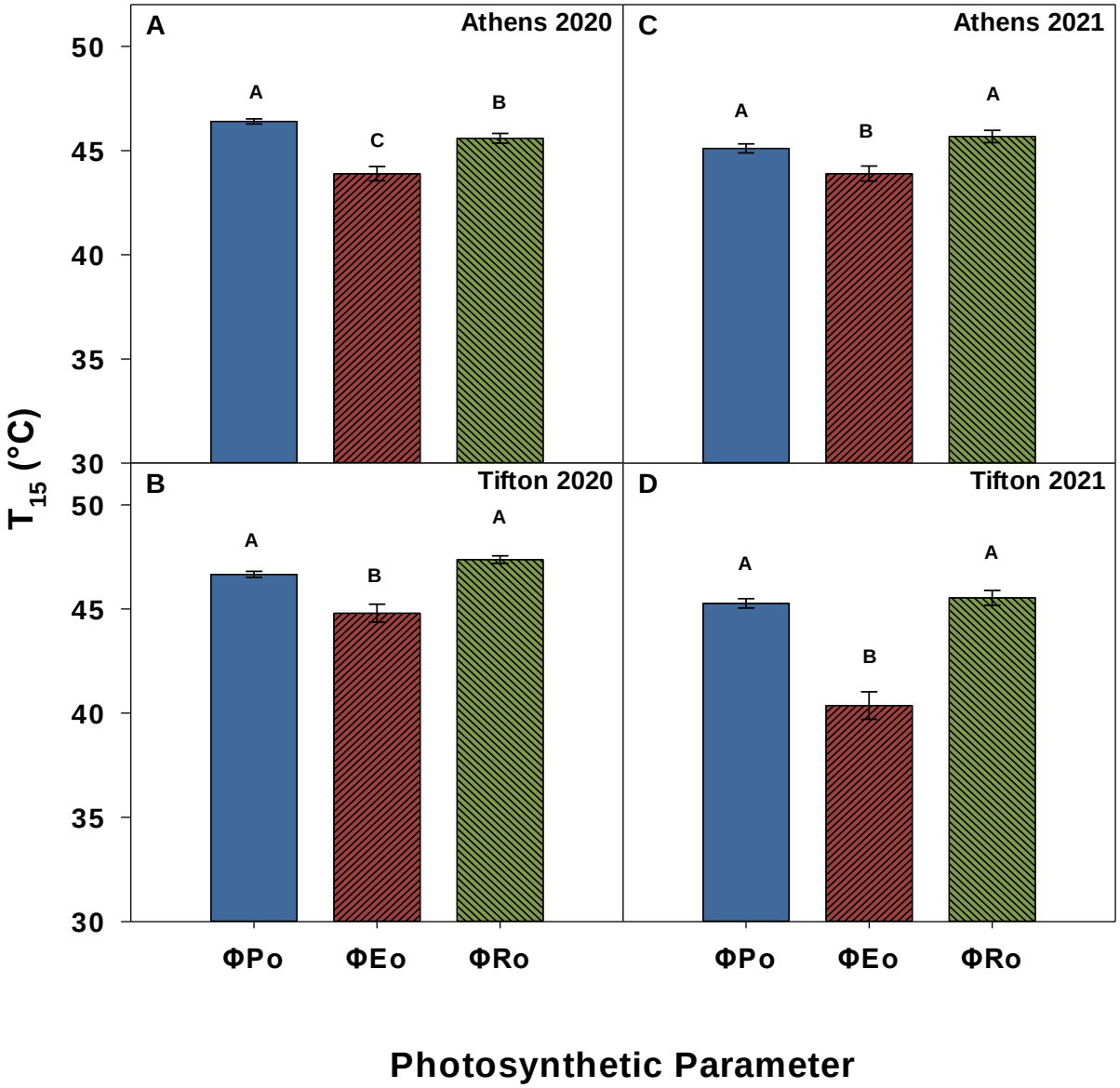

Figure 2.8. High temperature thresholds (T_{15}) for the efficiency of photosynthetic parameters (Φ_{Po}, Φ_{Eo} and Φ_{Ro}) at both locations and years. Data are means ± standard error [n = 80 in 2020 (A and B); n = 88 in 2021 (C and D)] and bars not sharing a common letter within a given site-

year are significantly different ($p < 0.05$). Means were generated by combining data across all genotypes and replicates within a given site year.

2.3.3. Thermotolerance Plasticity of Intersystem Electron Transport

Because intersystem electron transport was observed to be the most heat sensitive component, genotype mean $T_{15}(\Phi_{Eo})$ values (Average of eight replicate plots for a given variety) were plotted against the environment $T_{15}(\Phi_{Eo})$ values (Average of all genotypes and replicates) for each site year and slopes were compared for all the upland cotton genotypes. Notable differences were observed among genotypes in their responsiveness to environment. Specifically, DP 1646 was observed to have the highest slope (2.157), and Tamcot Sphinx, DG 3615, T0018MDN and ST 5020 were statistically comparable to DP 1646. In contrast, T0246BC3MDN, MDN0101 (GH191), Acala Maxxa, UA 48 and DES 56 had the lowest slopes (Average slope = 0.474), indicating that photosynthetic thermotolerance in these genotypes was least responsive to environment. Among the most thermotolerance-stable genotypes, some were among the most heat tolerant in all environments (e.g. MDN0101 (GH191)), whereas others were among the most heat tolerant in a low $T_{15}(\Phi_{Eo})$ environments and among the least heat tolerant in a high $T_{15}(\Phi_{Eo})$ environment (Acala Maxxa).

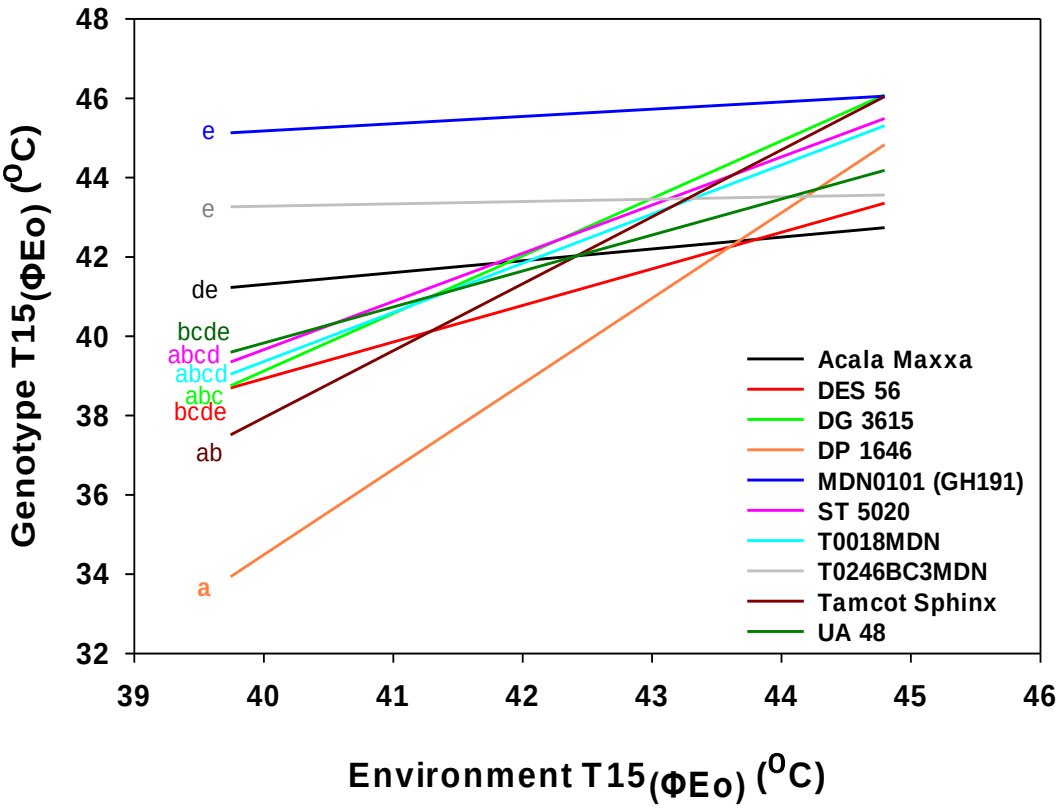

Figure 2.9. Genotype mean $T_{15}(\Phi_{Eo})$ for 10 upland cotton genotypes versus the environment mean $T_{15}(\Phi_{Eo})$ value of all genotypes within a given site year. Lines represent linear functions and those lines not sharing a common letter exhibit significantly different slopes ($p < 0.05$).

2.4. DISCUSSION

Climate change is expected to increase the duration, severity, and intensity of heat wave events, which will likely have negative implications for crop production. For cotton, high temperature reduces mainstem growth, leaf area (Reddy et al., 1992), and photosynthetic rates (Cottee et al., 2010; Snider et al., 2010). Heat stress also negatively affects a number of reproductive processes, (Reddy et al., 1999, Loka and Oosterhuis 2010, Snider et al., 2009; Ton 2011), leading to declines

in yield (Lewis et al., 2000; Oosterhuis, 2002). As a result, previous authors have utilized numerous methods to screen cotton genotypes for heat tolerance (Cottee et al., 2010; Bibi et al., 2008; Wu et al., 2014; Liu et al., 2006; Snider et al., 2010, 2011). These efforts require controlled environment facilities or specially-built structures for heat tolerance assessments to be performed in the field, limiting heat tolerance screening under field conditions. Several studies have combined sample collection from plants grown under identical conditions with chlorophyll fluorescence assessments (OJIP transient) at a range of temperature conditions to develop high temperature thresholds for specific photosynthetic processes and genotypes (Chastain et al., 2016; Hu et al., 2018; Snider et al., 2010, 2013, 2015a, 2015b, Brestic et al., 2012). The aforementioned method is potentially promising for heat tolerance screening in field-grown cotton. The three quantum efficiencies (Φ_{Po}, Φ_{Eo}, and Φ_{Ro}) used in this study are interdependent (Strasser et al., 2010), yet they provided an opportunity to assess thermotolerance differences in the efficiency of energy trapping at PSII, intersystem electron transport and PSI end electron acceptor reduction). The first hypothesis of the present was that diverse cotton genotypes would exhibit significant differences in thermotolerance for specific thylakoid processes. In support of this hypothesis, the diverse collection of cotton genotypes evaluated here showed significant differences in heat tolerance, but these differences were dependent on site-year and the thylakoid process evaluated (Figures 2.2-2.7).

First, the effects of genotype on the quantum efficiencies of each photosynthetic process was determined at different incubation temperatures. In attention to exhibiting innate differences in quantum efficiencies even at optimal temperatures (Figure 2.2-2.4), significant genotype effects were observed at high temperature extremes. For example, T0246BC3MDN, MDN0101 (GH191) and DG 3615 exhibited the highest values for Φ_{Po}, Φ_{Eo}, and Φ_{Ro} for the Tifton site in 2020 when

samples were incubated at 50 °C. In contrast, no significant genotypic differences were observed for any of the aforementioned thylakoid processes at 50 °C for the Athens site in 2020. The Pima cotton (*Gossypium barbadense* L.) genotype DP 341 was added to the diverse set of genotypes in 2021 at both locations. With the inclusion of this genotype, similar to 2020, significant genotypic differences in the three quantum efficiencies at 50 °C were only observed at the Tifton site. For this location, DP 341 and Tamcot Sphinx had the highest values for all three quantum efficiencies when incubated at 50 °C. Furthermore, the temperature causing a 15% decline in photosynthetic efficiency (T_{15}) was estimated and used as a standardized measure of heat tolerance for each sample. For T_{15} (Φ_{Po}) and T_{15} (Φ_{Eo}), genotypic differences were only observed for T_{15} in 2021 at the Tifton location (Figure 2.5-2.6), where DP 341, T0246BC3MDN and Acala Maxxa exhibited the highest T_{15} values for both these parameters. For $T_{15}\Phi_{Ro}$, differences were only observed in 2020 at the Tifton location (Figure 2.7), where DP 1646, T0018MDN, DG 3615, T0246BC3MDN, Acala Maxxa, DES 56, ST 5020 and UA 48 were the most heat tolerant genotypes. These results show that heat tolerance of thylakoid components is strongly-dependent on environment and the photosynthetic component evaluated. For site-years where significant variations were observed, the difference between the most heat tolerant and least tolerant genotypes for the efficiency of energy trapping by Photosystem II [T_{15} (Φ_{Po})] was 2.2 °C whereas the difference was 5.5 °C for intersystem electron transport [T_{15} (Φ_{Eo})] and just 1.4 °C for photosystem I end electron acceptor reduction [T_{15} (Φ_{Ro})]. Thus, there was substantially greater genotypic variation in thermotolerance for electron transport than for the other processes. The response of PSII to growth temperature has been used previously to screen for differences in heat tolerance among diverse cotton genotypes in field and controlled-environment settings (Bibi et al., 2004; Cottee et al., 2010; Wu et al., 2014). Furthermore, our laboratory has previously used PSII-based T_{15} estimates to identify cotton

genotype differences in PSII heat tolerance and heat tolerance plasticity for only two advanced cotton cultivars (Snider et al., 2010, 2013, 2015). However, this is the first experiment that the authors are aware of to document genotypic variability in thermotolerance for specific thylakoid processes using OJIP fluorescence in field-grown cotton. As noted above, the genotypic responses we observed were highly-dependent on site-year, which may be a function of environmental variability. For example, T_{15} can be influenced by growth temperature and water availability for field-grown cotton (Chastain et al., 2016; Snider et al., 2013; Hu et al., 2018). Moffatt et al., (1990) observed that thermotolerance rankings for different wheat genotypes changed depending on whether plants were grown under controlled environment or field conditions, further indicating that chlorophyll fluorescence is sensitive to environmental conditions. Because species-specific differences in thermotolerance indicate differences in ability to acclimate to diverse environmental conditions (Knight and Ackerly, 2002), it is possible that the genotypes chosen for the current study exhibit differences in thermotolerance acclimation. As a result, we attempted to related T_{15} with weather variables such as average daily maximum temperature, minimum temperature, average daily temperature or the highest temperature observed in the two weeks preceding each sampling date for the entire data set (as described previously in Snider et al., 2013). The T_{15} values for energy trapping by photosystem II were significantly and positively correlated with the average maximum temperature ($r = 0.620$) and the highest temperature observed prior to each sample date ($r = 0.536$). T_{15} values for photosystem I end electron acceptor reduction were only positively correlated with the highest temperature observed prior to each sample date ($r = 0.445$). Previous research conducted in Arkansas and Georgia, USA has shown that T_{15} values for PS II in cotton were strongly related with the average daily maximum temperature of a given environment (Snider et al., 2013). Conversely, significant correlations between temperature and heat toleranvce were

not observed for any quantum efficiency when considered within each cultivar separately. There is strong evidence that T_{15} can be affected by a number of other factors such as stage of plant growth and plant water status (Havaux, 1992; Chastain et al., 2016; Snider et al., 2013, 2015). Because of this, clear relationships between T_{15} and air temperature measures are not always obtainable.

It was also hypothesized in the current study that specific component processes of the thylakoid reactions would differ significantly in thermotolerance under field conditions. In support of this hypothesis, we observed that quantum yield of energy trapping by photosystem II (PSII) was the most heat tolerant process in all four site-years of the study, where T_{15} values ranged from 47.4 °C in 2020 to 43.8 °C in 2021 at Tifton. Early reports (Berry and Bjorkman 1980) indicated that photosystem II was among the most heat sensitive components of the photosynthetic apparatus; however, a growing body of evidence from more recent studies have suggested that PSII is exceptionally tolerant to high temperatures that would normally inhibit other photosynthetic processes (Gombos et al., 1994; Haldimann and Feller 2005; Salvucci and Crafts-Brandner 2004; Snider et al., 2013; Wise et al., 2004). Furthermore, the ability of PSII to acclimate to high temperature has led other authors to suggest that Upland cotton plants rarely experience high temperatures that would appreciably inhibit PSII function (Hu et al., 2018; Snider et al., 2013; 2015). Reduction of photosystem I (PSI) end electron acceptors was also a consistently heat tolerant process, being equally heat tolerant to PSII in all but one site-year (Athens 2020; Figure 2.8). Studies documenting the thermotolerance of PSI in cotton are fewer than for PSII, but recently conducted, controlled-environment research has documented comparable levels of thermotolerance for PSI and PSII (Hu et al., 2018), which is consistent with our current observations for a diverse collection of field-grown Upland cotton. In contrast, quantum yield of

inter-system electron transport was consistently the most heat sensitive process across all site years, where T_{15} values ranged from 46.4 °C in 2020 at Athens to 34.2 °C in 2021 at Tifton (Figure 2.8). Previous research has suggested that electron transport may be one of the most important functional limitations to photosynthesis under high temperature stress in Pima cotton (Wise et al., 2004; Schrader et al., 2004). For Upland cotton, Hu et al. (2018) evaluated a single cotton cultivar at contrasting growth temperature conditions, and found that PSII and PSI were consistently the most heat tolerant thylakoid components, whereas intersystem electron transport was the most heat-sensitive process. These observations indicate that among the thylakoid reactions, intersystem electron transport is the most heat sensitive process, irrespective of environment or genotype evaluated.

The third hypothesis of this study was that diverse upland cotton genotypes will exhibit differences in their thermotolerance plasticity for the most heat-sensitive thylakoid specific process. We observed from the previous objective that intersystem electron transport was the most heat sensitive process. To test the thermotolerance plasticity of intersystem electron transport for the upland cotton genotypes, the slopes from their linear regression lines were compared. Substantially variation in thermotolerance plasticity was observed among the diverse collection of cotton genotypes evaluated here. Importantly, DP 1646, Tamcot Sphinx, DG 3615, T0018MDN and ST 5020 had the highest slopes, indicating the highest thermotolerance plasticity. This indicates that these genotypes may acclimate more readily to environmental change than other genotypes (Knight and Ackerly, 2002). In contrast, heat tolerance of intersystem electron transport for the genotypes T0246BC3MDN, MDN0101 (GH191), Acala Maxxa, UA 48 and DES 56 were the least responsive to environment. However, some of these genotypes were also the most heat tolerant in all environments (e.g. MDN0101 (GH191). Despite the fact that these cultivars

exhibited significant differences in the response of heat tolerance to environment, the specific environmental variable driving heat tolerance plasticity in responsive genotypes could not be determined. As noted above, correlations with ambient temperature variables were not observed when considered within a single genotype. Although water deficit can affect heat tolerance of photosystem II (Snider et al., 2013; Chastain et al., 2016), it is not possible to determine if plant water status contributed to genotypic variation in thermotolerance of Φ_{E0} in the current study. For example, all field sites were irrigated according to recommendations for field grown cotton, and plant water status was not measured. Thus, future research should determine specific drivers (drought, high temperature, etc.) of environment-induced variation in thermotolerance using controlled environment studies. Furthermore, it should be determined if high thermotolerance plasticity or innately high and stable thermotolerance is a more advantageous trait in production environments characterized by high levels of abiotic stress (Snider et al., 2015).

2.5. CONCLUSION

The objectives of the current study were to 1) assess genotypic variation in thermotolerance of thylakoid component processes for diverse cotton genotypes, 2) assess differences in heat tolerance for specific photosynthetic components of the thylakoid reactions and 3) quantify genotypic differences in thermotolerance plasticity of the most heat sensitive thylakoid component in upland cotton. Among the diverse cotton genotypes evaluated, significant genotypic variation in the thermotolerance of photosystem II, intersystem electron transport, and photosystem I were observed in some site-years. Thermotolerance rankings among genotypes were also strongly dependent on the photosynthetic process evaluated. Specifically, genotypes that exhibited the most thermostable energy trapping by photosystem II and intersystem electron transport in one

environment were the least heat tolerant for PSI end electron acceptor reduction in other environments. We also conclude that intersystem electron transport is more heat-sensitive than photosynthetic processes occurring at PSII or PSI, which are the most heat tolerant thylakoid components. The comparison of slopes of the upland cotton genotypes for the intersystem electron transport showed that the genotypes differed significantly in thermotolerance plasticity of intersystem electron transport. Identifying the weakest link in photosynthetic tolerance to high temperature will facilitate future heat tolerance selection efforts by focusing on the most heat-susceptible processes. Given the environmental dependence of our results, future research will need to evaluate genotypic variation in high temperature acclimation potential of specific processes.

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CHAPTER 3

GENOTYPIC VARIATION IN FUNCTIONAL CONTRIBUTORS TO YIELD FOR FIELD-GROWN COTTON

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ABSTRACT

Yield improvement in cotton could be accelerated through selection for functional yield drivers such as interception of photosynthetically active radiation (∑IPAR), radiation use efficiency (RUE), and harvest index (HI). However, information on the extent to which these traits vary in cotton in the southeastern US is limited. In the current study, it was hypothesized that functional yield drivers would vary significantly within a diverse cotton collection. This study was conducted in Tifton, GA and Athens, GA, and included a diverse collection of 10 cotton genotypes in 2020 and 11 genotypes in 2021. The results indicate that lint yield, total biomass production, ∑IPAR and HI were all affected by genotype. Even among the highest-yielding genotypes, values for these traits differed significantly, indicating that high yields can be achieved by differentially manipulating these underlying traits. However, when considered for all genotypes, only HI exhibited a significant quadratic relationship with yield, where HI for peak lint production was 0.325. Boll production and intra-boll yield components were also affected by genotype. Boll density and lint percent were important contributors to lint yield. Genotypes differed in their individual seed surface area, lint weight per seed, and the number of seeds per boll, and the highest yielding genotypes had the most seed per boll. The genotypes evaluated in the current study achieve high lint production per boll and lint yields by manipulating different yield components.

Key words: *Gossypium hirsutum*; *Gossypium barbadense*; Physiological contributors; Intra-boll components; Lint yield

3.1. INTRODUCTION

Cotton refers to any plant species within the genus Gossypium that produces spinnable fibers as epidermal outgrowths of their seed coat. The most widely cultivated species of cotton is *Gossypium hirsutum* L. which is commonly known as Upland cotton. It is a high-yielding, widely-adapted cotton species that accounts for more than 90% of cotton production around the world (Jabran et al., 2020). The other cultivated species of cotton are *G. barbadense* L. (Egyptian cotton), *G. herbaceum* L. (Arabian/Levant cotton) and *G. arboreum* L. (tree cotton) (Lee and Fang, 2010). The United States is one of the top cotton producing countries in the world, following only India and China in total production. During the year 2019/2020, the US produced 922 kg ha^{-1} of cotton on 4.7 million hectares (USDA 2021).

Globally, cotton has wide genetic diversity, where wild relatives of cotton are substantially different from the current, improved cultivars in many ways (Abdurakhmonov et al., 2004; Mammadov et al., 2018). In their native habitats, wild cotton plants are indeterminate perennials resembling shrubs or small trees. In contrast, cotton is widely grown as an annual crop. *G. hirsutum* has been found to be more genetically diverse within the species than the other three major cultivated species of cotton (Abdurakhmonov et al., 2008). However, after the domestication of cotton, breeding efforts were made within smaller sets of genetic materials through crossing and re-selection, which led to losses in genetic diversity for modern cotton (Wendel et al., 1992, Brubaker et al., 1999). May et al., (1995) documented that in the late 1980s, reselection and repeated crossing within genetically related material was done to develop proprietary cultivars. Bowman et al., (1996) also reported low genetic variation among the Upland cotton cultivars released between 1970 and 1990. Conaty and Constable (2020) reported in Australian breeding programs that selection for improved cultivars has led to increased total dry matter production and

carbon assimilation rates, but increases in lint percent (the fraction of seedcotton accounted for by fiber) have been the dominant driver of yield improvement. Similar observations have been made for public breeding programs in the United States, where yield improvement has been positively associated with higher lint percent (Campbell et al., 2011). Selection for increases in lint percent has also led to reductions in individual seed size (Campbell et al., 2011), which has negative implications for seedling vigor (Snider et al., 2014, 2015, 2018; Virk et al., 2019, 2020). Furthermore, small seed size could potentially lead to seed fragment contamination of cotton fiber during the ginning process (Pearson, 1955). Thus, there is a need to exploit other functional yield-driving traits in future selective breeding efforts in cotton.

Crop yield can be calculated as $\sum IPAR \times RUE \times HI$ (Earl and Davis, 2003), where $\sum IPAR$ is cumulative photosynthetically active radiation intercepted by the crop canopy during a growing season, RUE is the dry matter produced per unit of solar radiation absorbed (radiation use efficiency), and harvest index is the fraction of total above-ground dry matter accounted for by the economically valuable portion of the crop. Vargas et al. (2002) suggested that PAR interception is mainly governed by green leaf area, its duration of exposure to light and canopy architecture. Bai et al. (2016), evaluated ten cotton varieties and documented significant differences in $\sum IPAR$, yet biomass production was not strongly associated with $\sum IPAR$ when considered across three years of the study. Sultana et al. (2023), while working with diverse cotton cultivars, reported that cultivars with higher light interception also had higher yields. In contrast, previous research in Australia has indicated that genetic yield improvement has been associated negatively with light interception by the canopy (Conaty and Constable, 2020), but similar efforts to evaluate the contribution of IPAR to lint yield in diverse genotypes are limited. RUE is mainly governed by the photosynthetic capacity of the plant but also by canopy structure (Vargas et al., 2002).

Regarding RUE, Rosenthal and Gerik (1991) demonstrated a significant cultivar effect on radiation use efficiency (during the reproductive phase) in three different cotton cultivars (Acala SJ-2, Deltapine 50, and Tamcot CD3H), mainly due to differences in boll number among the selected cultivars. Conaty and Constable (2020) documented that yield improvement in Australian breeding programs was positively associated with total biomass production along with higher single-leaf photosynthetic rates. Bange and Milroy (2000) observed that greater RUE and light interception contributed towards more dry matter production in cotton cultivars. Thus, it will be important for the research here to evaluate both IPAR and RUE as both these parameters drive total biomass production.

Harvest index can be increased in cotton by increasing the ratio of reproductive dry weight to total dry weight (Wells and Meredith, 1984), or by altering within-boll components without a co-occurring change reproductive to vegetative dry matter partitioning (Conaty and Constable, 2020). Harvest index was reported as the dominant contributor to genotypic variability in lint yield for modern versus obsolete cultivars in Australia (Conaty and Constable, 2020). Genotypes with early flowering and fruiting will typically produce less vegetative growth and a higher fraction of total biomass can be partitioned toward reproductive organs (Wells and Meredith, 1984). Meredith and Wells (1989) showed that obsolete cotton cultivars had 24% lower lint yields than modern cultivars and the increase in lint yields was due to more dry matter distribution to reproductive structures than vegetative growth. Improvements in HI and lint yield over the years is mainly attributed to increases in lint percent (Campbell et al., 2011, Bridge and Meredith, 1971, Bridge et al., 1971, Culp and Green, 1992). Campbell et al. (2011) reported that there was a 9% increase in lint percent of PeeDee germplasm cultivars over the span of 70 years. Bridge and Meredith (1971) evaluated obsolete and modern cultivars of cotton and observed an increase in lint percent. They

suggested that lint yield is closely associated with lint percent and it might be a major contributor to the improvement of lint yield. Importantly, harvest index is also governed by yield components such as boll number, boll weight, seed number per boll, seed surface area and lint weight per seed (Worley et al., 1974, Smith and Coyle, 1997, Bednarz et al., 2007). Boll number per unit land area (boll density) has been reported as an important contributor to genotypic lint yield variation in cotton (Zeng and Meredith 2009; Wu et al., 2004). Boll density is also strongly influenced by environment (Hu et al., 2018; Lee et al., 2023; Snider et al., 2019; 2021), whereas intra-boll yield components are more consistently driven by genotype (Virk et al., 2023). Harrel and Culp (1976) suggested that a greater number of seeds per boll should increase within-boll seed surface area available for fiber production. Additionally, these authors indicated that cotton seeds with higher surface area have more potential for lint production per seed, which is another important contributor to genotypic variation in lint yield. These traits and other within-boll yield drivers have been suggested as selection criteria for increased lint yield over the past several decades (Miller and Rawlings 1967; Bridge et al., 1971; Bednarz et al., 2006; Campbell et al., 2011; Virk et al., 2023).

As noted previously, yield improvement programs in cotton should utilize diverse cotton genotypes for the selection of valuable traits such as functional drivers of yield. However, the extent to which the physiological drivers of yield vary among diverse cotton genotypes has received limited attention, particularly in the southeastern United States. The identification of traits that contribute to higher yields can guide breeding programs in selecting and developing cotton varieties with improved yield potential. Thus, in the current study, it was hypothesized that functional yield drivers would vary significantly among diverse cotton genotypes. As a result, the

objective of the current study was to assess genotypic variation in physiological (∑IPAR, RUE, and HI) and yield component contributors to yield in a diverse set of field-grown cotton genotypes.

3.2. MATERIALS AND METHODS

3.2.1. Study site and plant material

The present study was carried out at two University of Georgia research farms over two growing seasons: Lang-Rigdon Research Farm, Tifton, GA and Iron Horse Farm, Athens, GA. The planting dates were June 2 in 2020 at both the locations, May 10, 2021 at Tifton, and June 18, 2021 at Athens. The soil at the Tifton location is characterized as a Tifton sandy loam soil and at the Athens site as a Pacolet sandy loam soil. Small plot experiments were conducted with 10 cotton genotypes in 2020 and 11 different genotypes in 2021. The experiment at each site was arranged as a randomized complete block design with 8 replications of each genotype. Plots were 3.05 m long, single-row plots with a 1.83 m inter-row spacing and each replicate of plots separated by 3 m bare soil alleys. Seeds were sown at a rate of 11 seeds per linear meter of row. Recommendations of the University of Georgia Cooperative Extension Service for the production of high-yielding cotton (1681 kg ha^{-1} lint yield goal) were followed for soil fertility, irrigation, and pest management practices in both years of the study (Whitaker et al., 2019). Weather data were obtained from a nearby weather station (University of Georgia Weather Network) for the 2020 and 2021 growing seasons (http://www.georgiaweather.net/), and average daily maximum temperature, minimum temperature, average daily relative humidity, cumulative total solar radiation, and cumulative total rainfall during the growing season for each site year are provided in Table 3.1.

Table 3.1: The average daily maximum temperature (T_{max}), minimum temperature (T_{min}), average daily relative humidity (RH), cumulative total solar radiation, and cumulative total rainfall for each location and growing season.

Location	Year	T_{max} (°C)	T_{min} (°C)	RH (%)	Total Solar Radiation (MJ/m^2)	Total Rainfall (mm)
Tifton	2020	29.5	18.8	76.1	3841	607
Athens	2020	26.5	15.1	77.3	3784	774
Tifton	2021	30.1	19.2	76.6	3497	771
Athens	2021	26.7	15.0	77.0	3828	680

The Upland cotton (*Gossypium hirsutum* L.) genotypes used in this study were selected from different breeding programs across the United States. DES 56 was characterized as an early-maturing and high-yielding cultivar developed in the Mississippi Delta by crossing PD 2164 and Stoneville 213 (Bridge and Chism 1978; Bridge and Meredith 1983). A large proportion of commercially-grown cotton cultivars have this genotype as a part of their pedigrees (Van Esbroeck et al., 1998). Acala Maxxa was developed in 1975 by USDA Cotton Research Station, Shafter, California by crossing T7538 and S4959. This cultivar shows characteristics like improved yield and fiber quality (CPCSD 1990). Tamcot Sphinx was developed under the Texas Multi-Adversity Resistance (MAR) Genetic Improvement Program by crossing the strain MAR-CDP37HPIH-1-1-86 and a selection from Paymaster 145 and released in 1995 by the Texas Agricultural Experiment Station (El-Zik and Thaxton 1996). Tamcot Sphinx shows high resistant to reniform nematode and is characterized with a cylindrical growth habit and storm-resistant bolls (El-Zik and Thaxton 1996). UA 48 is a conventional cultivar of cotton developed by crossing Arkot 8712 and FM 966

and released in November 2010 by the Arkansas Agricultural Experiment Station (Bouland and Jones 2012). UA 48 is an early maturing cultivar with resistance to bacterial blight, and outstanding fiber quality and high yield (Bourland and Jones 2012). T0018MDN, T0246BC3MDN and MDN0101 (GH191) are exotic genotypes of Upland cotton characterized as late flowering with extensive vegetative growth at the expense of reproductive growth (Jiang et al., 2018). The elite, industry checks included in the current study were DP 1646 B2XF (Bayer Crop Science), DG 3615 B3XF (Nutrien Ag Solutions) and ST 5020 GLT (BASF-Stoneville cotton). DP 1646 B2XF was the most widely grown cultivar in the US at the start of this project. DG 3615 B3XF was selected because it exhibited the highest lint yields in the University of Georgia on-farm variety trials (www.ugacotton.com). ST 5020 GLT was selected for its unique root anatomical traits and greater seedling vigor than most commercial cultivars tested in previous experiments (Snider et al., 2022). Lastly, in 2021, a commercially-available Pima cotton (*Gossypium barbadense* L.) cultivar (DP 341 RF) was included in the experiment at both field sites. Pima cotton is commonly grown in the arid Southwestern United States.

3.2.2. Physiological Measurements

3.2.2.1.Canopy light interception and radiation use efficiency

Light interception was measured approximately every two weeks, beginning during the squaring stage of development and continuing until the earliest maturing plots had reached physiological maturity as defined in Gwathmey et al. (2016). Measurements of photosynthetically active radiation (PAR) were taken from above and below the canopy simultaneously between 1200 h and 1400 h using a ceptometer (AccuPAR LP-80; METER Environment, Pullman, WA). Two below canopy measurements were done per plot at each sample time with the line PAR sensor

placed perpendicular across the row at two different positions within the plot and an external PAR sensor in full sunlight immediately adjacent to the plot of interest. The average fraction of photosynthetically active radiation intercepted by the canopy ($IPAR_f$) was calculated from these measurements. An average $IPAR_f$ was obtained for all measurements throughout the growing season and then multiplied by incoming incident PAR (48% of total solar radiation) for the entire season to get ∑IPAR (MJ m^{-2}).

For estimating the total above-ground biomass, three plants per plot were removed at agronomic maturity but prior to harvest maturity. The number of harvestable sympodial and monopodial bolls per plant were counted, and these data were used in the yield and boll density calculations described in subsequent sections. Definitions of agronomic maturity, harvest maturity, and harvestable bolls are provided in Gwathmey et al. (2016). Plants were then dried in a forced-air oven for 48 h to obtain total dry matter per plant which was then corrected for plant density to get total dry matter per plot and thereafter, total dry matter per hectare (kg $hectare^{-1}$) was quantified. Dry biomass per unit land area was then divided by ∑IPAR to estimate radiation use efficiency (RUE) in g MJ^{-1}.

3.2.2.2. Lint yield and harvest index

A sample of 50 bolls was harvested by hand and seedcotton weight was measured at harvest maturity to estimate seedcotton weight per boll. The seedcotton was then ginned using a table top gin to obtain lint weight per boll and lint percent. The total number of harvestable bolls per plant was multiplied by lint weight per boll to estimate lint yield per plant. Lint yield per plant was extrapolated to lint yield in kg ha^{-1} by accounting for plant densities. Harvest Index (HI) was calculated as the ratio of lint yield (kg ha^{-1}) to the total above-ground biomass (kg ha^{-1}).

3.2.2.3. Boll density and intra-boll components

From the harvestable boll counts noted in section 2.2.1, sympodial and monopodial boll density (boll number per hectare) were calculated. After the harvesting and ginning of seedcotton, the average boll mass, lint percent, lint weight and seed weight per boll was obtained. Then, one hundred seeds from each plot were weighed to estimate seed index (g 100 seed^{-1}) and average individual seed weight. Then, the number of seeds per boll was calculated as below,

$$seeds\ boll^{-1} = \frac{seed\ weight\ boll^{-1}}{single\ seed\ weight}$$

Average seed surface area was calculated from the formula given by Groves and Bourland (2010),

$$SSA = 35.74 + 6.59 * seed\ index$$

Lastly, the lint weight per seed was calculated from lint weight per boll and seeds per boll as given below,

$$Lint\ weight\ seed^{-1} = \frac{Lint\ weight\ boll^{-1}}{seeds\ boll^{-1}}$$

3.2.3. Statistical Analysis

Statistical analysis was performed within each growing season separately due to the different number of genotypes in each year (10 genotypes in 2020 and 11 in 2021). However, the data were pooled across both locations within each year because an initial analysis of variance indicated that there was no interaction between genotype and location for lint yield. Thus, the effect of genotype on yield and all yield contributors of interest was assessed using a mixed-effects analysis of variance, where physiological parameter of interest was the dependent variable, block x year was

considered a random effect, and genotype was the fixed effect of interest. Post hoc analysis for means separation was conducted using Fisher's Protected LSD.

3.3. RESULTS

3.3.1. Lint Yield and Total Biomass

The ANOVA results for the genotype effect for lint yield, total biomass and other physiological yield contributors and yield components for two growing seasons are provided in Table 3.2. Lint yields were significantly affected by genotype in both growing seasons (Table 3.2 and Figure 3.1A and 3.1B). In 2020, Acala Maxxa, DG 3615, T0018MDN, DP 1646, Tamcot Sphinx, ST 5020, UA 48 and DES 56 had the highest lint yields (1385 kg ha^{-1}) whereas MDN0101 (GH191) and T0246BC3MDN had the lowest lint yields (880 kg ha^{-1}). In 2021, DG 3615 was the highest yielding genotype (2044 kg ha^{-1}), whereas MDN0101 (GH191), T0018MDN and DP 341 had the lowest lint yields (846 kg ha^{-1}). Total aboveground biomass was significantly affected by genotype in 2020 only (Figure 3.1C). During this growing season, T0246BC3MDN, T0018MDN, MDN0101 (GH191), DG 3615 and DES 56 produced the most biomass (6124 kg ha^{-1}), whereas Tamcot Sphinx, UA 48, ST 5020, DP 1646 and Acala Maxxa and DES 56 produced the least biomass (4788 kg ha^{-1}). One cultivar (DES 56) produced statistically equivalent biomass to cultivars with the highest and the lowest levels of biomass production. There were no significant genotypic differences in total biomass in 2021 and the average total biomass was 5565 kg ha^{-1} (Figure 3.1D).

Table 3.2: Mixed effects ANOVA results for the effect of genotype on multiple yield-determining traits for two growing seasons. NS indicates P > 0.05.

Parameter	Growing year	p-value
Lint yield	2020	0.0149
	2021	<.0001
Total biomass	2020	0.0152
	2021	NS
Cumulative IPAR (∑IPAR)	2020	<.0001
	2021	<.0001
Radiation use efficiency	2020	NS
	2021	NS
Harvest index (HI)	2020	<.0001
	2021	<.0001
Total boll density	2020	NS
	2021	0.0196
Sympodial boll density	2020	0.0011
	2021	0.0002
Monopodial density	2020	NS
	2021	NS
Boll mass	2020	0.0248
	2021	<.0001
Lint percent	2020	<.0001
	2021	<.0001
Lint weight/boll	2020	<.0001
	2021	<.0001
Seeds/boll	2020	NS
	2021	<.0001
Seed surface area (SSA)	2020	<.0001
	2021	<.0001
Lint weight/seed	2020	<.0001
	2021	<.0001

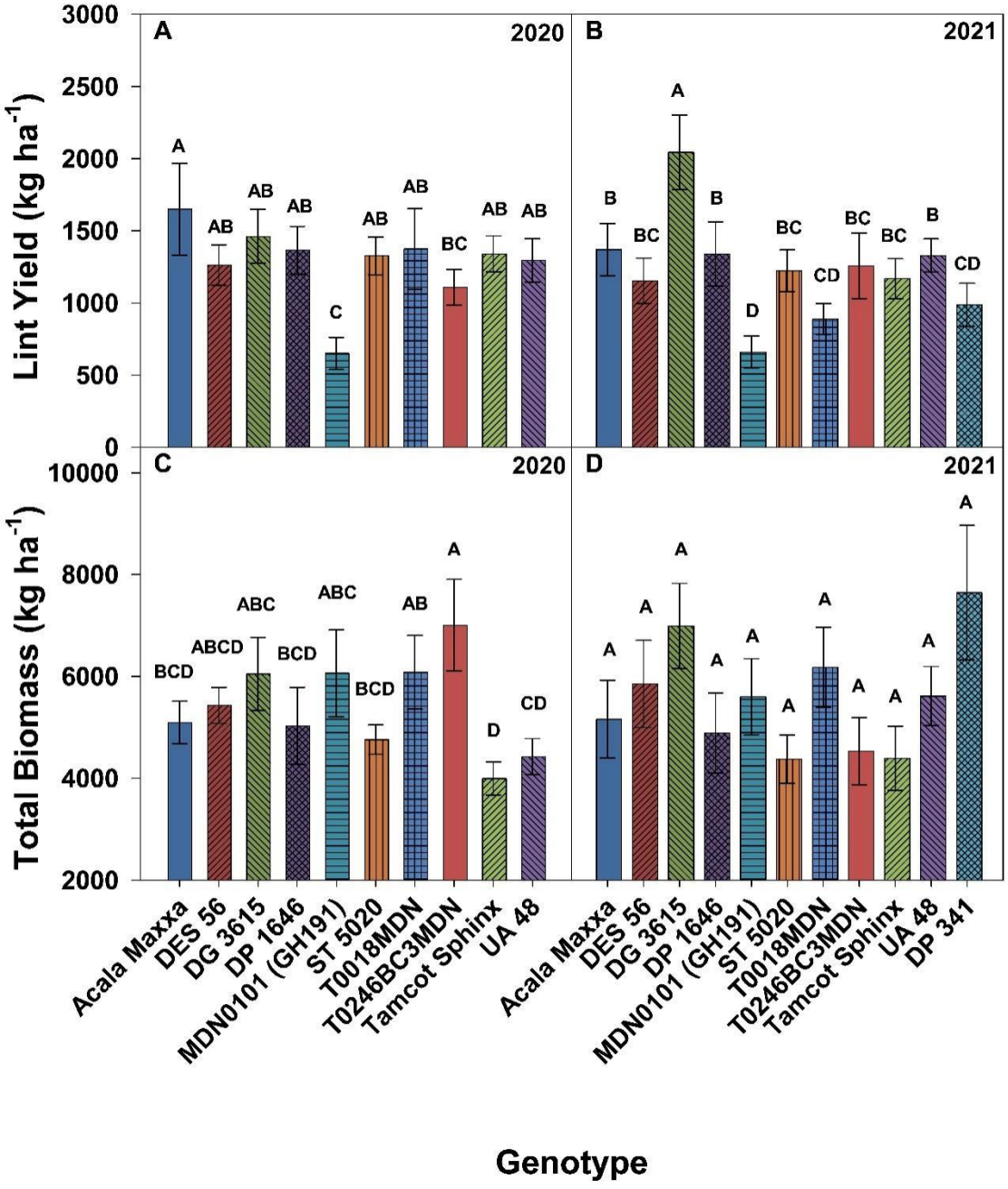

Figure 3.1. Lint yield (A and B) and total biomass (C and D) for 10 cotton genotypes in 2020 (A and C) and 11 in 2021 (B and D) at field sites in Athens and Tifton, Georgia. Data are means ± standard error (n = 16), and bars not sharing a common letter within a given year are significantly different ($p < 0.05$). Data were combined across both locations prior to analysis.

3.3.2. Intercepted Photosynthetically Active Radiation, Radiation Use Efficiency and Harvest Index

∑IPAR was significantly affected by cotton genotype in both years (Figure 3.2A and 3.2B). In 2020 (Figure 3.2A), T0246BC3MDN, T0018MDN, MDN0101 (GH191), DG 3615, Acala Maxxa and Tamcot Sphinx intercepted the most solar radiation (716MJ m^{-2}), whereas DP 1646 and ST 5020 intercepted the least solar radiation (644 MJ m^{-2}). However, in 2021 (Figure 3.2B), DP 341, MDN0101 (GH191), UA 48 and T0018MDN had the highest values for ∑IPAR (698 MJ m^{-2}), and DP 1646, T0246BC3MDN had the lowest values for ∑IPAR (587 MJ m^{-2}). Radiation use efficiency (RUE) was unaffected by genotype in either year (Figure 3.2C and 3.2D).

Harvest index (HI) was also significantly affected by cotton genotype in both years (Figure 3.2E and 3.2F). In 2020 (Figure 3.2E), Tamcot Sphinx, Acala Maxxa, UA 48, DP 1646 and ST 5020 incorporated the highest fraction of total biomass into lint yield (0.314) whereas MDN0101 (GH191) and T0246BC3MDN had the lowest HI (0.153). In 2021 (Figure 3.2F), DG 3615, Tamcot Sphinx, ST 5020, DP 1646, Acala Maxxa, T0246BC3MDN and UA 48 had the highest HI (0.292) whereas MDN0101 (GH191), DP341 and T0018MDN had the lowest HI (0.139).

When the average lint yield of the 10 upland cotton genotypes in two years was plotted against cumulative IPAR, RUE and HI, a significant relationship was observed between lint yield and harvest index only (Figure 3.3A, B and C). Specifically, a quadratic response was observed between HI and lint yield, where lint yield increased from a low of 711 kg ha^{-1} to a maximum of 1435 kg ha^{-1} as harvest index increased from 0.119 to 0.325 (Figure 3.3C). Increases in HI beyond 0.325 did not result in appreciably higher lint yields.

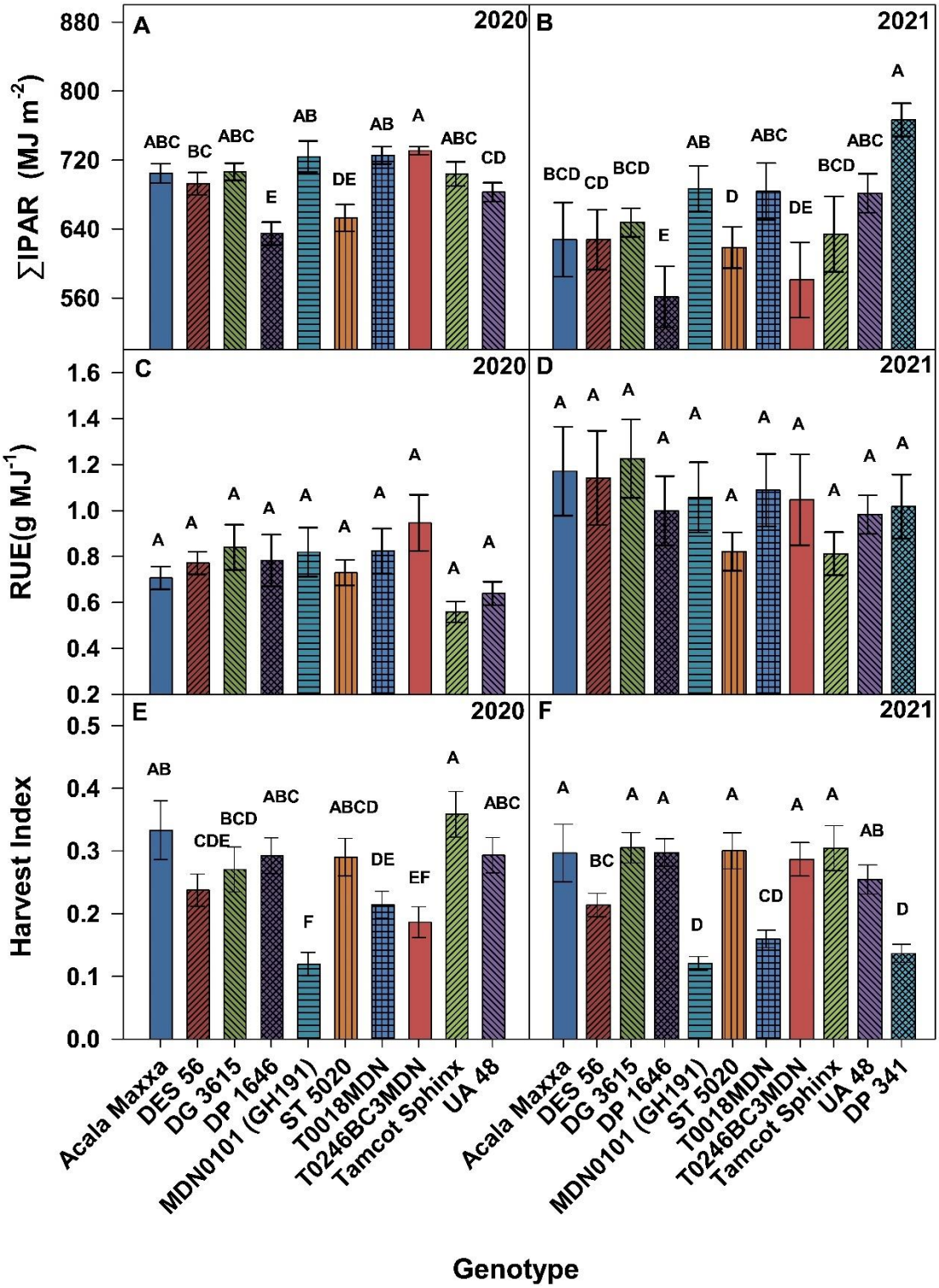
A
2020
B
2021
C
2020
D
2021
E
2020
F
2021
∑IPAR (MJ m-2)
RUE(g MJ-1)
Harvest Index
Genotype
Acala Maxxa
DES 56
DG 3615
DP 1646
MDN0101 (GH191)
ST 5020
T0018MDN
T0246BC3MDN
Tamcot Sphinx
UA 48
DP 341

Figure 3.2. ∑IPAR (A and B), radiation use efficiency (RUE; C and D) and harvest index (E and F) for 10 diverse cotton genotypes in 2020 (A, C and E) and 11 in 2021 (B, D and F). Data are means ± standard error (n = 16), and bars not sharing a common letter within a given year are significantly different ($p < 0.05$). Data were combined across both locations prior to analysis.

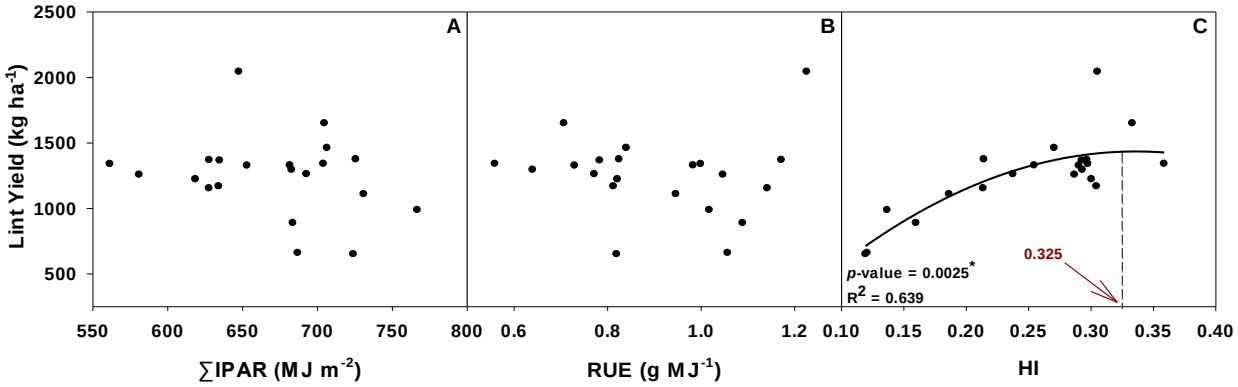

Figure 3.3. Lint yield responses to cumulative IPAR (∑IPAR; A), radiation use efficiency (RUE; B) and harvest index (C). Data points are the mean of 16 replicate plots for a single upland cotton cultivar across two locations (Tifton and Athens, Georgia) in two growing seasons. There was no significant association between (∑IPAR and lint yield (A); and RUE and lint yield (B). The equation for the quadratic function given in C is as follows: Lint Yield = 668.1 + 2612.2(HI) – 15372.1(HI-0.25)^2.

3.3.3. Boll Densities

The total boll density was significantly affected by genotype only in 2021 (Figure 3.4B) and no significant differences were observed in 2020 (Figure 3.4A). The average boll density of all the genotypes in 2020 was 644937 bolls ha^{-1} (Figure 3.4A). However, in 2021 (Figure 3.4B), total boll

density ranged from 435514 bolls ha^{-1} to 831293 bolls ha^{-1}. Six genotypes (DP 341, DG 3615, Acala Maxxa, DP 1646, UA 48 and T0246BC3MDN) had statistically equivalent boll densities to the highest boll density, and six genotypes (MDN0101 (GH191), T0018MDN, Tamcot Sphinx, DES 56, ST 5020 and T0246BC3MDN) had statistically equivalent boll densities to the lowest boll density. One genotype (T0246BC3MDN) produced statistically equivalent bolls ha^{-1} to genotypes with the highest and the lowest boll densities.

The number of sympodial bolls was significantly affected by genotype in both 2020 and 2021 (Figure 3.4C and 3.4D). In 2020 (Figure 3.4C), T0018MDN, ST 5020, Acala Maxxa, T0246BC3MDN, DES 56, Tamcot Sphinx, DP 1646, DG 3615 and UA 48 had the highest number of sympodial bolls (482816 bolls ha^{-1}) and MDN0101 (GH191) had the lowest number of sympodial bolls (294735 bolls ha^{-1}). In 2021 (Figure 3.4D), DP 341 produced the highest number of sympodial bolls (683195 bolls ha^{-1}), whereas MDN0101 (GH191) and T0018MDN had the lowest number of sympodial bolls (279997 bolls ha^{-1}). There were no significant genotypic differences in the number of monopodial bolls in any of the growing seasons. The genotypes had an average of 180928 monopodial bolls ha^{-1} in 2020 and 160990 monopodial bolls ha^{-1} in 2021 (Figure 3.4E and 3.4F).

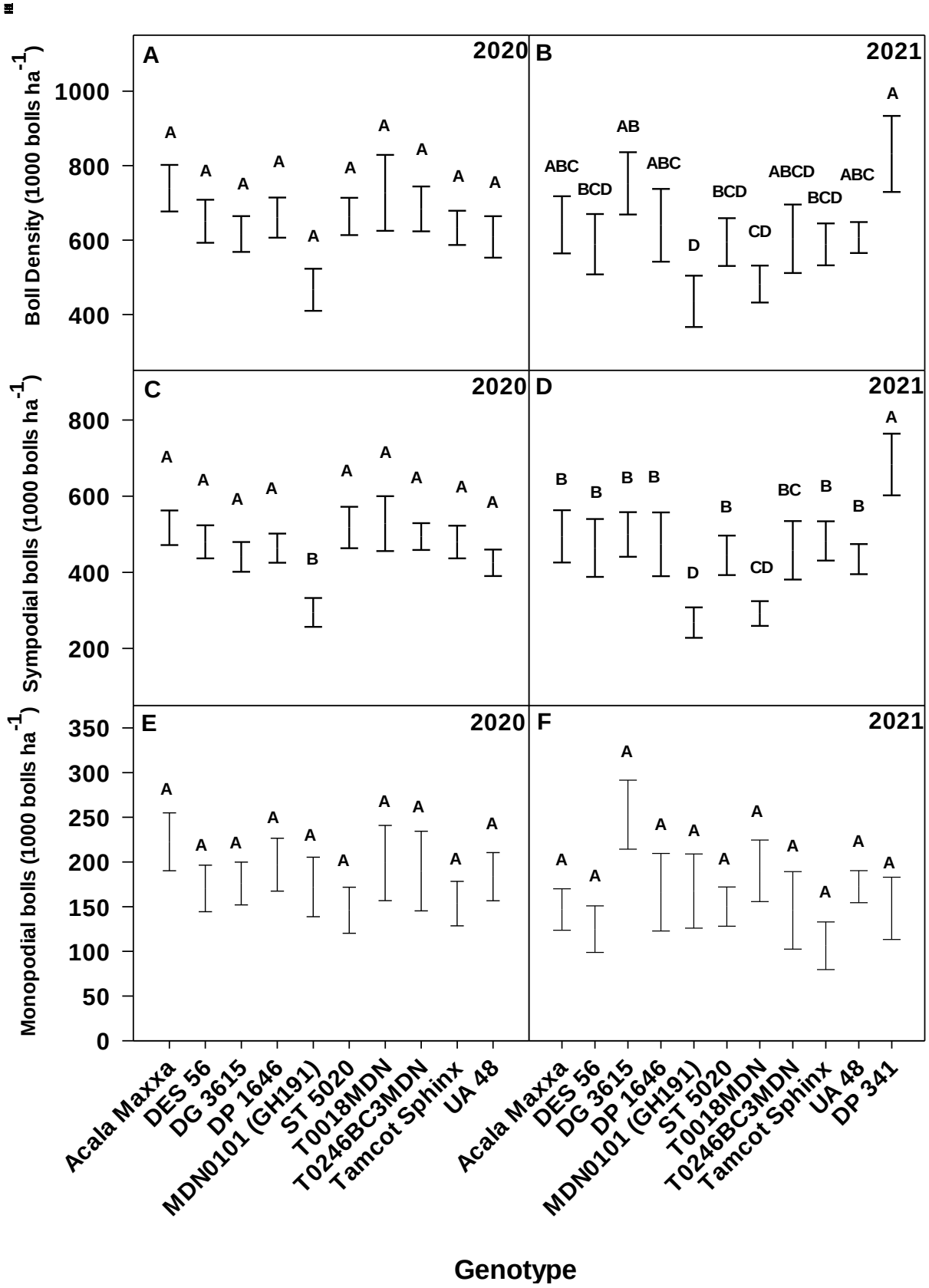
A
2020
B
2021
C
2020
D
2021
E
2020
F
2021
Boll Density (1000 bolls ha-1)
Sympodial bolls (1000 bolls ha-1)
Monopodial bolls (1000 bolls ha-1)
1000
800
600
400
200
350
300
250
200
150
100
50
0
Acala Maxxa
DES 56
DG 3615
DP 1646
MDN0101 (GH191)
ST 5020
T0018MDN
T0246BC3MDN
Tamcot Sphinx
UA 48
DP 341
Genotype

Figure 3.4. Boll density (A and B), number of sympodial bolls per ha (C and D) and number of monopodial bolls per ha (E and F) for 10 diverse cotton genotypes in 2020 (A, C and E) and 11 in 2021 (B, D and F). Data are means ± standard error (n = 16) and bars not sharing a common letter within a given year are significantly different ($p < 0.05$). Data were combined across both locations prior to analysis.

3.3.4. Intra-boll components

Boll mass was significantly different among the genotypes in both years (Figure 3.5A and 3.5B). In 2020 (Figure 3.5A), UA 48, Tamcot Sphinx, DG 3615 had the highest boll mass (5.18 g seedcotton boll^{-1}), whereas MDN0101 (GH191), DP 1646, DES 56, T0246BC3MDN and ST 5020 had the lowest boll mass (4.41 g seedcotton boll^{-1}). In the year 2021 (Figure 3.5B), DG 3615 and UA 48 had the highest boll mass (5.83 g seedcotton boll^{-1}), and DP 341 had the lowest boll mass (3.01 g seedcotton boll^{-1}). Lint percent was also significantly different among the genotypes in both years (Figure 3.5C and 3.5D). In both the years, DP 1646 and DG 3615 had highest lint percent (46.02% in 2020 and 44.95% in 2021), whereas MDN0101 (GH191) had the lowest lint percent (30.63% in 2020 and 30.86% in 2021).

Lint weight per boll was also significantly affected by genotype in both seasons (Figure 3.5E and 3.5F). In 2020 (Figure 3.5E), DG 3615, UA 48, Tamcot Sphinx, Acala Maxxa and DP 1646 had highest lint weight per boll (2.10 g lint boll^{-1}), whereas MDN0101 (GH191) had the lowest lint weight (1.30 g lint boll^{-1}). However, in 2021 (Figure 3.5F), DG 3615 had the highest lint weight (2.66 g lint boll^{-1}) and DP 341 had the lowest lint weight per boll (1.14 g lint boll^{-1}).

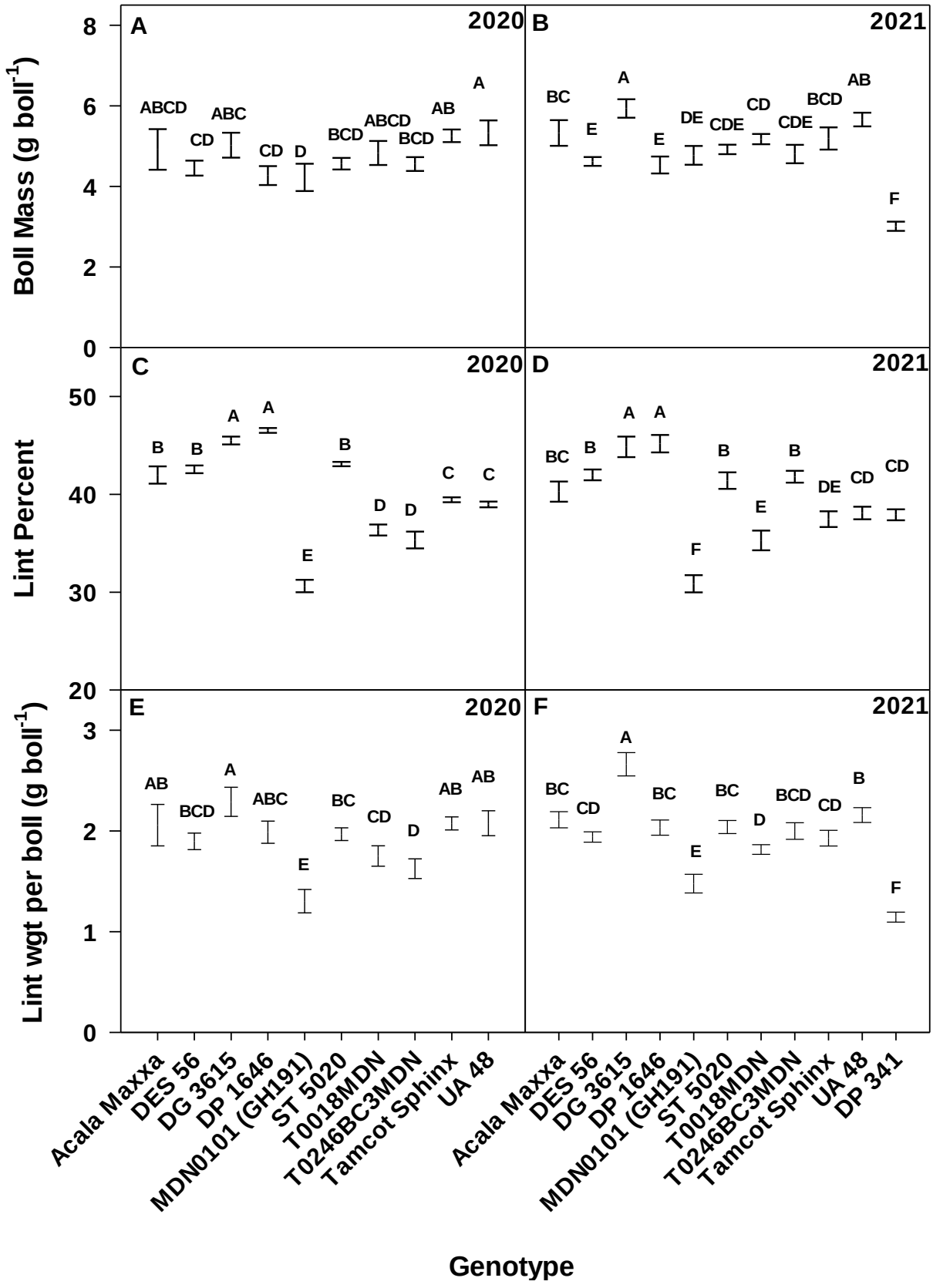
A
2020
B
2021
C
2020
D
2021
E
2020
F
2021
Boll Mass (g boll-1)
Lint Percent
Lint wgt per boll (g boll-1)
Genotype
Acala Maxxa
DES 56
DG 3615
DP 1646
MDN0101 (GH191)
ST 5020
T0018MDN
T0246BC3MDN
Tamcot Sphinx
UA 48
DP 341

Figure 3.5. Boll mass (A and B), lint percent (C and D) and lint weight per boll (E and F) for 10 diverse cotton genotypes in 2020 (A, C and E) and 11 in 2021 (B, D and F). Data are means ± standard error (n = 16) and bars not sharing a common letter within a given year are significantly different ($p < 0.05$). Data were combined across both locations prior to analysis.

The number of seeds per boll was significantly affected by genotype in 2021 only (Figure 3.6B). There were no significant differences in 2020 and the average number of seeds for all the genotypes was 29.2 seeds boll^{-1} (Figure 3.6A). However, in 2021 (Figure 3.6B), DG 3615, MDN0101 (GH191), UA 48, T0018MDN, DP 1646, T0246BC3MDN and Acala Maxxa had the highest number of seeds in a single boll (31.6 seeds boll^{-1}), whereas DP 341 had the lowest number of seeds (16.3 seeds boll^{-1}).

Seed surface area (SSA) was significantly affected by genotype in both growing seasons (Figure 3.6C and 3.6D). In 2020 (Figure 3.6C), MDN0101 (GH191), UA 48 and Tamcot Sphinx had the most seed surface area (106.7 mm^2), whereas DP 1646 had the least surface area per seed (84.6 mm^2). In 2021 (Figure 3.6D), DP 341, UA 48, Tamcot Sphinx, T0018MDN and Acala Maxxa had the most seed surface area (105.4 mm^2), whereas DP 1646 had the least seed surface area (86.8 mm^2).

Similarly, lint weight per seed was significantly different among the selected genotypes in both 2020 and 2021 (Figure 3.6E and 3.6F). In both the years, DG 3615 had the highest lint weight per seed (0.088 g lint seed^{-1} in 2020 and 0.083 g lint seed^{-1} in 2021), whereas MDN0101 (GH191) had the lowest lint weight per seed (0.048 g lint seed^{-1} and 0.047 g lint seed^{-1} in 2021).

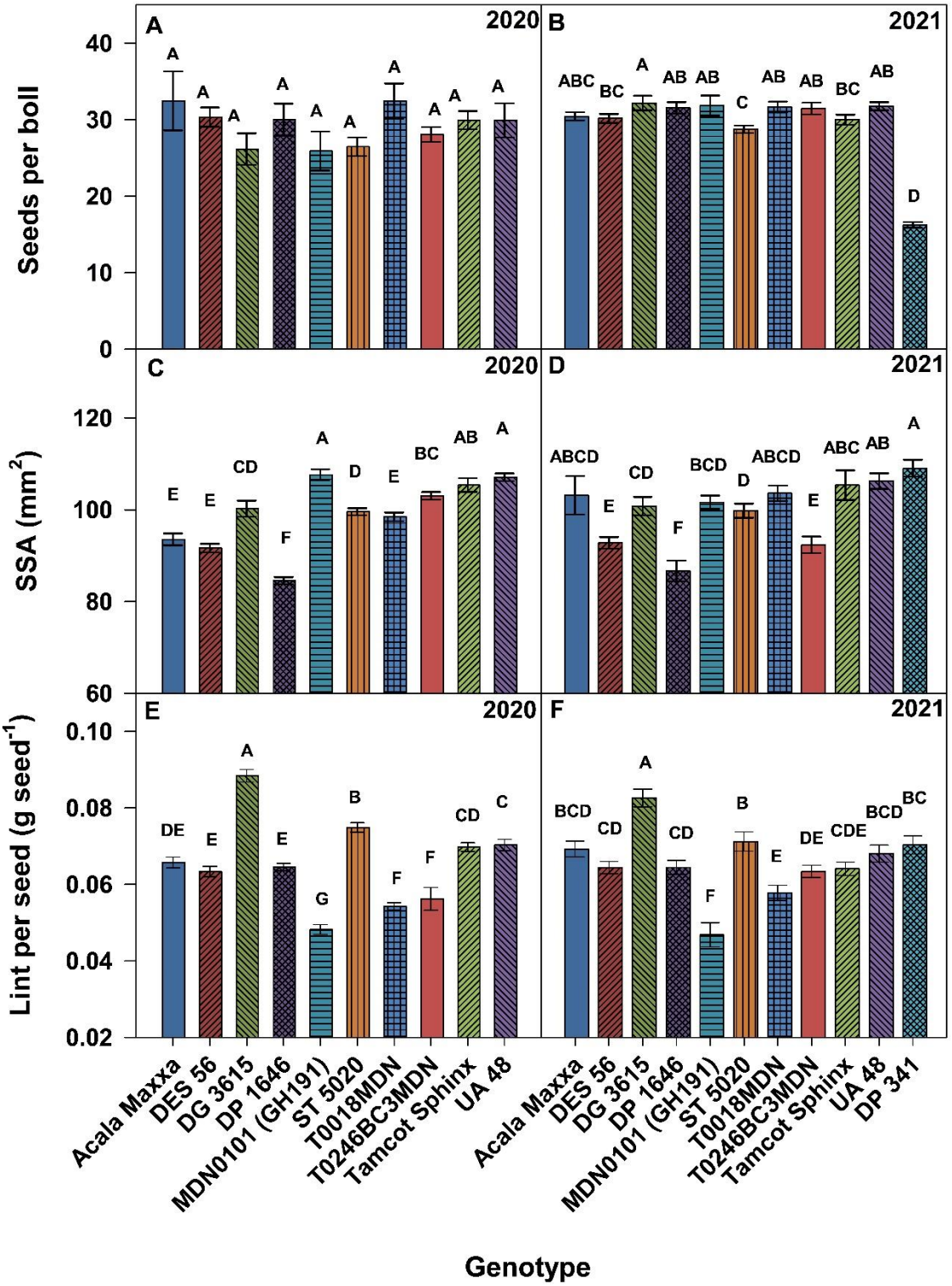
A
2020
B
2021
C
2020
D
2021
E
2020
F
2021
Seeds per boll
SSA (mm^2)
Lint per seed (g seed^{-1})
Genotype
Acala Maxxa
DES 56
DG 3615
DP 1646
MDN0101 (GH191)
ST 5020
T0018MDN
T0246BC3MDN
Tamcot Sphinx
UA 48
DP 341

Figure 3.6. Seeds per boll (A and B), seed surface area (C and D) and lint weight per seed (E and F) for 10 diverse cotton genotypes in 2020 (A, C and E) and 11 in 2021 (B, D and F). Data are means ± standard error (n = 16) and bars not sharing a common letter within a given year are significantly different ($p < 0.05$). Data were combined across both locations prior to analysis.

3.4. DISCUSSION

It is well-established that crop yield is the product of biomass production and the fraction of that biomass that is converted into the economically valuable portion of the crop (harvest index). Biomass production is further governed by canopy light interception and radiation use efficiency (Monteith, 1977). Harvest index in cotton is further governed by boll production and alterations in intra-boll yield components (Conaty and Constable, 2020). Despite the importance of these physiological traits in determining yield, breeding efforts have focused on the selection of a small number of agronomically desirable traits, which has led to a reduction in genetic diversity for modern cotton genotypes (Wendel et al., 1992; Bowman et al., 1996; Brubaker et al., 1999). Evaluating the functional drivers of yield in a diverse collection of cotton genotypes could 1) identify the extent to which these traits vary in cotton and 2) elucidate the traits that are most important for yield maximization. Conaty and Constable (2020) reported that increases in total biomass production and harvest index were important contributors to yield improvement in Australia. However, similar efforts to evaluate the contribution of these physiological parameters to yield in diverse genotypes are limited for the southeastern US. Therefore, in the current study, it was hypothesized that physiological contributors to yield will vary significantly as a function of genotype.

Among the key yield drivers proposed by Monteith (1977), we can conclude that total biomass production was highly dependent on genotype. However, neither biomass nor light interception were significantly associated with lint yield when considered across all the genotypes evaluated. In contrast, harvest index was highly predictive of genotypic variation in lint yield across the diverse collection of cotton genotypes evaluated here. For example, the variation in biomass production ranged from the a low of 3994 kg ha^{-1} to a high of 7005 kg ha^{-1} in 2020 where significant genotypic differences were observed. Biomass production is dependent on both ∑IPAR and RUE (Monteith, 1977). However, in the present study, we did not find any significant genotypic effect for RUE in either growing season (Table 3.2). In contrast, ∑IPAR was significantly affected by genotype in both growing years, where it ranged from 635 MJ m^{-2} to 726 MJ m^{-2} in 2020 and from 561 MJ m^{-2} to 767 MJ m^{-2} in 2021. Variation in PAR interception is governed by differences in either leaf area development or canopy structure, including leaf shape, thickness, angle and spatial orientation on the plant in different genotypes (Vargas et al., 2002, Boote and Loomis 1991). Bai et al. (2016) documented variation in canopy-intercepted solar radiation in different cotton varieties mainly due to different growth habits and LAI among the varieties. Bai et al. (2016) also reported a positive relationship between the interception of solar radiation and production of total biomass. In the current study, we also observed that some of the highest yielding varieties had low biomass and some of the lowest yielding varieties had high biomass. For example, the lowest yielding varieties in 2020, MDN0101 (GH191) and T0246BC3MDN, were among the genotypes that produced the most biomass. Conaty and Constable (2020) have suggested that increases in biomass do not always improve lint yield due to the indeterminate growth habit of the cotton crop and its source-sink properties. Hence, total biomass might not be the best factor for explaining variation in cotton lint yield because some of

the higher biomass producing genotypes likely diverted resources towards vegetative sinks rather than reproductive sinks.

Among the three yield-determining traits, the fraction of total biomass accounted for by yield (harvest index or HI) was the most reliable indicator of yield. HI ranged from a low of 0.12 to a high of 0.36 in 2020 and from 0.12 to 0.31 in 2021. Both the lowest yielding varieties in 2020, MDN0101 (GH191) and T0246BC3MDN, had the lowest HI. Similarly, the Pima cultivar (DP 341) in 2021 was among the lowest yielding genotypes with the highest light interception and the lowest HI. Meredith and Wells (1989) reported differences in lint yields of obsolete and modern cultivars which were associated with differences in dry matter partitioning between the vegetative and reproductive plant organs. Genotypes with higher reproductive/vegetative dry matter ratio were also characterized by having less vegetative growth and early flowering and fruiting. Conaty and Constable (2020) identified HI as the primary factor contributing to the variation in lint yield between modern and obsolete cotton cultivars in Australia through the modification of within-boll components, mainly lint percent. In their report, yield improvement was not associated with dry matter distribution to reproductive and vegetative tissues. When relationships between yield and key physiological yield drivers are assessed across all genotypes, we can conclude that ∑IPAR and RUE are not significantly related with lint yield. In contrast, a strong quadratic relationship between HI and yield is observed, where peak HI for lint production is 0.325. However, even among genotypes with the highest yields, HI and biomass production can vary substantially. For example, in 2020, DG 3615 and Tamcot sphinx exhibited statistically comparable yields. However, Tamcot Sphinx is an early maturing cultivar (El-zik and Thaxton 1996; El-zik and Thaxton 1998), and it produced the lowest biomass levels with the highest harvest index. By

comparison, DG 3615 achieved high yields in this same season by maximizing biomass production and having an intermediate HI value relative to other genotypes (Figs 3.1 and 3.2).

Yield and harvest index can be influenced by genotypic variation in boll production and intra-boll yield components. We can conclude that genotype affected boll densities and all intra-boll yield components. Total boll production trends were similar to trends in the number of sympodial bolls among the genotypes (Figure 3.4). In 2021, boll densities ranged from 435514 bolls ha^{-1} to 831293 bolls ha^{-1} across all genotypes. The genotype with the highest number of sympodial bolls also had the highest boll density and vice-versa. Among the upland cotton genotypes, those with the lowest boll density also had the lowest yields. A study by Zeng and Meredith (2009) reported boll density to be the primary determinant of lint yield in exotic cotton germplasm. Similarly, Worley et al. (1974) and Sharma et al. (2015) found that number of bolls per unit land area had a significant impact on the overall contribution to lint yield. Wells and Meredith, (1984) observed a linear increase in lint yields of obsolete and modern cultivars which was mainly associated with increased boll numbers. Campbell et al. (2011) also showed a linear increase in boll number following seventy years of genetic improvements in yield in the Southeastern US. In contrast, the Pima cotton cultivar in 2021, DP 341, produced the highest boll densities, but was the lowest yielding cultivar (Figs. 3.1 and 3.4). This is because individual boll size and seed production per boll were substantially lower in Pima cotton than any of the upland cultivars evaluated (Figs. 3.5 and 3.6). Pima is a cultivar mainly grown in Southwestern US where the conditions are more favorable for its growth. A recent study by Holladay et al. (2022) reported that when Pima genotypes were grown in the Southeastern US, they yielded 50% lower and had smaller bolls with lower lint percent than upland cotton.

Boll mass and lint percent were strongly affected by genotype in both years of the study, and genotypic variation in these two traits was highly predictive of lint yield. The genotypes with lower boll mass and/or lint percent also had lower lint yields. In 2020, DP 1646 and DG 3615 were among the highest yielding genotypes and had the highest lint percent. However, boll mass was significantly different between these two genotypes, illustrating that high yields can be achieved by altering either one of these traits. Similarly, there were two genotypes with statistically lower yields than the maximum, and these two were in the same category as genotypes with the lowest boll mass and lint percent. As noted above, in 2021, the Pima cultivar was among the genotypes with highest boll numbers, but it had the lowest average boll mass, ultimately resulting in the lowest lint yields (Fig. 3.5). As noted elsewhere, lint weight per boll is an important yield component and is driven by boll mass and lint percent (Hu et al., 2018). Previous research has documented that seedcotton mass per boll was somewhat predictive of genotypic variation in lint yield in upland cotton (Zeng and Meredith, 2009). Furthermore, lint percent is strongly governed by genotype (Snider et al., 2013; Virk et al., 2023) and has been used to drive genetic yield improvements through selective breeding for decades (Campbell et al., 2011, Bridge and Meredith, 1971, Bridge et al., 1971, Culp and Green, 1992). Campbell et al. (2011) reported that there was a 9% increase in lint percent of PeeDee germplasm cultivars over the span of 70 years.

Differential manipulation of seed-specific yield components was also a likely contributor to genotypic variation in lint yield. The number of seeds per boll and lint weight per boll govern the amount fiber produced in a single boll, and seed surface area is an important indicator of how many fibers can be produced on a single seed (Harrel and Culp 1976; Worley et al., 1976; Ruan 2013). However, increases in total seed surface area can be brought about through increases in seed number or increases in seed size (Harrel and Culp, 1976). The number of seeds per boll was

not affected by genotype in 2020, but it was in 2021. Previous studies have shown that the number of seeds produced per boll can be significantly affected by environmental conditions (Kohel and Benedict 1984; Pettigrew 2008; Ekinci et al., 2017; Hu et al., 2018), which may explain the differences in results observed between the two years. When a genotype effect was observed for this trait in 2021, genotypes with the highest lint yields had the most seed per boll and genotypes with the lowest lint yields had the fewest seed per boll. However, even among the highest yielding upland genotypes, significant differences were observed in the number of seeds per boll (Figs. 3.1 and 3.6). Regarding individual seed surface area, the highest lint yields were achieved by cultivars having the highest seed surface area (Tamcot Sphinx and UA 48) and lowest seed surface area (DP 1646). Lint weight per seed exhibited nearly identical trends in both growing seasons, where DG 3615 produced the highest lint weight per seed and MDN 0101 (GH191) produced the lowest lint weight per seed. Previous research has shown that seed surface area and lint weight per seed are more strongly influenced by genotype than environment (Virk et al., 2023). Thus, these and other intra-boll yield components could be used as selection criteria for yield improvement (Miller and Rawlings 1967; Bridge et al., 1971; Worley et al., 1974, Smith and Coyle, 1997, Bednarz et al., 2006; Bednarz et al., 2007; Campbell et al., 2011; Virk et al., 2023). However, we conclude that the cultivars evaluated in the current study achieve high lint production per boll and lint yields by manipulating different yield components. The higher yielding varieties that achieve these yields by manipulating different yield components can be crossed with each other to breed for higher yields.

3.2. CONCLUSIONS

The objective of the current study was to assess genotypic variation in physiological (∑IPAR, RUE, and HI) and yield component contributors to yield in a diverse set of field-grown cotton

genotypes. It was observed that lint yield, total biomass production, ∑IPAR and HI were all significantly affected by genotype. The most productive genotypes achieved high lint yields in different ways, where some produced high biomass and light interception by the canopy but an intermediate HI value. Other genotypes had among the lowest levels of biomass production, yet the highest average HI. However, when considering relationships between functional yield drivers and yield for all genotypes combined, neither biomass nor light interception showed a significant association with lint yield, and harvest index was the strongest predictor of lint yield. Boll production and intra-boll yield components were found to be influenced by genotype, with boll density, mass, and lint percent varying among the highest yielding genotypes and playing a significant role in lint yield. Differences in individual seed surface area, lint weight per seed, and the number of seeds per boll were observed among genotypes. Knowledge of genotypic variation in physiological yield drivers and yield components could accelerate yield improvement by combining traits conducive to high biomass production with underlying yield component traits that optimize HI.

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CHAPTER 4

GENOTYPIC VARIATION IN GROWTH, SINGLE LEAF PHYSIOLOGY, AND ACCLIMATION POTENTIAL OF THYLAKOID PROCESSES IN COTTON EXPOSED TO HIGH TEMPERATURE EXTREMES

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ABSTRACT

The cotton crop already experiences yield-limiting high temperatures during the growing season, which are predicted to worsen in a changing climate. This makes the identification of heat tolerant cultivars that can acclimate to high temperature conditions a necessity. The objective of this study was to assess the effects of growth temperature and genotype on plant growth, single-leaf physiology, and thermotolerance of thylakoid processes for cotton exposed to optimal and supra-optimal temperature conditions. A diverse collection of cotton cultivars was selected based on previously documented differences in thylakoid-specific thermotolerance, and they were exposed to optimal (30/20 C) and two supra-optimal growth temperatures (35/25 and 40/30 °C). There was a significant interaction between genotype (G) and growth temperature (T) for all growth measures (leaf area, mainstem node production, and plant dry weight), photosynthetic rates, nighttime respiration and stomatal conductance, and thermotolerance of photosystem II. Greater relative growth (plant dry matter) under high temperature was strongly and positively correlated with leaf area and strongly and negatively correlated with nighttime respiration and stomatal conductance. In contrast, photosynthetic parameters were not significantly associated with genotypic variation in growth-specific thermotolerance. Thermotolerance of the thylakoid reactions was not consistently associated with more thermotolerant growth. We conclude that genotypes with heat tolerant vegetative growth have higher relative leaf area and lower relative values for nocturnal respiration and stomatal conductance under high temperature conditions.

Keywords: *Gossypium hirsutum*; *Gossypium barbadense*; Thermotolerance; Heat acclimation; Photosynthesis; Thylakoid reactions

4.1. INTRODUCTION

Cotton, *Gossypium spp,* is the most important fiber crop grown worldwide. The most widely cultivated species of cotton is *Gossypium hirsutum* L. which is commonly known as Upland cotton. It is a high-yielding cotton species and accounts for more than 90% of cotton production around the world (Jabran et al., 2020). Oosterhuis (2000) suggested that high temperature (>35 °C) was a contributing factor to yield variability for cotton grown in the Mississippi River Delta region of the US. India, Pakistan and a few other South Asian countries also commonly experience high temperatures (up to 48°C) during the growing season that can result in yield limitations (Gur et al., 2010). Furthermore, heat wave events are expected to increase in intensity along with increases in daily mean temperature due to a changing climate (Meehl and Tebaldi, 2004). As a result, heat-induced yield losses will likely only be exacerbated in the future.

In addition to causing declines in productivity, high temperatures can also negatively affect vegetative growth and a number of underlying physiological processes in cotton. For example, previous studies have reported decreased germination and emergence rates, mainstem growth, leaf area production, and photosynthetic rates at high temperature extremes (Arndt 1945; Reddy et al., 1992a; Cottee et al., 2010). Reddy et al. (1992a,b) observed reductions in plant height, the number of mainstem nodes, leaf area and dry weight for cotton seedlings after 3 weeks when grown at higher than optimum temperatures. Raphael et al. (2017) also reported a decrease in germination and higher percentages of abnormal seedlings at temperatures above 40 °C. Camejo et al. (2005) observed a 50% decline in photosynthesis of a heat-sensitive tomato cultivar when it was transferred from 25 °C to 45 °C temperature conditions. In another study on a tropical rainforest tree, Pons and Welschen (2003) reported a decrease in net photosynthesis (A_N) when the air temperatures were increased from 28 to 38 °C. A number of other studies have also documented

significant reductions in A_N and have addressed the mechanisms contributing to heat-induced photosynthetic inhibition (Crafts-Brandner and Salvucci, 2000; Salvucci and Crafts-Brandner, 2004; Wise et al., 2004; Snider et al., 2010; Hu et al., 2018).

Photosystem II (PSII) is associated with the oxygen evolving complex which catalyzes the photolysis of water and production of molecular oxygen (Taiz and Zeiger 2010). The oxidation-reduction attributes of PS II acceptors can be disrupted under high temperatures and can affect overall electron transport (Mathur et al., 2014). PS II photoinhibition (Berry and Bjorkman 1980) and inhibition of its repair (Akhverdiev et al., 2008) can occur at moderately high temperatures, whereas severe heat stress can inactivate the oxygen evolving complex (Murata et al., 2006). The maximum quantum yield of photosystem II (F_v/F_m) has been reported to significantly decrease at 40°C relative to optimal temperature conditions (30°C) (Hejnak et al., 2015; Westhuizen et al., 2020). However, the recovery of PS II after being exposed to high temperatures in spinach leaves (Agarwal and Jajoo 2021), and its ability to acclimate to prevailing temperature conditions in cotton (Snider et al., 2013; Hu et al., 2018) has also been reported. Several authors have reported no declines in F_v/F_m under moderately high temperatures that significantly limit photosynthesis (Law and Crafts-Brandner, 1999; Snider et al., 2010, 2013, 2015a,b). Thus, other studies have suggested that either limitations to electron transport, at sites other than PSII (Schrader et al., 2004; Wise et al., 2004) or inactivation of rubisco activase (Feller et al., 1998; Law and Crafts-Brandner, 1999; Salvucci and Crafts Brandner, 2004) are likely the main limitations to photosynthesis in cotton.

Selective breeding has utilized a number of valuable traits from wild cotton accessions, but continued selection for agronomically desirable traits has led to a reduction in genetic diversity for modern cotton genotypes (Wendel et al., 1992; Bowman et al., 1996; Van Esbroeck et al., 1998;

Brubaker et al., 1999; Lu and Myers 2002; Paterson et al., 2004). Other potentially valuable traits such as tolerance to environmental stresses, have not been intentionally selected for in most breeding programs (Paterson et al., 2004; Guo et al., 2008). Even more concerning is the possibility that declines in genetic diversity through selective breeding may have made cotton more genetically vulnerable to environmental stresses (Paterson et al., 2004) at a time when climate change is expected to increase the frequency and intensity of extreme weather events (heat waves, drought periods, rainfall events; Meehl et al., 2004; Tebaldi et al., 2006).

Increased thermotolerance following high temperature exposure is known as heat acclimation and improves plant performance under subsequent high temperature exposure (Sethar et al., 2002). Heat acclimation has been observed in many plant species, including soybean, potato and tomato (Chen et al., 1982). Burke et al. (1976) suggested that different plant species or even different genotypes within the same species can differ in acclimation potential. Heat-acclimated plants may show changes in their morphological as well as their physiological characteristics such as temperature responses of photosynthesis and respiration or alterations in dry matter distribution when compared to non-acclimated plants (Sethar et al., 2002). According to Sethar et al. (2002), an exposure to sub-lethal high temperatures may increase the optimum temperature for photosynthesis by altering the temperature-sensitive thylakoid membranes and photosynthetic apparatus of many plants. According to Burke (2001), when cotton seedlings are exposed to temperatures higher than the optimum, they acquire certain levels of thermotolerance and these levels are at maximum at the temperatures 37.7-40 °C after which they start declining (Singh et al., 2007). Adaptive changes in PS II were observed by Sethar et al. (2002) in two Pakistani cotton cultivars after exposing the leaves to a sub-lethal high temperature prior to heat stress.

One approach to identify genotypic differences in heat tolerance acclimation is to collect leaf samples from different thermal environments and use chlorophyll fluorescence assessments to document photosynthetic efficiencies at a range of incubation temperatures (Froux et al., 2004; Snider et al., 2013; Hu et al., 2018). Specifically, temperature-fluorescence response curves can be used to identify the temperature causing a 15% decline in photosynthetic efficiency (T_{15}), which has been used as a standardized measure of heat tolerance as described previously (Froux et al., 2004; Snider et al., 2010, 2013, 2015a,b; Chastain et al., 2016; Hu et al., 2018). OJIP fluorescence or rapid induction measurements can be used to estimate the quantum yield of multiple processes such as energy trapping at photosystem II (ϕ_{P0}), inter-photosystem electron transport (ϕ_{E0}), and PSI end electron acceptor reduction (ϕ_{R0}), among many other thylakoid specific processes (Strasser et al., 2010). Combining this technique with the T_{15} approach above under a range of high temperature conditions could be used to determine the genetic capacity for acclimation of specific thylakoid processes to high temperature extremes in cotton. In many cotton growing regions, the crop is commonly exposed to growth-limiting high temperatures, and in such environments, cultivars identified as potentially heat tolerant would be advantageous. Several studies have reported genotypic differences in heat tolerance in cotton using a number of different assessments, including chlorophyll fluorescence, membrane integrity assays, and enzyme activity assays (Bibi et al., 2008; Snider et al., 2009; Cottee et al., 2010; Snider et al., 2010, 2011; Snider and Oosterhuis, 2011; Wu et al., 2014; Jaconis et al., 2021). However, differences in the ability of cotton cultivars to acclimate to prevailing heat stress conditions has received minimal attention. To address this knowledge gap, the current study utilized five cotton genotypes, previously shown to differ in *in vitro* photosynthetic thermotolerance, to assess the effects of growth temperature on plant growth, leaf-level physiology, and acclimation potential of photosynthetic components of the thylakoid

reactions. The current study tested two hypotheses. 1) The cotton genotypes evaluated in the current study would exhibit significant differences in vegetative growth response to temperature (thermotolerance). 2) Differences in growth-specific thermotolerance would be associated with variation in single-leaf physiological processes and heat acclimation of thylakoid processes. Therefore, the objective of this study was to assess the effects of growth temperature and genotype on plant growth, single-leaf physiology, and thermotolerance of thylakoid processes for cotton exposed to optimal and supra-optimal temperature conditions.

4.2. MATERIALS AND METHODS

4.2.1. Plant material and experimental design

The experiment was conducted in three large walk-in Conviron CG-72 (72 ft^2) growth chambers at The Georgia Envirotron, University of Georgia, Griffin, GA. Growth temperature regimes included an optimal growth temperature (30/20 °C), and two above-optimum growth temperatures (35/25 & 40/30 °C) (Virk et al., 2021). All the growth conditions except the growth temperature were kept identical in all three chambers, where the maximum daily photosynthetically active radiation was 700 µmol m^{-2} s^{-1}, 14/10 h was the day/night photoperiod duration, and relative humidity varied from 50 % at midday to ~90 % during the night. Pots with 2 L volume were filled with Pro-Mix HP high porosity growing medium with 65-70% peat moss. The growing medium also contains horticulture grade perlite, dolomitic and calcitic limestone, wetting agent and mycorrhizae. The potting mix was fertilized with 50 mL of a 0.8% w/v solution of a water-soluble fertilizer (P.F.I. 20-20-20 multipurpose plant food with micronutrients) at the start of the experiment. Beginning a week after emergence, the application frequency was increased to every 3-4 days to avoid nutrient deficiency (Ennahli and Earl, 2005). Four seeds of each genotype were sown in each pot at a depth of 2.5 cm and later thinned to one

plant per pot after germination. The pots were kept well-watered and the frequency of watering varied with temperature regime until the end of growth period (four weeks). The experiment was arranged as a split-plot, randomized complete block design with experimental run treated as a temporal block, growth temperature as the whole-plot factor and genotype as the sub-plot factor. Each experimental run had five replicates. Prior to the current study, a genetically diverse collection of 11 cotton genotypes were evaluated for thermotolerance of thylakoid specific processes under field conditions during the 2020 and 2021 growing seasons. Based on the observed differences in heat tolerance (Chapter 2) the following five genotypes were utilized for the current study because they represented the broadest range in PSII thermotolerance variability in the field. Genotypes included two exotic lines [T0246BC3MDN, MDN0101 (GH191)], a conventional cultivar with desirable agronomic traits (UA 48), a commercial check (DP 1646), and a Pima (*G. barbadense*) cultivar (DP 341).

4.2.2. Gas exchange, fluorescence and growth assessments

Gas exchange and fluorescence measurements were conducted on the uppermost fully expanded leaf (fourth node below the plant terminal) prior to destructive sampling for growth analysis and thermotolerance assays. Data were obtained using an LI-6800 (Li-COR Biosciences, Lincoln, NE, USA) portable photosynthesis system for dark and light adapted measurements with system settings comparable to those described in Chastain et al. (2016) for cotton. The dark-adapted measurements were done pre-dawn on the same sample date as midday measurements (2 hours before the lights were turned on). The LI-6800 chamber settings included 400 µmol mol^{-1} of reference CO_2, air flow rate of 500 µmol s^{-1} and 60±10% relative humidity, and the temperature was set according to the growth chamber temperature. The chamber was clamped onto the fully expanded leaf until steady state respiration was observed (~120s). The parameters that were

recorded during pre-dawn measurements included mitochondrial respiration (R_D), stomatal conductance (g_{sdark}), intrinsic water use efficiency (iWUE), leaf temperature, and maximum quantum yield of photosystem II (F_v/F_m). The daytime measurements were done once the plants were fully light-adapted (2 hours after the lights were turned on). The leaf chamber was maintained at a 1500 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), 400 µmol mol^{-1} of reference CO_2, air flow rate of 500 µmol s^{-1} and 60 ± 10% relative humidity. Temperature was set to equal growth chamber temperature at the time of measurement. The chamber was clamped onto the leaf until steady state A_N was observed (60 to 120s). Recorded parameters of interest included A_N, stomatal conductance (g_s), iWUE, photosynthetic electron transport rate through photosystem II (ETR), and leaf temperature. In all instances where iWUE was reported, it was calculated by dividing A_N by stomatal conductance (Gilbert et al., 2011). Leaf to air temperature differential (T_{leaf} - T_{air}) was calculated by subtracting air temperature inside the leaf chamber at the time of measurement from leaf abaxial surface temperature.

Immediately following physiological assessments, whole plants were destructively sampled and put in the Ziploc bags lined with moistened paper towels and then transported to the lab for growth analysis. The total number of mainstem leaf nodes were determined, and the shoots were then separated into leaves and stems. Leaf area per plant, in cm^2, was determined using a table-top leaf area meter (LI-COR, LI-3100C). Thereafter, plant tissues were dried at 80 °C for 48 h and weighed using a laboratory scale (Mettler Toledo, PG2002-S) to determine sample dry weight.

4.2.3. Heat tolerance assessments of individual leaf samples

Uppermost fully-expanded leaves from each pot (from the fourth mainstem node below the terminal) were collected at the end of the growth period (4 weeks) and kept in plastic bags containing moist paper towels to prevent desiccation. To ensure dark adaptation, which is a

requirement for OJIP assessments (Strasser et al., 2010), the leaves were kept in dark conditions overnight in an insulated box at room temperature (Khan et al., 2021, Hu et al., 2018, Snider et al., 2015b; Virk et al., 2021; Fan and Jespersen 2022). For temperature incubation, leaf discs of ~1cm diameter were taken from each leaf sample and placed on moist filter paper in direct contact with a large thermal gradient table described extensively elsewhere (Chastain et al., 2016). Leaves were initially incubated at 30 °C for five minutes prior to the first measurement. Thereafter samples were progressively incubated at 35, 40, 45, and 50 °C for five minutes at each temperature prior to measurements as described elsewhere (Epron 1997; Ladjal et al., 2000; Froux et al., 2004; Snider et al., 2010a,b; Bordignon et al., 2019). An Opti-Sciences OS5p fluorometer was used to obtain OJIP fluorescence traces at each incubation temperature. Parameters of interest included quantum yield of energy trapping by photosystem II ($\boldsymbol{\phi_{Po}}$), quantum yield of electron transport between photosystem II and photosystem I ($\boldsymbol{\phi_{Eo}}$), and quantum yield of PSI end electron acceptor reduction ($\boldsymbol{\phi_{Ro}}$). Calculation of these three quantum efficiencies was done according to Strasser et al. (2010). In order to quantify heat tolerance of each process, for each sample, the photosynthetic component of interest was plotted versus incubation temperature, and third-degree polynomial regression was utilized to interpolate the temperature at which a 15% decline in efficiency was observed relative to the optimum (T_{15}) as described extensively elsewhere (Froux et al., 2003; Gimeno et al., 2009; Snider et al., 2010a, 2015).

4.2.4. Statistical analysis

The effect of growth temperature (T), genotype (G), and interactive effects [T × G] on plant growth, single-leaf physiology, and thermotolerance of specific thylakoid processes was assessed using a two-way, mixed-effects analysis of variance, where growth indicators, physiological parameters, and high temperature thresholds were the dependent variables of interest.

Experimental run × growth temperature and experimental run × growth temperature × block were considered random effects, and growth temperature and genotype were the fixed effects of interest. Post hoc analysis for means separation was conducted using Fisher's Protected LSD ($p < 0.05$). Relative values under heat stress were calculated as a ratio of genotype mean at 40/30 °C growth temperature regime to genotype mean at 30/20 °C regime for the parameters that showed G × T interactions. Afterwards, pairwise correlation analysis was performed in order to determine if any associations existed between these parameters. All statistical analyses were conducted using JMP Pro 15 software.

4.3.RESULTS

4.3.1. G × T interaction effects on growth, gas exchange, and fluorescence

Table 4.1: ANOVA results for the effect of genotype, growth temperature, and genotype × growth temperature interaction for select early plant growth indicators, daytime leaf measurements, nighttime leaf measurements, and photosynthetic thermotolerance indicators.

Plant Response	P-Value		
	Genotype (G)	Growth Temperature (T)	G x T
Growth Indicators			
Nodes	**< 0.0001**	0.2119	**0.0127**
Leaf Area	**0.0315**	**0.0332**	**0.0009**
Dry Weight	**< 0.0001**	**0.0149**	**0.0178**
Daytime Leaf Measurements			
A_N	**< 0.0001**	0.7229	**0.0190**
g_s	**0.0001**	0.2500	0.1765
ETR	**< 0.0001**	0.4365	**0.0222**
T_{leaf} - T_{air}	**0.0035**	0.6481	0.1105
iWUE	**0.0012**	0.3049	0.0945
Nighttime Leaf Measurements			
R_D	**0.0091**	0.1776	**0.0126**
g_{sdark}*	**< 0.0001**	0.1415	**< 0.0001**
F_v/F_m	**0.0009**	0.0748	0.0502
Photosynthetic thermotolerance			

T_{15} (Φ_{Po})	0.0828	0.1406	**0.0035**
T_{15} (Φ_{Eo})	**0.0147**	0.2532	0.0705
T_{15} (Φ_{Ro})	**0.0269**	0.1453	0.0819

*Negative nighttime g_s values (not biologically possible) excluded prior to analysis.

A_N: Net photosynthesis; g_s: stomatal conductance; ETR: Electron Transport Rate; T_{leaf} - T_{air}: air temperature differential; iWUE: Intrinsic Water Use Efficiency; R_D: Nocturnal Respiration; g_{sdark}: nighttime stomatal conductance; F_v/F_m: Maximum quantum yield of PSII; T_{15} (Φ_{Po}): T_{15} for PSII; T_{15} (Φ_{Eo}): T_{15} for electron transport; T_{15} (Φ_{Ro}): T_{15} for PSI.

At four weeks after planting, all plant growth indicators except the number of mainstem nodes were significantly affected by growth temperature (T), all growth indicators were significantly affected by genotype (G), and a significant interaction between genotype and growth temperature (G × T) was observed for all three growth parameters (Table 4.1). Since G × T interactions could be indicative of innate differences in plant tolerance to high growth temperature conditions, only the growth data showing a significant G × T interaction are presented in this paper. The number of mainstem nodes remained unchanged when the growth temperature increased from 30/20 °C to 35/25 °C but increased significantly with an increase in growth temperature from 35/25 °C to 40/30 °C for the genotype MDN0101 (GH191). In contrast, node number increased from 30/20 °C to 35/25 °C but remained unchanged when the growth temperature increased from 35/25 °C to 40/30 °C for UA 48, DP 1646 and T0246BC3MDN. For the genotype DP 341, the number of mainstem nodes increased as growth temperature regime shifted from 30/20 °C to 35/25 °C but decreased at the 40/30 °C growth temperature regime (Figure 4.1A).

All genotypes showed a decrease in leaf area per plant when the growth temperatures were increased. At 30/20 °C, DP 341, DP 1646 and T0246BC3MDN produced the highest leaf area per plant. The genotypes did not show significant differences in leaf area at 35/25 °C; however, at 40/30 °C, DP 1646, UA 48 and MDN0101 (GH191) produced the most leaf area per plant, and DP

341 and T0246BC3MDN produced the least leaf area per plant (Figure 4.1B). Furthermore, reductions in leaf area as chamber temperatures were increased from 30/20 °C to 40/30 °C ranged from 58 % for UA 48 to 74 % for DP 341. For total dry weight, the only Pima cultivar evaluated, DP 341, was in the same statistical grouping as upland cultivars producing the highest dry weights at the 30/20 °C growth temperature regime. However, the same cultivar produced the lowest dry weights at 40/30 °C. By comparison, dry weight in the upland genotypes except, T0246BC3MDN, either increased from 30/20 °C to 35/25 °C or remained stable, thereafter remaining in the highest dry weight category at 40/30 °C (Figure 4.1C). The dry weight of T0246BC3MDN showed a similar trend to Pima, exhibiting significant reductions in dry weight at each successive increase in growth temperature.

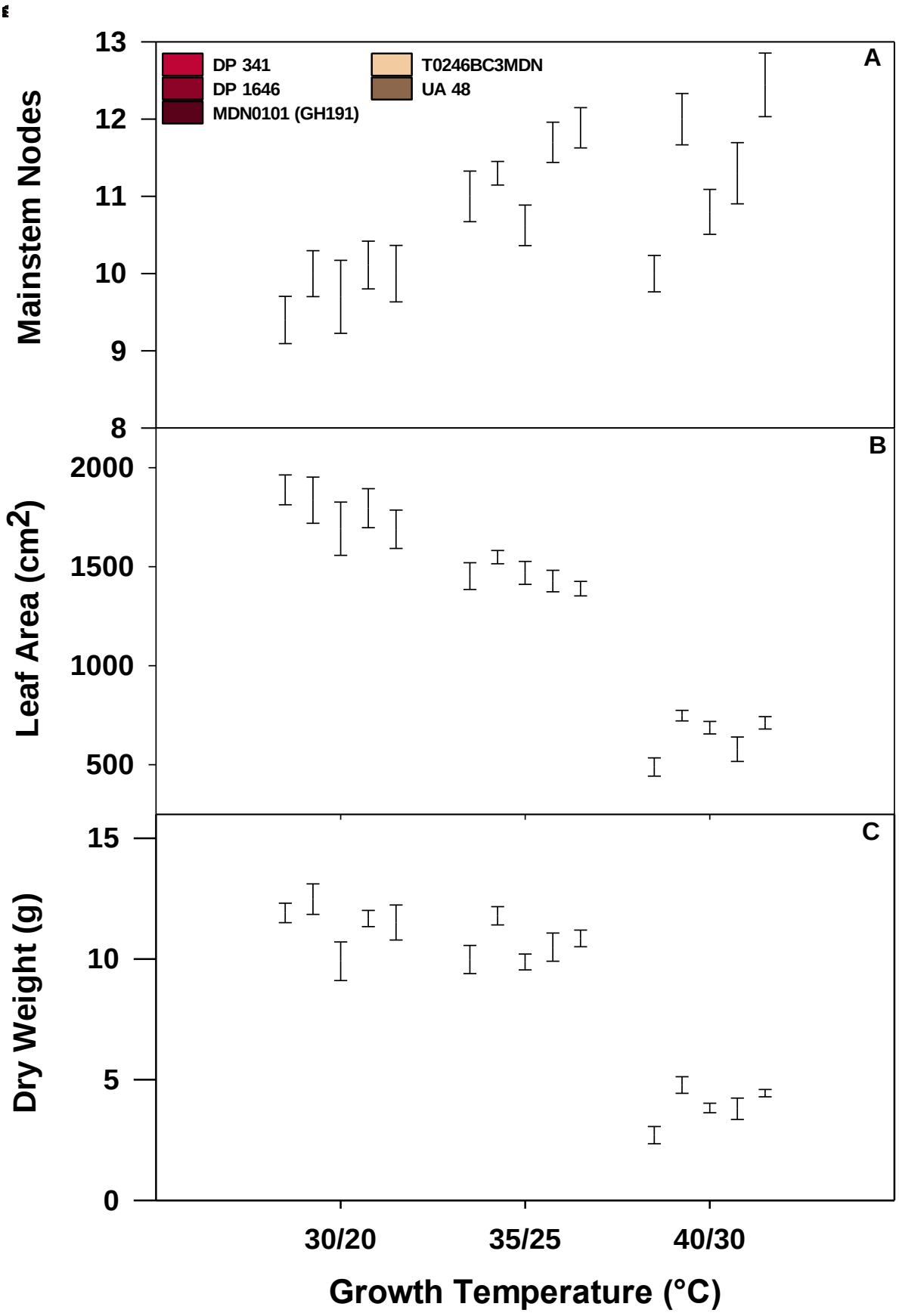
DP 341
DP 1646
MDN0101 (GH191)
T0246BC3MDN
UA 48
A
B
C
Mainstem Nodes
Leaf Area (cm2)
Dry Weight (g)
13
12
11
10
9
8
2000
1500
1000
500
15
10
5
0
30/20
35/25
40/30
Growth Temperature (°C)

Figure 4.1. Number of mainstem nodes (A), leaf area per plant (B) and plant dry weight (C) of cotton seedlings at four weeks after planting for five cotton genotypes grown under three different growth temperature regimes. Values are means (n = 10) ± standard errors, and bars not sharing a common letter are significantly different ($p < 0.05$).

Genotype significantly affected every daytime, leaf-level response evaluated as well as nighttime respiration and stomatal conductance. Furthermore, there was a significant interaction between genotype and growth temperature regime for A_N, ETR, R_D and g_{sdark}. For DP 341, DP 1646, and T0246BC3MDN, A_N was unaffected by growth temperature. For UA 48, A_N was lower at the 35/25 °C temperature regime than either of the other two temperature regimes. In contrast, MDN0101 (GH191) showed an increase in A_N as growth temperature increased, being 15.8 % higher at the 40/30 °C temperature regime than the 30/20 °C regime. Regarding genotype effects at each growth temperature, under optimal conditions, DP 1646, T0246BC3MDN, and UA 48 exhibited the highest A_N values. DP 1646 and T0246BC3MDN had the highest net assimilation rates at both 35/25 °C and 40/30 °C regimes. ETR also increased or remained stable as growth temperatures increased. ETR increased for DP 341, DP 1646, MDN0101 (GH191) and T0246BC3MDN when the growth temperature increased from 30/20 °C to the 35/25 °C regime but remained unchanged from the 35/25 °C to the 40/30 °C regime. For UA 48, ETR increased by 11.9 % when the growth temperature increased from 30/20 °C to 40/30 °C. Regarding the genotype effects within specific growth temperatures, DP 1646, T0246BC3MDN and UA 48 showed the highest ETR at optimum growth temperatures as well as the highest growth temperature regime (40/30 °C). At the 35/25 °C regime, only DP 1646 and T0246BC3MDN had the highest ETR. MDN0101 (GH191) had the lowest ETR at all growth temperatures (Figure 4.2B).

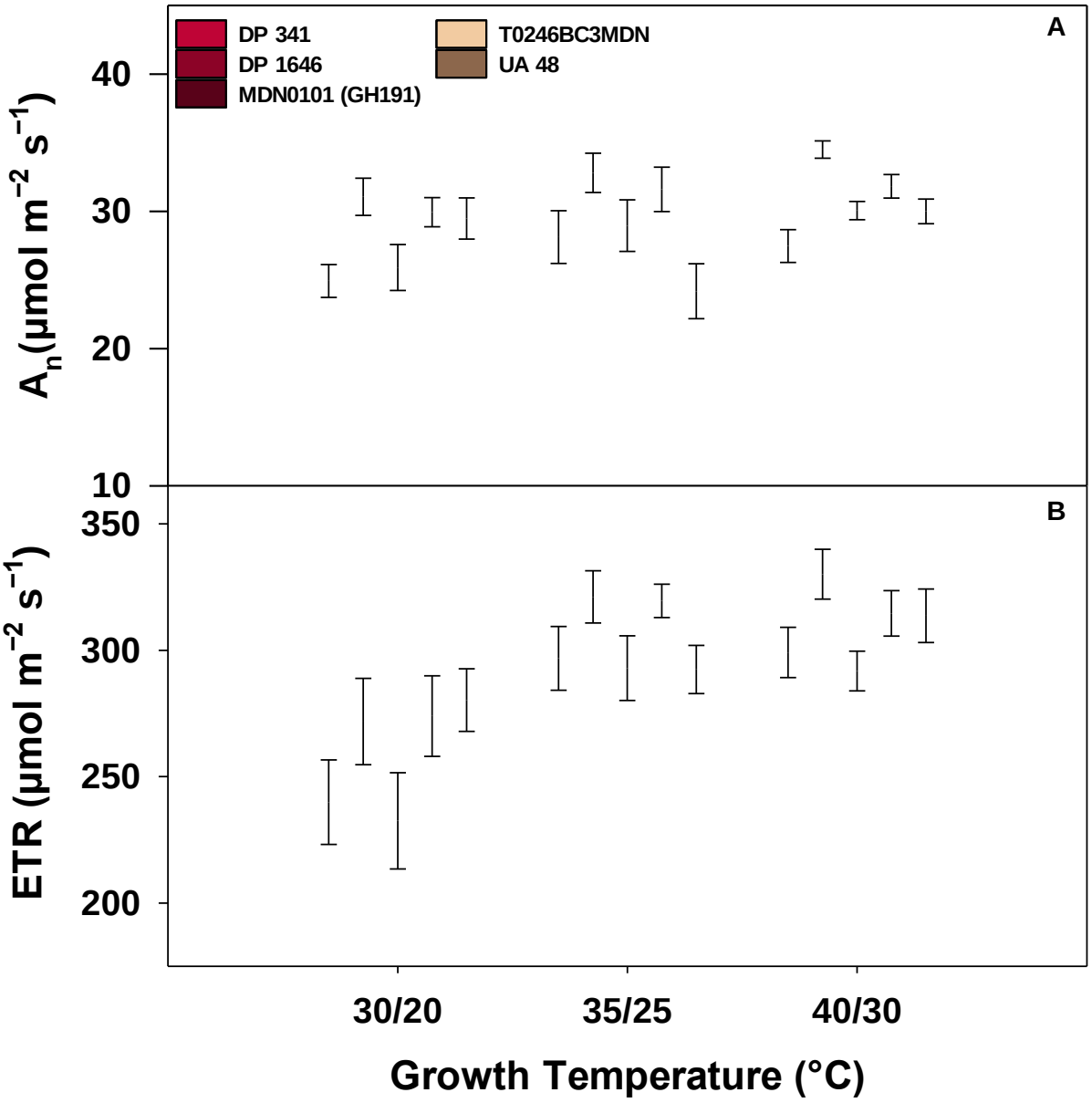

Figure 4.2. Net photosynthetic rate (A_N; A) and electron transport rate through PSII (ETR; B) of cotton seedlings at four weeks after planting for five cotton genotypes grown under three different growth temperature regimes. Values are means (n = 10) ± standard errors, and bars not sharing a common letter are significantly different ($p < 0.05$).

As noted above, there was a significant interaction between genotype and growth temperature for dark respiration (R_D) rates. The Pima cultivar (DP 341) and one of the exotic upland cultivars (T0246BC3MDN) exhibited an increase in nighttime respiration rates with each increase in growth temperature. In contrast, DP 1646, MDN0101 (GH191), and UA 48 had stable R_D rates when the growth temperature increased from 30/20 °C to 35/25 °C but the rates increased when the temperature regime shifted from 35/25 °C to 40/30 °C. There were no significant differences among the genotypes at the optimum temperature regime; however, at the 35/25 °C regime, DP 341 had the highest R_D rates and both DP 341 and T0246BC3MDN had the highest R_D rates at the 40/30 °C regime (Figure 4.3A). All the genotypes except DP 341 showed a decrease in nighttime stomatal conductance (g_{snight}) when growth temperature was increased from 30/20 °C to 35/25 °C but remained unchanged as temperatures increased to a 40/30 °C regime (Figure 4.3B). For DP 341, g_{snight} decreased when the growth temperature increased from 30/20 °C to 35/25 °C but showed a significant increase when the temperature regime shifted from 35/25 °C to 40/30 °C. Significant genotypic effects on g_{snight} were observed at the optimum temperature and the highest temperature regime. At the optimum temperature, DP 1646 had the highest g_{snight} and DP341, T0246BC3MDN, and UA 48 had the lowest g_{snight} values, whereas at the 40/30 °C regime, DP 341, DP 1646 and MDN0101 (GH191) and UA 48 had the lowest g_{snight}.

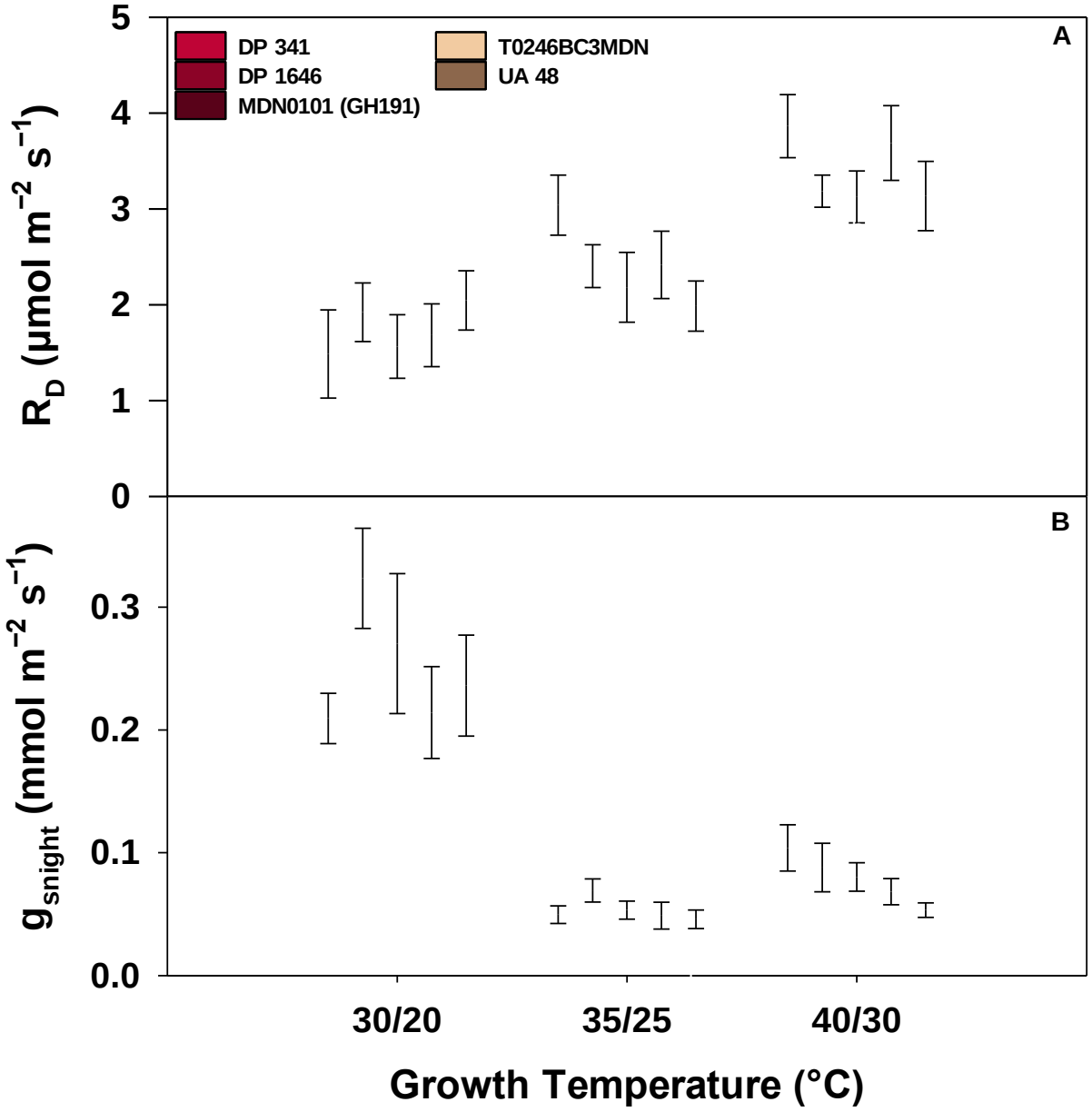

Figure 4.3. Nighttime respiration rates (R_D; A) and nighttime stomatal conductance (g_{snight}; B) of cotton seedlings at four weeks after planting for five cotton genotypes grown under three different growth temperature regimes. Values are means (n = 10) ± standard errors, and bars not sharing a common letter are significantly different ($p < 0.05$).

4.3.2. Genotype effects on g_s (daytime), iWUE, $T_{leaf} - T_{air}$ and F_v/F_m

Across all growth temperatures, daytime stomatal conductance (g_s), intrinsic water use efficiency (iWUE), leaf to air temperature differential ($T_{leaf} - T_{air}$) and the maximum quantum yield of PS II (F_v/F_m) were affected by genotype (Figure 4.4). DP 1646, MDN0101 (GH191) and T0246BC3MDN had the highest stomatal conductance (g_s = 0.851 mol H_2O m^{-2} s^{-1}), whereas UA 48 and DP 341 had the lowest stomatal conductance during the daytime (g_s = 0.712 mol H_2O m^{-2} s^{-1}) (Figure 4.4A). For iWUE, UA 48 and T0246BC3MDN had the highest intrinsic water use efficiency (iWUE = 52.15 µmol mol^{-1}) whereas MDN0101 (GH191), DP 341 and DP 1646 had the lowest values of iWUE (iWUE = 41.96 µmol mol^{-1}) (Figure 4.4B). $T_{leaf} - T_{air}$ followed similar trends as daytime stomatal conductance, where the genotypes with higher g_s had cooler leaves and the genotypes with lower g_s had warmer leaves. DP 1646, MDN0101 (GH191) and T0246BC3MDN had the largest differences between leaf temperature and air temperature ($T_{leaf} - T_{air}$ = -2.16 °C), whereas UA 48 and DP 341 had the lowest differences between leaf temperature and air temperature during the daytime ($T_{leaf} - T_{air}$ = -1.82 °C) (Figure 4.4C). The highest values of maximum quantum yield of PS II (F_v/F_m) were observed in DP 341 and T0246BC3MDN (F_v/F_m = 0.826), whereas the lowest values were observed in UA 48, DP 1646, MDN0101 (GH191) and T0246BC3MDN (F_v/F_m = 0.823) (Figure 4.4D).

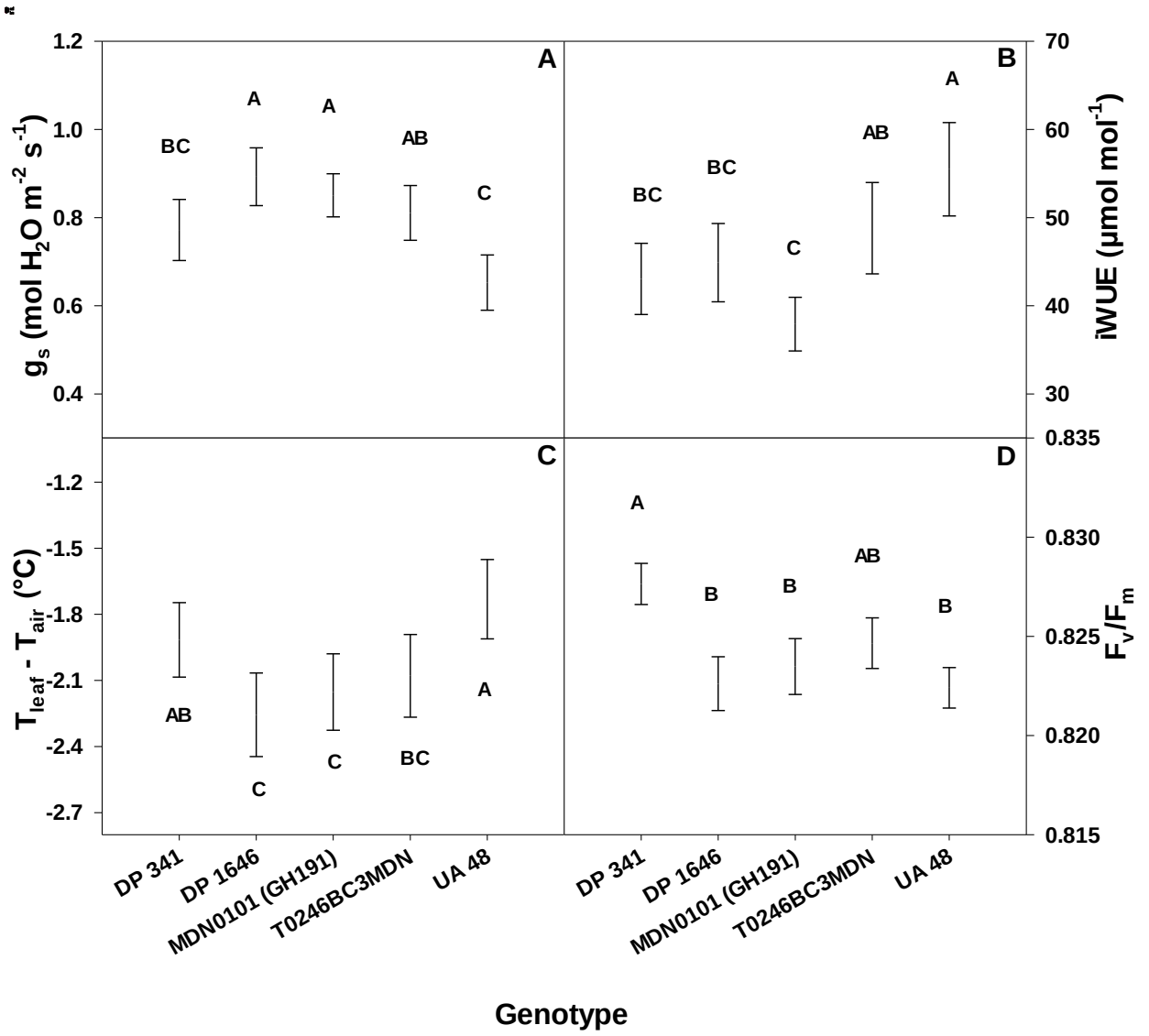

Figure 4.4. Daytime stomatal conductance (g_s; A), intrinsic water use efficiency (iWUE; B), leaf minus air temperature differential ($T_{leaf} - T_{air}$; C) and maximum quantum yield of PS II (F_v/F_m; D) for cotton seedlings at four weeks after planting for five cotton genotypes across all three growth temperature regimes combined. Values are means (n = 30) ± standard errors, and genotypes not sharing a common letter in a given graph are significantly different ($p < 0.05$).

4.3.3. Photosynthetic thermotolerance

A G × T interaction was observed in thermotolerance of Photosystem II, quantified as a 15% decline in the maximum quantum yield of photosystem II relative to the optimum. DP 341, T0246BC3MDN and UA 48 had stable T_{15} (Φ_{Po}) values when the growth temperature changed from a 30/20 °C regime to a 35/25 °C regime, but showed a significant increase in T_{15} (Φ_{Po}) when growth temperature increased to 40/30 °C. For DP 1646, T_{15} (Φ_{Po}) increased with each increase in growth temperature. For MDN0101 (GH191), T_{15} (Φ_{Po}) increased when the growth temperature shifted from a 30/20 °C regime to a 35/25 °C regime but remained stable with further increases in growth temperature. At 30/20 °C, DP 341, MDN0101 (GH191), T0246BC3MDN and UA 48 had the highest T_{15} (Φ_{Po}) values [44.5 °C], whereas DP 1646 and UA 48 had the lowest T_{15} (Φ_{Po}) values [44 °C]. At 35/25 °C, MDN0101 (GH191) had the highest T_{15} (Φ_{Po}) [46.3 °C], whereas the remaining genotypes had the lowest T_{15} (Φ_{Po}) values [45.2 °C]. However, at 40/30 °C, UA 48 and T0246BC3MDN exhibited the greatest heat tolerance of PSII [T_{15} (Φ_{Po}) = 47.6 °C] and DP 341 and MDN0101 (GH191) had the lowest T_{15} (Φ_{Po}) values [46.5 °C] (Figure 4.5). The average T_{15} (Φ_{Po}) across all the genotypes increased from 44.35 °C at 30/20 °C to 47.03 °C at 40/30 °C.

Thermotolerance of intersystem electron transport and photosystem I end electron acceptor reduction [T_{15} (Φ_{Eo}) and T_{15} (Φ_{Ro})] were only significantly affected by genotype. For intersystem electron transport, MDN0101 (GH191) and DP 341 were significantly more heat tolerant [T_{15} (Φ_{Eo}) = 42.2 °C] than DP 1646 and UA 48 [T_{15} (Φ_{Eo}) = 41.2 °C] (Figure 4.6A). Similarly, for photosystem I, MDN0101 (GH191) and DP 341 were the most heat tolerant genotypes [T_{15} (Φ_{Ro}) = 42.8 °C], and were significantly more thermotolerant than UA 48 [T_{15} (Φ_{Ro}) = 41.3 °C] (Figure 4.6B).

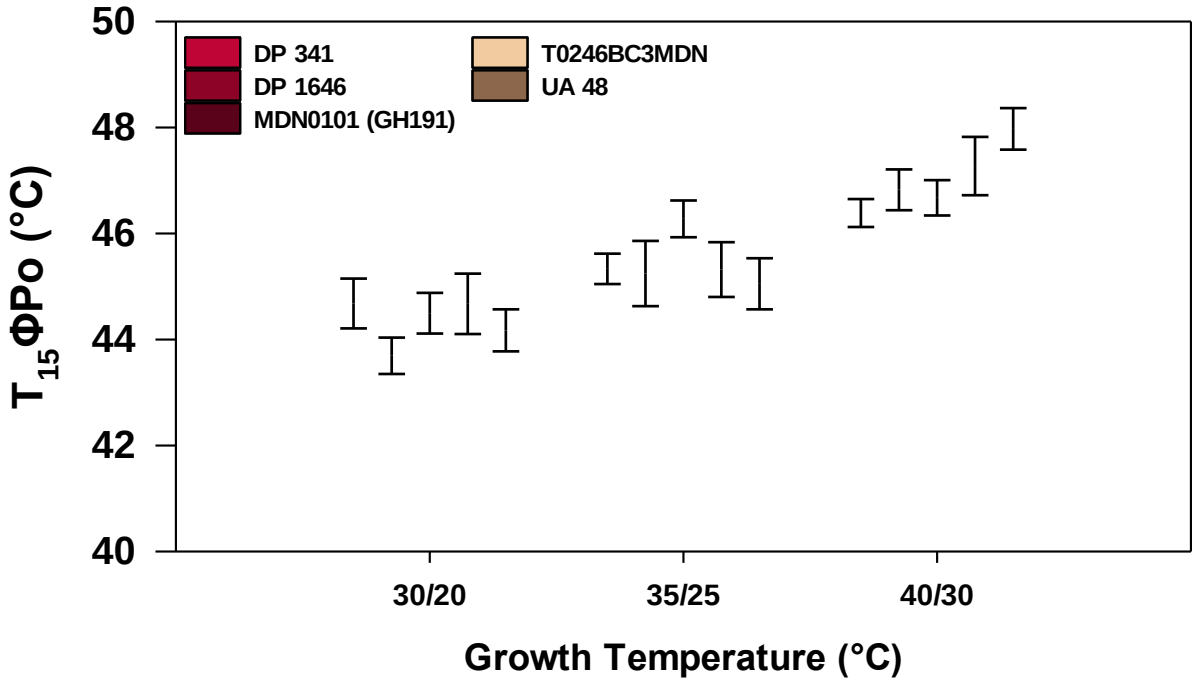

Figure 4.5. High temperature thresholds for energy trapping by Photosystem II [T_{15} (Φ_{Po})] of cotton seedlings at four weeks after planting for five cotton genotypes grown under three different growth temperature regimes. Values are means (n = 10) ± standard errors, and bars not sharing a common letter are significantly different ($p < 0.05$).

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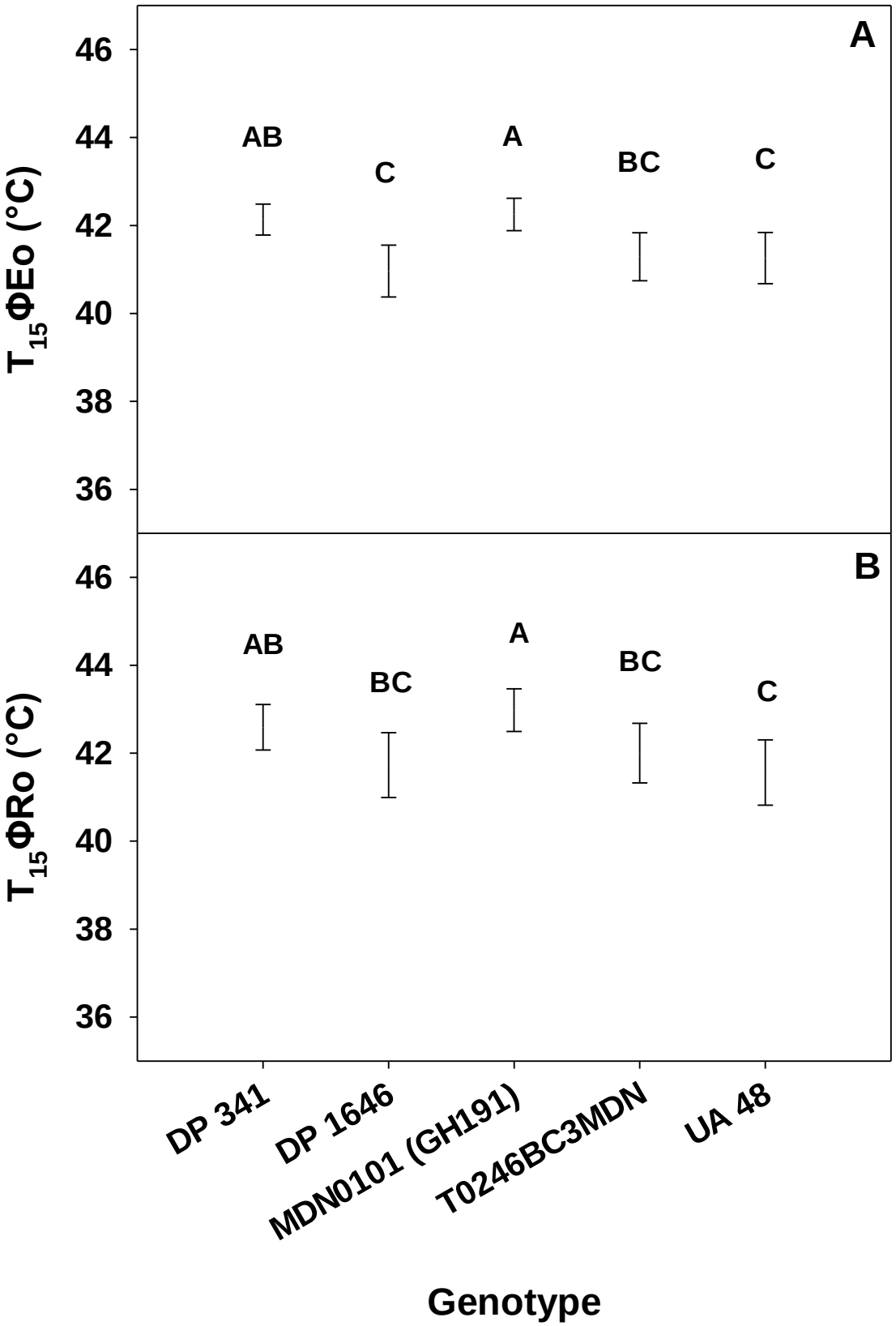
A
B
46
44
42
40
38
36
AB
C
A
BC
C
T_{15}ΦEo (°C)
T_{15}ΦRo (°C)
DP 341
DP 1646
MDN0101 (GH191)
T0246BC3MDN
UA 48
Genotype

Figure 4.6. High temperature thresholds for intersystem electron transport ($T_{15}\Phi_{Eo}$) (A) and photosystem I end electron acceptor reduction ($T_{15}\Phi_{Ro}$) (B) for cotton seedlings at four weeks after planting for five cotton genotypes. Values are means ± standard errors (n = 30; $p < 0.05$).

4.3.4. Correlation Analysis

Pairwise correlation analysis was performed in order to determine any existing associations between genotype-specific relative values under high temperature for mainstem nodes, leaf area, plant dry weight, A_N, ETR, R_D, g_{snight} and $T_{15}(\Phi_{Po})$. Significant associations were observed between leaf area and plant dry weight; ETR and A_N; R_D and leaf area; R_D and plant dry weight; g_{snight} and leaf area; g_{snight} and plant dry weight, and g_{snight} and $T_{15}(\Phi_{Po})$. Leaf area and plant dry weight were positively correlated with a correlation coefficient of r = 0.994 (p=0.0006), and ETR was positively correlated with A_N with a correlation coefficient of r = 0.929 (p=0.0225). In contrast, R_D and leaf area; R_D and plant dry weight; g_{snight} and leaf area; g_{snight} and plant dry weight; g_{snight} and $T_{15}(\Phi_{Po})$ were negatively correlated, with correlation coefficients ranging from r = -0891 (p=0.0425) to -0.956 (p=0.0111).

Table 4.2. Pearson product-moment correlation coefficients for relationships between number of mainstem nodes, leaf area per plant (LA), plant dry weight (DW), net photosynthesis (A_N), electron transport rate (ETR), nighttime respiration (R_D), nighttime stomatal conductance (g_{snight}) and high temperature thresholds for energy trapping by PS II ($T_{15}\Phi_{Po}$). Genotype means were determined across both experimental runs prior to the calculation of relative values under heat stress (40/30 °C relative to 30/20 °C) and correlation analysis.

	LA	DW	A_N	ETR	R_D	g_{snight}	$T_{15}\Phi_{Po}$
Nodes	0.671	0.711	0.142	-0.083	-0.543	-0.707	0.756
LA	--	0.994**	-0.007	-0.28	-0.949*	-0.944*	0.719
DW		--	0.013	-0.286	-0.939*	-0.956*	0.741
A_N			--	0.929*	-0.251	0.259	-0.501
ETR				--	0.024	0.542	-0.706
R_D					--	0.806	-0.47
g_{snight}						--	-0.891*

* Correlation is significant at $P < 0.05$

4.4. DISCUSSION

According to Burke and Wanjura (2010), 28 ± 3 °C is considered to be optimum for growth and development of the cotton crop. However, average daily temperatures can reach well above the optimum in many cotton growing regions around the world, limiting crop growth and negatively affecting lint yields (Gur et al., 2010). High temperatures have also been reported as important contributors to year-to-year yield variability in cotton yields for the US mid-south (Oosterhuis 2000). In such environments, cultivars identified as potentially heat tolerant would be advantageous. Genotypic variation in heat tolerance in cotton has been reported by multiple authors using different methods of evaluation, including membrane integrity assays, chlorophyll fluorescence measurements, and enzyme activity assays (Bibi et al., 2008; Snider et al., 2009; Cottee et al., 2010; Snider et al., 2010, 2011; Snider and Oosterhuis, 2011; Wu et al., 2014; Jaconis et al., 2021). However, minimal attention has been given to the varying ability of cotton cultivars to acclimate to heat stress conditions. The current study addresses the effects of growth temperature on plant growth, leaf-level physiology, and acclimation potential of photosynthetic

components of the thylakoid reactions for five cotton genotypes that were previously shown to differ in *in vitro* photosynthetic thermotolerance.

The first hypothesis tested was that the cotton genotypes evaluated in the current study would exhibit significant differences in growth-specific thermotolerance. Data presented in Table 4.1 and Figure 4.1 support our hypothesis. First, there was significant interaction between growth temperature and genotype for all growth parameters evaluated. The number of mainstem nodes produced by the end of the growth period responded positively to high growth temperature for all genotypes except for the Pima cultivar (DP 341). For Pima, the number of mainstem nodes peaked at the 35/25 °C temperature regime and declined significantly at the highest growth temperature. Thus, node development in Pima cotton was more heat sensitive than in upland cotton. Studies simultaneously comparing the heat sensitivity of early season growth in Pima and upland cotton are non-existent. However, Reddy et al. (1992a) observed that the rate of mainstem node addition in Pima at different growth temperatures was similar to the rates for upland cotton reported in another study by Reddy et al. (1991). Genotypic variation in the production of mainstem nodes among advanced upland cotton breeding lines under high temperature conditions has been reported previously (Ekinci et al., 2017; Virk et al., 2021). The increase in mainstem node production with high growth temperature illustrates the positive effect of temperature on rate of development in upland cotton and has been reported in previous studies (Reddy et al., 1992a,b; Virk et al., 2021).

Leaf area development and dry weight production was much more sensitive to high temperature than the production of mainstem nodes, where all genotypes showed a decrease in leaf area per plant with each successive increase in growth temperature. However, the magnitude of leaf area reduction from the optimum temperature to the highest temperature ranged from 58 % for UA 48 to 74 % for DP 341. Similar to our current study, it has been shown leaf area

development is one of the most heat sensitive processes in cotton. Reddy et al. (1992b) reported that leaf area of upland cotton plants decreased by 50% at 40 °C relative to plants grown at 30 °C. Similarly, in Pima cotton, leaf area decreased by 40% as the temperature increased from 31.3 °C to 35.5 °C (Reddy et al., 1993). These temperature-induced reductions in leaf area were accompanied by an increase in the number of mainstem nodes, indicating that individual leaf area was especially thermosensitive. Virk et al. (2021) also showed genotypic differences in leaf area under high temperature for 20 advanced breeding lines of upland cotton.

For plant dry weight, notable differences in thermotolerance were observed among the genotypes evaluated. For example, dry weight declined significantly at each increase in growth temperature for DP 341 and T0246BC3MDN, whereas the remaining cultivars only showed significant reductions in dry weight at the highest growth temperature regime. This shows that DP 341 and T0246BC3MDN were less heat tolerant than the other cultivars evaluated. Reductions in plant dry weight under high temperature conditions have been reported in previous studies (Reddy et al. 1992b; Reddy et al., 1993; Virk et al., 2021). Notable genotypic differences in the heat sensitivity of dry matter production have been documented among upland cotton genotypes (Ashraf et al., 1994; Virk et al., 2021). Virk et al. (2021) documented a peak in seedling dry matter production at a 35/25 °C growth temperature regime for upland cotton at two weeks after planting. Genotypic differences in early season biomass production between Pima and upland cotton have not been previously documented.

The second hypothesis tested was that differences in growth-specific thermotolerance would be associated with variation in single-leaf physiological processes and heat acclimation of thylakoid processes. The data presented in Table 4.1 and Figure 4.2 show that there was a significant interaction between genotype and temperature for A_N and ETR. However, under the

highest temperature regime, these two processes were higher than the values under optimum temperature conditions or equivalent to values at the optimum for all cultivars. Correlation analysis revealed that relative values for plant dry weight under heat stress were not significantly correlated with either photosynthetic parameter (Table 4.2). Therefore, photosynthetic activity per unit leaf area was not a likely contributor to heat-induced reductions in plant growth. In contrast, genotypic variation in relative leaf area under high temperature was strongly associated with relative dry weight ($r = 0.994$; Table 4.2). We conclude that individual leaf area represents a more important predictor of early season thermotolerance than photosynthetic activity per unit leaf area. In contrast with the current study, reductions in A_N and ETR have been observed previously when cotton plants or leaves were exposed to temperatures above 35 °C (Crafts-Brandner and Salvucci 2000; Salvucci and Crafts-Brandner, 2004; Wise et al., 2004; Bibi et al., 2008; Snider et al., 2009, 2010). However, previously published research documented photosynthetic response to rapid onset, short term high temperature exposure, whereas plants in the current study were exposed to high temperature since planting. We speculate that morphological changes, such as decreased individual leaf area (less leaf area, more leaf nodes; Figure 4.1), likely led to higher specific leaf weight and higher specific leaf nitrogen. For example, Virk et al. (2021) showed that leaf area-based chlorophyll content increased concomitant with a decline in leaf area under high temperature for upland cotton. Previous studies have shown that increases in the aforementioned leaf traits lead to more stable A_N under abiotic stresses such as drought and temperature extremes (McDonald and Paulsen 1997; Kitao and Lei 2006; Xu et al., 2008).

Because respiratory carbon losses at night are important contributors to heat induced reductions in growth and yield worldwide (Mohammed and Tarpley 2009; Bahuguna et al., 2017; Sadok and Jagadish, 2020), nocturnal respiration rates were also evaluated in the current study.

The most heat sensitive cultivars (lowest relative dry weight under high temperature) showed significant increases in R_D with every increase in growth temperature above the optimum (Figure 4.3). Loka and Oosterhuis (2010) observed similar trends where respiration increased by 21-39% when cotton plants were exposed to long-term high nighttime temperatures. The remaining, most heat tolerant, cultivars only showed increases in R_D at the highest growth temperature. Furthermore, genotypic variation in relative R_D under high temperature was strongly and negatively associated with relative dry weight. Previous studies have suggested that increased nighttime respiration under high night temperatures can cause a shortage of carbohydrates available to drive growth (Oosterhuis 1999; Loka and Oosterhuis 2010). This indicates that genotypes with more stable nighttime respiration in response to increasing temperature exhibit more thermotolerant growth. Previous studies documenting this response in cotton are, to our knowledge, non-existent. Another notable observation is that cultivars with higher g_{snight} also had higher R_D and showed the least heat tolerant growth (Figure 4.3; Table 4.2). This is particularly important because previous research conducted by Fish and Earl (2009) showed that g_{snight} was strongly and negatively correlated with whole-plant water use efficiency in cotton. As a result, heat sensitive cotton genotypes may be inherently less water use efficient under high temperature extremes.

Other notable plant responses that were affected by cultivar but did not exhibit a significant G x T interaction were daytime g_s, leaf minus air temperature differential, intrinsic leaf-level water use efficiency (iWUE), and F_v/F_m. Cultivars having the highest g_s values had the lowest $T_{leaf} - T_{air}$. This is because g_s regulates transpirational water loss and the ability of the plant to cool below air temperature, as has been observed in numerous studies previously in both Pima and Upland cotton (Lu et al., 1994; Roche 2015; Chastain et al., 2016). In the current study, UA 48 and

T0246BC3MDN had the highest daytime iWUE. F_v/F_m exhibited only small differences between genotypes (approximately one percent difference from the highest to the lowest values) and was unaffected by increasing growth temperature. Several authors have reported no declines in F_v/F_m under high temperature extremes that significantly limit photosynthesis (Law and Crafts-Brandner, 1999; Snider et al., 2010, 2013, 2015a,b).

Regarding thermotolerance acclimation of thylakoid components, a G × T interaction was observed only for thermotolerance of Photosystem II [T_{15} (Φ_{Po})]. Indicating that there are significant genotypic differences in the ability of photosystem II to acclimation to high temperature. A number of studies have documented the ability of PS II to acclimate to high temperatures (Gombos et al., 1994; Wise et al., 2004; Salvucci and Crafts-Brandner 2004; Haldimann and Feller 2005; Snider et al., 2013, 2015; Hu et al., 2018). We suggest that the ability of PSII to acclimate to high temperature is a contributor to the stability of F_v/F_m across all growth temperatures observed here (Figure 4.4D). The current study is the first to document genotypic variation in PSII acclimation to high temperature in cotton.

The increase in T_{15} values for Photosystem II from the optimum temperature to the highest temperature ranged from 1.7 °C for DP 341 to 3.8 °C for UA 48 (Figure 4.5), which is evidence for genotypic differences in high temperature acclimation of PSII. The Pima cultivar, showed the lowest increase and was among the genotypes that exhibited the lowest thermotolerance of PS II at the highest temperature. This cultivar also had the most heat sensitive growth responses (Figure 4.1), suggesting that acclimation potential of PSII might be an important factor contributing to early season differences in heat tolerance. However, T0246BC3MDN was among the cultivars that acclimated the most to high temperature, yet it had the most heat sensitive growth among all upland genotypes evaluated. Therefore, other traits may be more indicative of innate differences in heat

tolerance for upland cotton. Thermotolerance of intersystem electron transport and photosystem I end electron acceptor reduction [T_{15} (Φ_{Eo}) and T_{15} (Φ_{Ro})] were only significantly affected by genotype. MDN0101 (GH191) and DP 341 were the most heat tolerant genotypes and UA 48 was the least tolerant genotype in terms of thermotolerance of intersystem electron transport and photosystem I end electron acceptor reduction (Figure 4.6A and 4.6B). MDN0101 (GH191) was among the genotypes that showed the most thermotolerance for early season growth, whereas Pima was among the most heat sensitive in terms of growth (Figure 4.1). Furthermore, two of the upland genotypes, DP 1646 and UA 48, despite being among the least thermotolerant for intersystem electron transport and photosystem I end electron acceptor reduction, demonstrated the most heat tolerant growth (Figure 4.1). The differences in heat tolerance of these processes were not consistently associated with early season differences in heat tolerance of different genotypes.

4.5. CONCLUSIONS

The objective of this study was to assess the effects of growth temperature and genotype on plant growth, single-leaf physiology, and thermotolerance of thylakoid components for cotton exposed to optimal and supra-optimal temperature conditions. There was significant interaction between growth temperature and genotype for all growth parameters evaluated, and leaf area development and dry weight production were more sensitive to high temperature than the production of mainstem nodes. There was a significant interaction between genotype and temperature for A_N and ETR; however, there was no correlation between relative growth and photosynthetic parameters. There was also a significant interaction between genotype and temperature for R_D and g_{sdark}. The cultivars with higher g_{snight} also had higher R_D and showed the least heat tolerant growth. The remaining single-leaf responses including daytime g_s, T_{leaf} - T_{air}, iWUE, and F_v/F_m did not exhibit a significant G × T interaction but were significantly affected by

genotype. A G × T interaction was observed in thermotolerance of Photosystem II, indicating genotypic variation in the ability of photosystem II to acclimate to high temperature. Thermotolerance of intersystem electron transport and photosystem I end electron acceptor reduction was affected by genotype only. Thermotolerance of thylakoid components was not consistently associated with thermotolerant growth. Genotypes displaying heat-tolerant vegetative growth exhibited a strong and positive association with leaf area and a strong and negative association with nighttime respiration and stomatal conductance, suggesting that genotypes with higher relative leaf area and lower relative values for nocturnal respiration and stomatal conductance tend to demonstrate greater relative growth in high temperature conditions.

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CHAPTER 5

CONCLUSIONS

Three experiments were conducted including two field experiments and one controlled environment experiment. The objectives of the first experiment were to 1) assess genotypic variation in thermotolerance of thylakoid component processes for diverse cotton genotypes, 2) assess differences in heat tolerance of thylakoid component processes, and 3) quantify differences in thermotolerance plasticity of the most heat sensitive thylakoid component process in upland cotton genotypes. We conclude that significant genotypic variation in the thermotolerance of photosystem II, intersystem electron transport, and photosystem I was present in the genotypes evaluated. However, genotype rankings for thermotolerance varied according to the photosynthetic processes evaluated and site year. The differences in genotypic responses between the site-years might be a function of environmental variability. Photosystem II and photosystem I were found to be relatively heat tolerant, while intersystem electron transport was the most heat-sensitive process. Comparing slopes of upland cotton genotypes revealed significant differences in thermotolerance plasticity for intersystem electron transport. These observations led to the conclusion that the cultivars evaluated may differ in heat acclimation for thylakoid specific processes.

The objective of the second field experiment was to assess genotypic variation in physiological ($\sum$IPAR, RUE, and HI) and yield component contributors to yield in a diverse set of field-grown cotton genotypes. Lint yield, total biomass production, light interception by the canopy, and harvest index were affected by genotype, however, when all the genotypes were considered, no

significant associations were observed between lint yield and biomass production or light interception. In contrast, harvest index showed a positive association with lint yield and was identified as a better predictor of lint yield. We also observed that after a peak value for harvest index (HI = 0.325), a yield plateau is reached and there is no further increase in lint yield with increase in harvest index. Boll production and intra-boll yield components including lint percent, and lint weight per boll, were also influenced by genotype. Boll density played a significant role in determining lint yield and genotypes with lower boll densities also exhibited lower yields. Additionally, boll mass and lint percent also influenced lint yields and genotypes with lower boll mass and/or lint percent tended to have lower lint yields. Genotypes differed in their individual seed surface area, lint weight per seed, and the number of seeds per boll, and genotypes with highest lint yields tended to have more seeds per boll but there were differences in number of seeds per boll even among the highest yielding genotypes.

The last experiment was conducted as a controlled-environment study. The objective of this study was to assess the effects of growth temperature and genotype on plant growth, single-leaf physiology, and thermotolerance of thylakoid processes for cotton exposed to optimal and supra-optimal temperature conditions. Growth temperature and genotype showed significant interactions for various growth parameters, with leaf area and dry weight production being more sensitive to high temperature. Interaction between growth temperature and genotype also influenced net photosynthesis, electron transport rate, nighttime respiration, and nighttime stomatal conductance. No interactions were found between photosynthetic parameters and growth characteristics. There was a strong positive relationship between relative plant dry weight and leaf area under high temperature, indicating that genotypes with heat-tolerant vegetative growth tend to have a higher relative leaf area. Conversely, there was a strong negative correlation between relative growth

under high temperature and nighttime respiration and stomatal conductance, suggesting that genotypes with heat tolerant vegetative growth exhibit lower relative values for nocturnal respiration and stomatal conductance under high temperature conditions. Genotypic differences were observed in the ability of photosystem II to acclimate to high temperature conditions, while thermotolerance of intersystem electron transport and photosystem I end electron acceptor reduction was only affected by genotype. Genotypes with highest photosynthetic thermotolerance were different in their acclimation ability in terms of growth. Variation in the heat tolerance of these thylakoid component processes may have a substantial impact on differences observed in early season heat tolerance, making them a valuable indicator of a plant's growth during the initial stages under high temperature conditions.

Lastly, these experiments highlight the importance of understanding genotypic variation in thermotolerance, heat acclimation potential, and yield-determining traits of diverse cotton genotypes. Identifying the most heat-sensitive processes and genotypes with higher acclimation abilities can aid in breeding and selecting cotton varieties with improved tolerance to heat stress. Deepening our knowledge of genotypic variation in yield-determining processes and yield components could facilitate in selecting and breeding cotton varieties with improved lint yields in the future.