

Variation in thermotolerance of photosystem II energy trapping, intersystem electron transport, and photosystem I electron acceptor reduction for diverse cotton genotypes

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ARTICLE INFO

Handling Editor: Dr K Kees Venema

Keywords:

Gossypium hirsutum
Gossypium barbadense
 Thermotolerance
 Photosynthesis
 Thylakoid reactions

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_{P_0}), intersystem electron transport (Φ_{E_0}), and photosystem I end electron acceptor reduction (Φ_{R_0}). Significant genotypic variation in photosynthetic thermotolerance was observed, but the response was dependent on location and photosynthetic parameter assessed. Φ_{E_0} was substantially more heat sensitive than Φ_{P_0} or Φ_{R_0} . Significant genotypic variation in thermotolerance plasticity of Φ_{E_0} 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.

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; Bitá 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,

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<https://doi.org/10.1016/j.plaphy.2023.107868>

Received 28 April 2023; Received in revised form 21 June 2023; Accepted 26 June 2023

Available online 28 June 2023

0981-9428/© 2023 Published by Elsevier Masson SAS.

respectively, than for seedlings grown at 32 °C. Reddy et al. (1992a,b) 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., 2010a,b; 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., 2007). 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) (Hejník et al., 2015; Van der Westhuizen et al., 2020). However, Agarwal and Jajoo (2021) reported that PS II recovered within 10 min 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 CO₂ assimilation (Law and Crafts-Brandner, 1999; Snider et al., 2010, 2013, 2015a, 2015b). 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, 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) (Gür 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_{P_0}), of inter-photosystem electron transport (Φ_{E_0}), of PSI end electron acceptor reduction (Φ_{R_0}), 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 *Triticum aestivum* (wheat). Chen et al. (2016) documented differences in thermotolerance plasticity among *Ageratina adenophora* (croftonweed) 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. Materials and methods

2.1. 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 the 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 US, where it is not unusual for daytime temperatures to exceed 42 °C during the summer months.

2.2. 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 1.

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 have 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 min to 1 h, likely because this represents the minimum amount of time required for all reaction centers to be in the open state (Jedrowski and Brüggemann, 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 ~1 cm 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; Lazár and Ilík, 1997; Froux et al., 2004; Burke, 1990; Gimeno et al., 2009). Leaf segments were first incubated at 30 °C for 6 min 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 6 min 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 2–30 min and assessed F_v/F_m every 2 min. It was observed that incubation times longer than 4 min 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 (5 min or less at each temperature) are commonly used for T₁₅ 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

Table 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

Table 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	$\frac{F_m - F_i}{F_m}$

genotypes, so variation in T_{15} accurately reflects relative differences in heat tolerance among processes, genotypes, or environments.

2.3. 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 ($3500 \mu\text{mol m}^{-2} \text{s}^{-1}$), 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 (Φ_{Po}), quantum yield of electron transport between photosystem II and photosystem I (Φ_{Eo}), and quantum yield of photosystem I end electron acceptor reduction (Φ_{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 assess 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).

2.4. 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. 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} ; Fig. 1). The T_{15} approach to assess heat tolerance is a widely utilized method in plant ecophysiology (Froux et al., 2004; Gimeno et al., 2009; Snider et al., 2010a,b; Snider et al., 2015). Thereafter, a mixed-effects analysis of

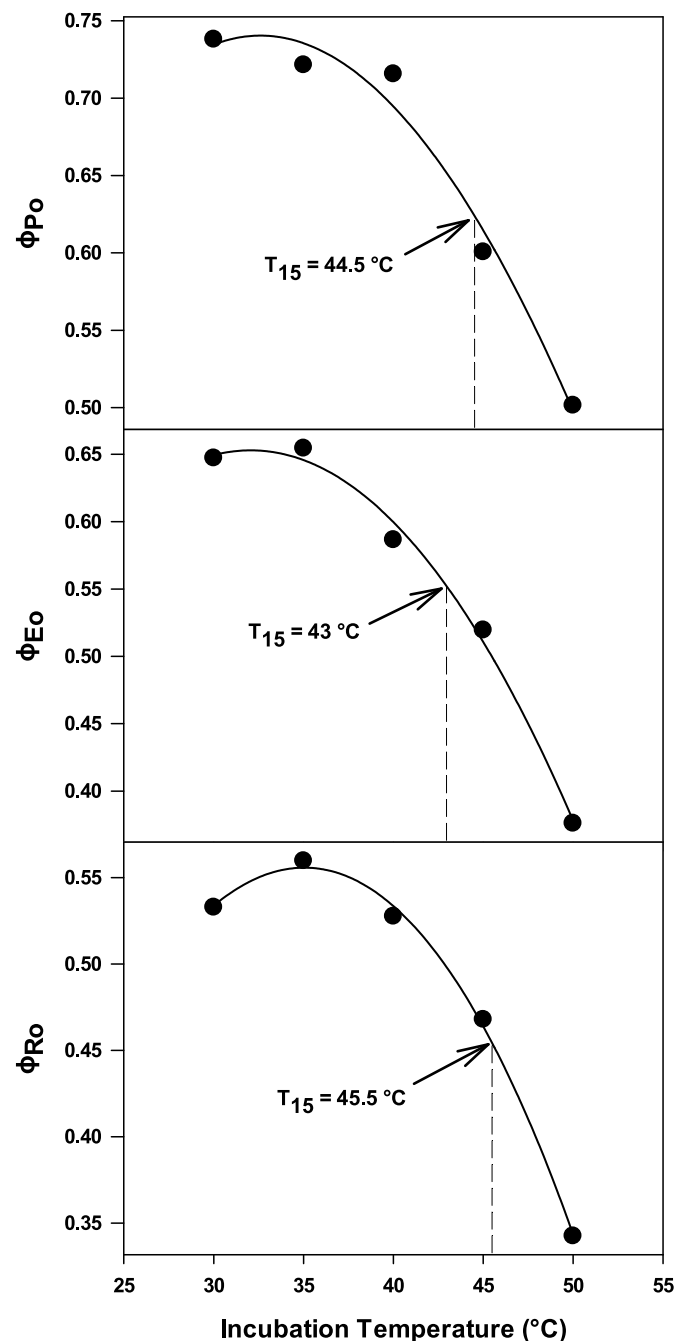


Fig. 1. An example graph illustrating how T_{15} was calculated for a single leaf and three different quantum efficiencies (defined in Table 2). A third-order polynomial function was fit to the quantum efficiency x incubation temperature data and the temperature causing a 15% decline in efficiency relative to 30 °C was interpolated from the resulting function. T_{15} is indicated with a vertical dashed line.

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 assess 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 $\times$ 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.

3. Results

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 Figs. 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 (Fig. 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 (Fig. 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 (0.678–0.452 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 (Fig. 2C) and at 30, 35, 40 and 50 °C in Tifton (Fig. 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 (Fig. 3A). For the Tifton location in 2020, the genotypes were significantly different in their Φ_{Eo} at all the incubation temperatures (Fig. 3B). MDN0101 (GH191), T0246BC3MDN, and DG 3615 were the genotypes

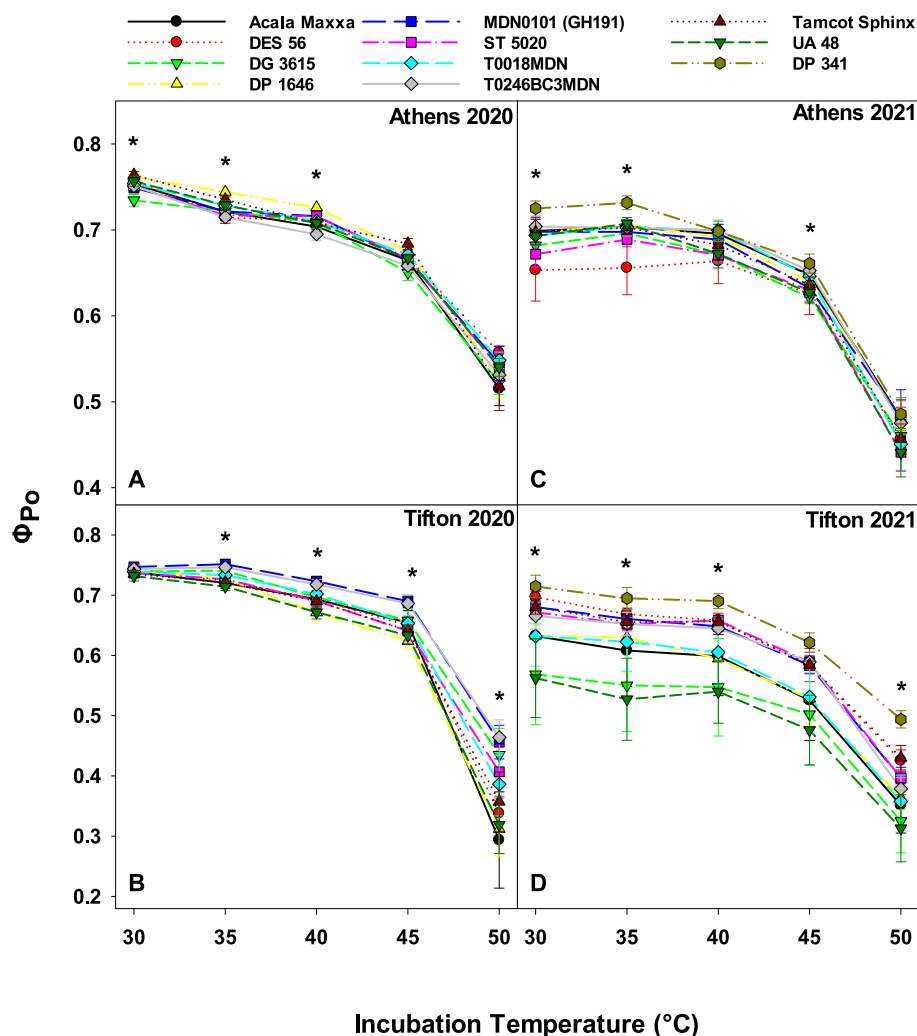


Fig. 2. The response of maximum quantum yield of energy trapping by photosystem II (Φ_{Po}) to increasing incubation temperatures for 10 cotton genotypes in 2020 (A and B) and 11 genotypes in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Each data point represents the means $\pm$ standard error of eight replications, and asterisks indicate a significant genotype effect at a given incubation temperature.

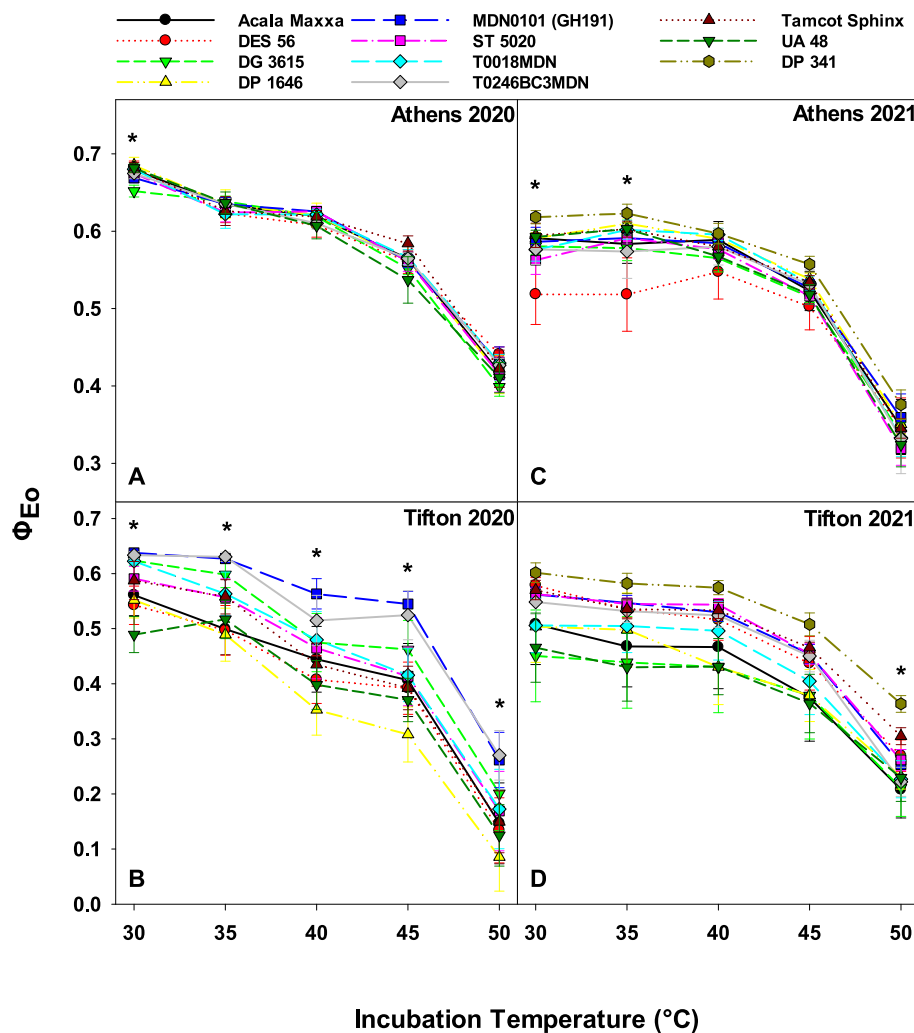


Fig. 3. The response of maximum quantum yield of electron transport between photosystem II and photosystem I (Φ_{Eo}) to increasing incubation temperatures for 10 cotton genotypes in 2020 (A and B) and 11 genotypes in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Each data point represents the means $\pm$ standard error of eight replications, and asterisks indicate a significant genotype effect at a given incubation temperature.

that exhibited consistently the highest Φ_{Eo} values at 45 and 50 °C (0.678–0.452). The lowest Φ_{Eo} values were exhibited by DP 1646, UA 48, DES 56, Tamcot Sphinx and Acala Maxxa at 45 and 50 °C (0.374–0.128) in 2020 at Tifton location. In 2021, significant differences were observed at 30 and 35 °C in Athens (Fig. 3C) and only at 50 °C in Tifton (Fig. 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 (Fig. 4A), whereas the genotypes were significantly different in their Φ_{Ro} at 40, 45 and 50 °C at the Tifton location in 2020 (Fig. 4B). At 45 and 50 °C, MDN0101 (GH191), T0246BC3MDN, and DG 3615 were the genotypes that exhibited consistently the highest Φ_{Ro} values (0.505–0.297 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 (Fig. 4C) and only at 50 °C in Tifton (Fig. 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.

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 Figs. 5–7 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 (Fig. 5A and B). 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 (Fig. 5C). However, thermotolerance of Φ_{Po} was significantly affected by genotype in 2021 at Tifton (Fig. 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 (Fig. 6A and C) or in 2020 at Tifton (Fig. 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 (Fig. 6D). DP 341, MDN0101 (GH191), T0246BC3MDN and Acala Maxxa (average T_{15} = 43.1 °C)

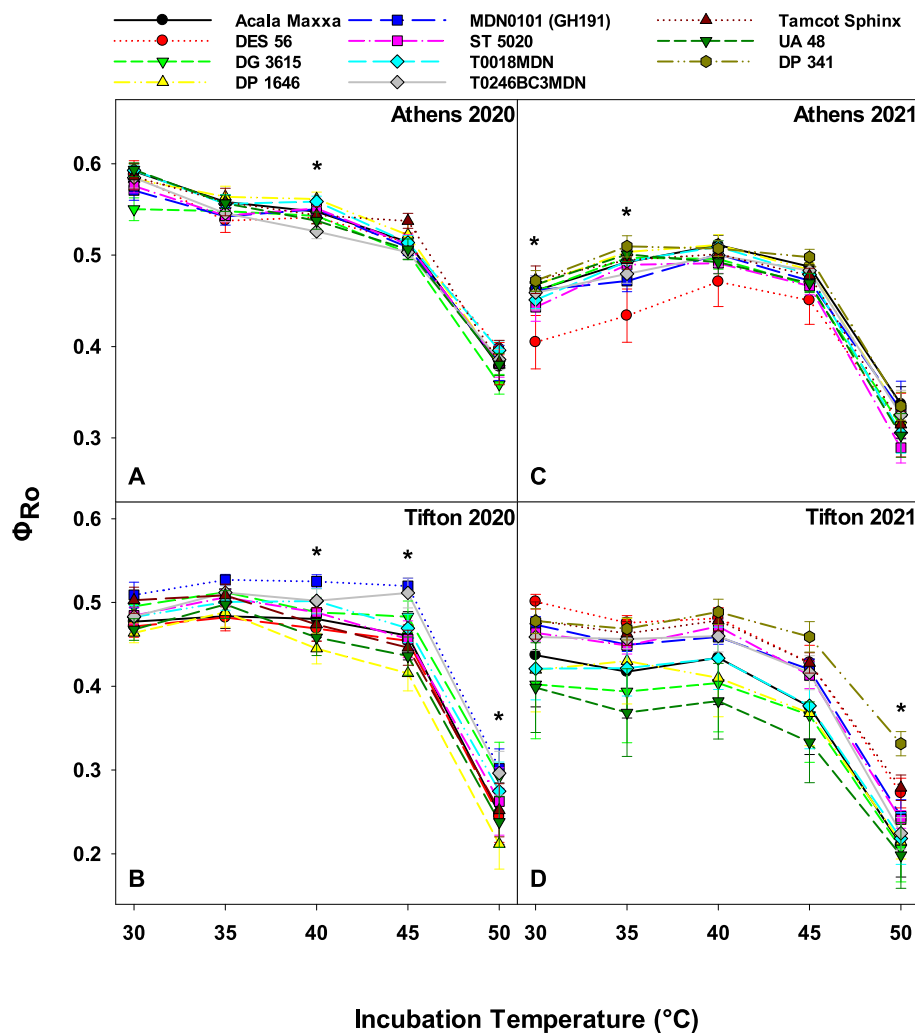


Fig. 4. The response of maximum quantum yield of photosystem I end electron acceptor reduction (Φ_{Ro}) to increasing incubation temperatures for 10 cotton genotypes in 2020 (A and B) and 11 genotypes in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Each data point represents the means $\pm$ standard error of eight replications, and asterisks indicate a significant genotype effect at a given incubation temperature.

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 (Fig. 7A and C) or for the 2021 season at Tifton (Fig. 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 (Fig. 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 (Fig. 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.

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 (Fig. 9). 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).

4. Discussion

Climate change is expected to increase the duration, severity, and intensity of heat wave events, which will likely have negative

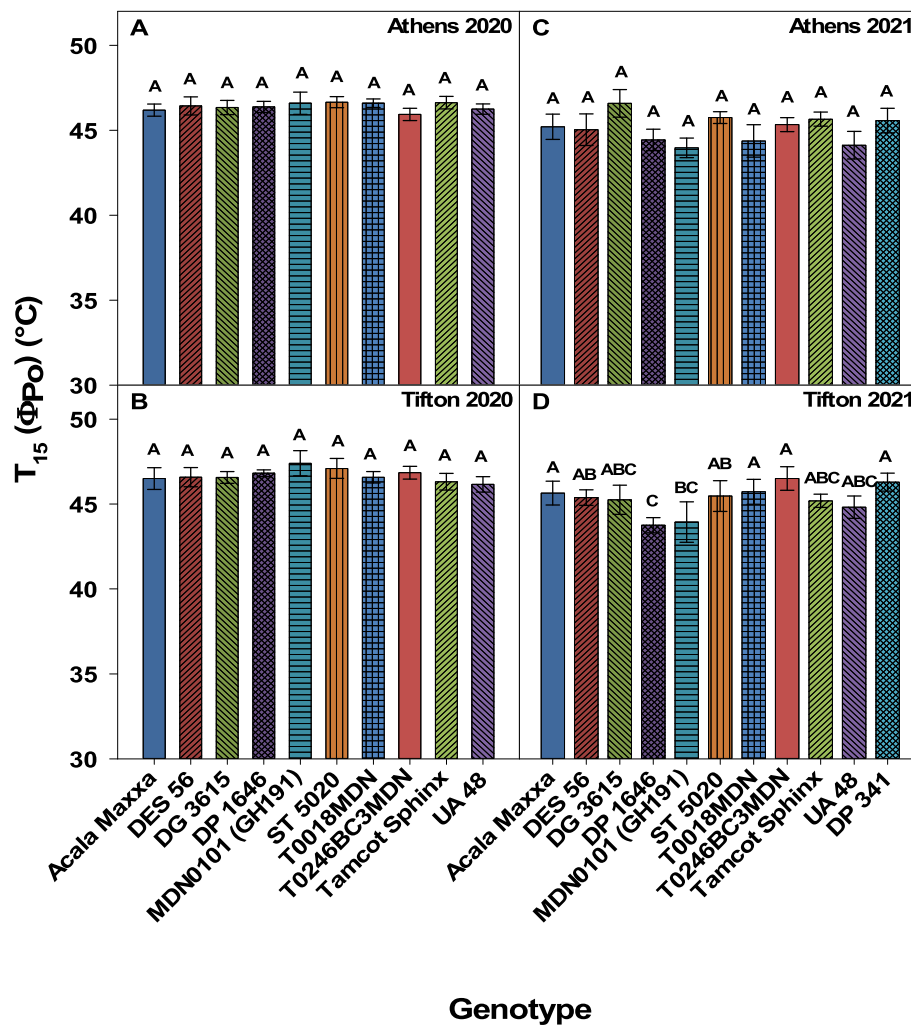


Fig. 5. High temperature thresholds (T_{15}) for the efficiency of energy trapping by Photosystem II [$T_{15} (\Phi_{Po})$] for 10 diverse cotton genotypes in 2020 (A and B) and 2021 (C and D) at field sites in Athens and Tifton, Georgia. Data are means $\pm$ standard error ($n = 8$) and bars not sharing a common letter within a given site-year are significantly different.

implications for crop production. For cotton, high temperature reduces mainstem growth, leaf area (Reddy et al., 1992a,b), and photosynthetic rates (Cottee et al., 2010; Snider et al., 2010a,b). 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., 2010a,b, 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., 2010a,b, 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 (Figs. 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 (Figs. 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 $T_{15} (\Phi_{Po})$ and $T_{15} (\Phi_{Eo})$, genotypic differences were only observed for T_{15} in 2021

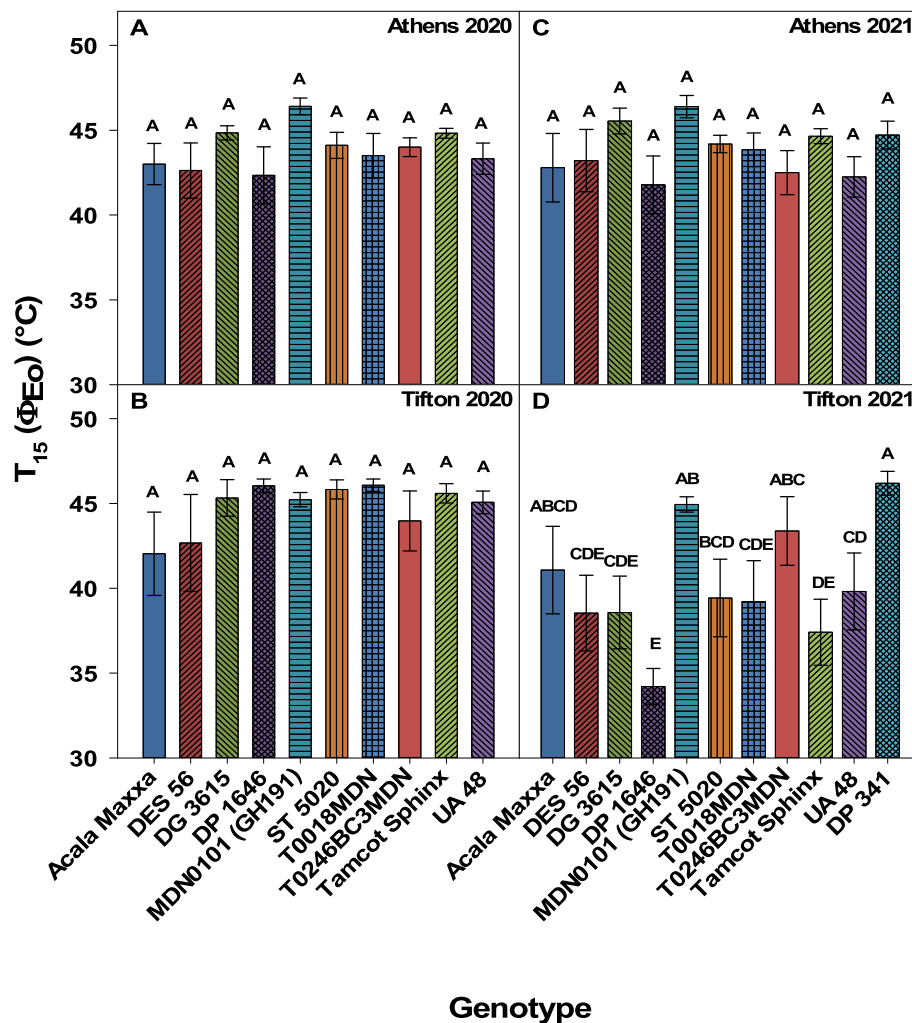


Fig. 6. High temperature thresholds (T_{15}) for the efficiency of intersystem electron transport [$T_{15}(\Phi_{E0})$] for 10 diverse cotton genotypes in 2020 (A and B) and 2021 (C and D) at field sites in Athens and Tifton, Georgia. Data are means $\pm$ standard error ($n = 8$) and bars not sharing a common letter within a given site-year are significantly different.

at the Tifton location (Figs. 5–6), where DP 341, T0246BC3MDN and Acala Maxxa exhibited the highest T_{15} values for both these parameters. For $T_{15}\Phi_{R0}$, differences were only observed in 2020 at the Tifton location (Fig. 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 [$T_{15}(\Phi_{P0})$] was 2.2 $^{\circ}\text{C}$ whereas the difference was 5.5 $^{\circ}\text{C}$ for intersystem electron transport [$T_{15}(\Phi_{E0})$] and just 1.4 $^{\circ}\text{C}$ for photosystem I end electron acceptor reduction [$T_{15}(\Phi_{R0})$]. 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., 2008; 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., 2010a,b, 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 ($r = 0.536$). The 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,

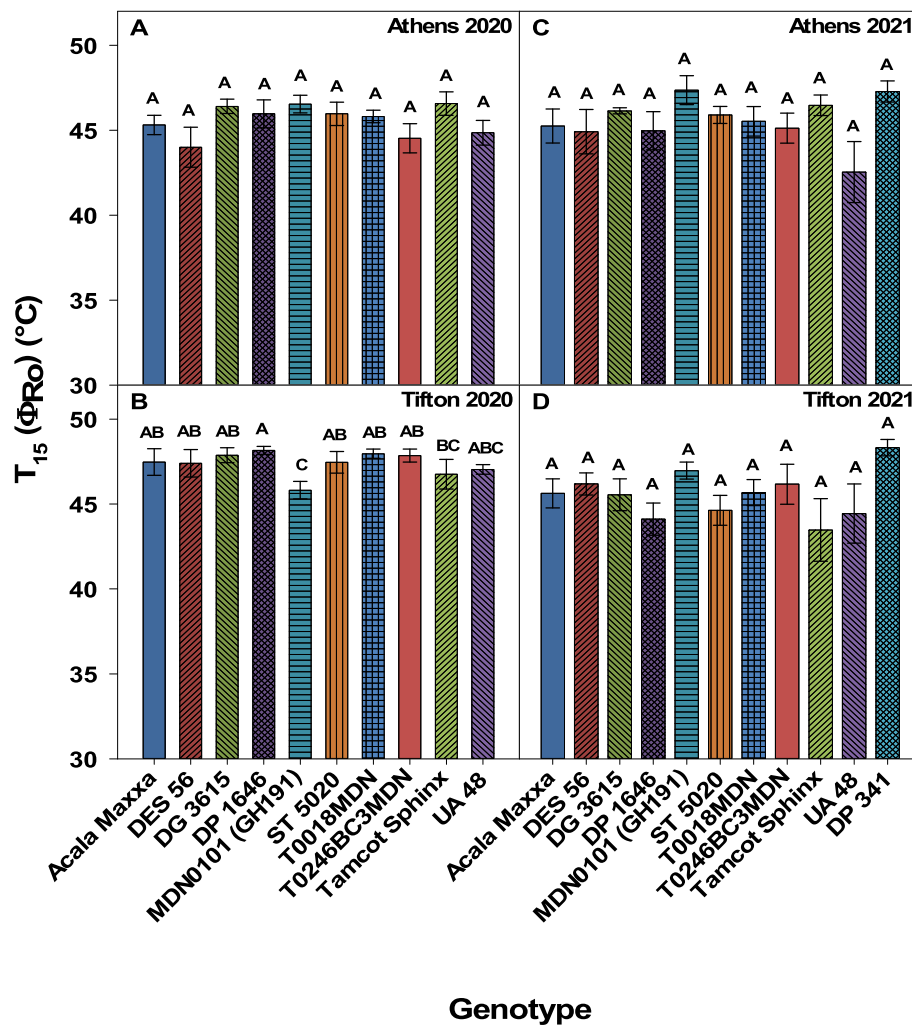


Fig. 7. High temperature thresholds (T_{15}) for the efficiency of photosystem I end electron acceptor reduction [$T_{15} (\Phi_{Ro})$] for 10 diverse cotton genotypes in 2020 (A and B) and 11 in 2021 (C and D) at field sites in Athens and Tifton, Georgia. Data are means $\pm$ standard error ($n = 8$) and bars not sharing a common letter within a given site-year are significantly different.

USA has shown that T_{15} values for PSII 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 tolerance 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, Fig. 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 (Fig. 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

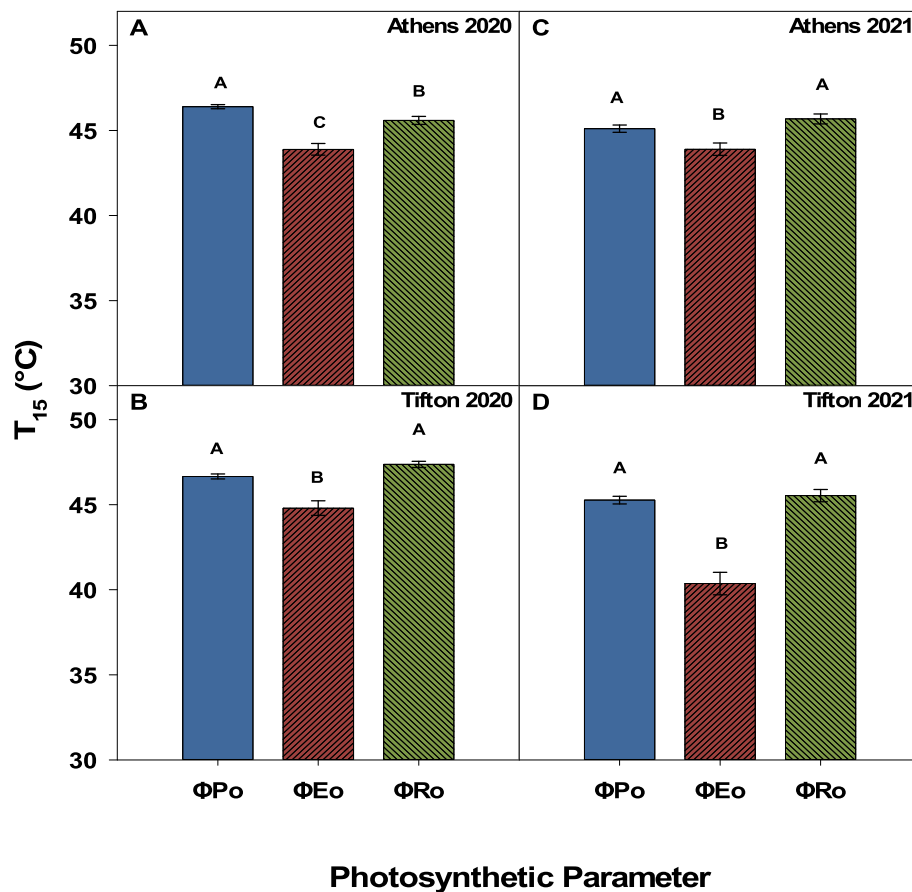


Fig. 8. High temperature thresholds (T_{15}) for the efficiency of photosynthetic parameters (Φ_{Po} , Φ_{Eo} and Φ_{Ro}) at both locations and years. Data are means $\pm$ standard error [$n = 80$ in 2020 (A and B); $n = 88$ in 2021 (C and D)] and bars not sharing a common letter within a given site-year are significantly different. Means were generated by combining data across all genotypes and replicates within a given site year.

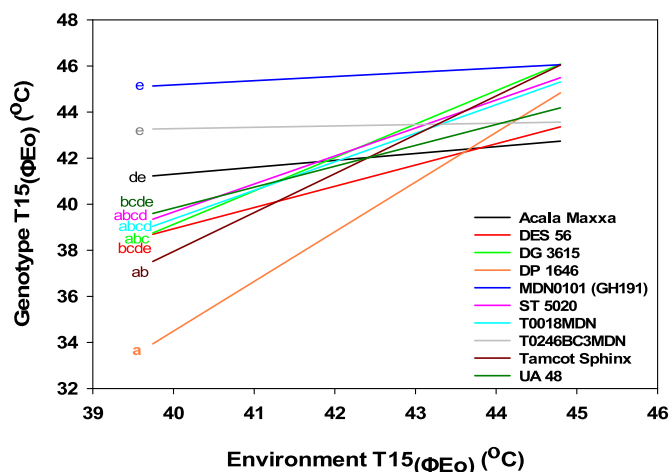


Fig. 9. Genotype mean $T_{15}(\Phi_{Eo})$ for 10 upland cotton genotypes versus the environment mean $T_{15}(\Phi_{Eo})$ value of all genotypes within a given site year. Lines represent linear functions and those lines not sharing a common letter exhibit significantly different slopes.

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_{Eo} 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).

5. Conclusions

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 and 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.

CRediT authorship contribution statement

Navneet Kaur: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **John L. Snider:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **Andrew H. Paterson:** Resources, Writing – review & editing. **Timothy L. Grey:** Resources, Writing – review & editing. **Changying Li:** Resources, Writing – review & editing. **Gurpreet Virk:** Investigation, Writing – review & editing. **Ved Parkash:** Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: John Snider reports financial support was provided by Georgia Cotton Commission. John Snider reports financial support was provided by National Science Foundation.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to acknowledge the Georgia Cotton Commission (Project number 18–148 GA), and the National Science Foundation (Award number 1934481) for their financial support for this research. We would also like to thank Lola Sexton, Will Vance and the Cotton Physiology team members.

References

Ashley, D.A., 1972. C-labelled photosynthate translocation and utilization in cotton plants 1. *Crop Sci.* 12 (1), 69–74.

- Azhar, F.M., Ali, Z., Akhtar, M.M., Khan, A.A., Trethowan, R., 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). *Plant Breed.* 128 (4), 356–362.
- Berry, J., Björkman, O., 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.* 31 (1), 491–543.
- Bibi, A.C., Oosterhuis, D.M., Gonias, E.D., 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. *J. Cotton Sci.*
- Bitá, C.E., Gerats, T., 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci.* 4, 273.
- Bordignon, L., Faria, A.P., França, M.G., Fernandes, G.W., 2019. Osmotic stress at membrane level and photosystem II activity in two C4 plants after growth in elevated CO₂ and temperature. *Ann. Appl. Biol.* 174 (2), 113–122.
- Bourland, F.M., Jones, D.C., 2012. Registration of ‘UA48’ cotton cultivar. *J. Plant Registrations* 6 (1), 15–18.
- Brestic, M., Zivcak, M., Kalaji, H.M., Carpentier, R., Allakhverdiev, S.I., 2012. Photosystem II thermostability in situ: environmentally induced acclimation and genotype-specific reactions in *Triticum aestivum* L. *Plant Physiol. Biochem.* 57, 93–105.
- Burke, J.J., 1990. Variation among species in the temperature dependence of the reappearance of variable fluorescence following illumination. *Plant Physiol.* 93 (2), 652–656.
- Burke, J.J., Wanjura, D.F., 2010. Plant Responses to Temperature Extremes. In *Physiology of Cotton*. Springer, Dordrecht, pp. 123–128.
- Bussotti, F., Gerosa, G., Digrado, A., Pollastrini, M., 2020. Selection of chlorophyll fluorescence parameters as indicators of photosynthetic efficiency in large scale plant ecological studies. *Ecol. Indic.* 108, 105686.
- Chastain, D.R., Snider, J.L., Choinski, J.S., Collins, G.D., Perry, C.D., Whitaker, J., et al., 2016. Leaf ontogeny strongly influences photosynthetic tolerance to drought and high temperature in *Gossypium hirsutum*. *J. Plant Physiol.* 199, 18–28.
- Chen, S., Yang, J., Zhang, M., Strasser, R.J., Qiang, S., 2016. Classification and characteristics of heat tolerance in *Ageratina adenophora* populations using fast chlorophyll a fluorescence rise OJIP. *Environ. Exp. Bot.* 122, 126–140.
- Constable, G.A., Rawson, H.M., 1980. Effect of leaf position, expansion and age on photosynthesis, transpiration and water use efficiency of cotton. *Funct. Plant Biol.* 7 (1), 89–100.
- Constable, G.A., Reid, P.E., Thomson, N.J., 2001. Approaches Utilized in Breeding and Development of Cotton Cultivars in Australia.
- Cottee, N.S., Tan, D.K.Y., Bange, M.P., Cothren, J.T., Campbell, L.C., 2010. Multi-level determination of heat tolerance in cotton (*Gossypium hirsutum* L.) under field conditions. *Crop Sci.* 50 (6), 2553–2564.
- Crafts-Brandner, S.J., Salvucci, M.E., 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO₂. *Proc. Natl. Acad. Sci. USA* 97 (24), 13430–13435.
- El-Zik, K.M., Thaxton, P.M., 1996. Registration of ‘Tamcot sphinx’ cotton. *Crop Sci.* 36 (4), 1074–1074.
- Epron, D., 1997. Effects of drought on photosynthesis and on the thermotolerance of photosystem II in seedlings of cedar (*Cedrus atlantica* and *C. libani*). *J. Exp. Bot.* 48 (10), 1835–1841.
- Fan, Q., Jespersen, D., 2023. Assessing heat tolerance in creeping bentgrass lines based on physiological responses. *Plants* 12 (1), 41.
- Feller, U., Crafts-Brandner, S.J., Salvucci, M.E., 1998. Moderately high temperatures inhibit ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. *Plant Physiol.* 116 (2), 539–546.
- Froux, F., Ducrey, M., Epron, D., Dreyer, E., 2004. Seasonal variations and acclimation potential of the thermostability of photochemistry in four Mediterranean conifers. *Ann. For. Sci.* 61 (3), 235–241.
- Gimeno, T.E., Pias, B., Lemos-Filho, J.P., Valladares, F., 2009. Plasticity and stress tolerance override local adaptation in the responses of Mediterranean holm oak seedlings to drought and cold. *Tree Physiol.* 29 (1), 87–98.
- Gombos, Z., Wada, H., Murata, N., 1994. The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane lipids: a mechanism of chilling tolerance. *Proc. Natl. Acad. Sci. USA* 91 (19), 8787–8791.
- Gupta, R., 2019. Tissue specific disruption of photosynthetic electron transport rate in pigeonpea (*Cajanus cajan* L.) under elevated temperature. *Plant Signal. Behav.* 14 (6), 1601952.
- Gür, A., Demirel, U., Özden, M., Kahraman, A., Çopur, O., 2010. Diurnal gradual heat stress affects antioxidant enzymes, proline accumulation and some physiological components in cotton (*Gossypium hirsutum* L.). *Afr. J. Biotechnol.* 9 (7), 1008–1015.
- Haldimann, P., Feller, U.R.S., 2005. Growth at moderately elevated temperature alters the physiological response of the photosynthetic apparatus to heat stress in pea (*Pisum sativum* L.) leaves. *Plant Cell Environ.* 28 (3), 302–317.
- Havaux, M., 1992. Stress tolerance of photosystem II in vivo: antagonistic effects of water, heat, and photoinhibition stresses. *Plant Physiol.* 100 (1), 424–432.
- Hejnak, V., Tatar, Ö., Atasoy, G.D., Martinková, J., Çelen, A.E., Hnilická, F., Skalický, M., 2015. Growth and photosynthesis of Upland and Pima cotton: response to drought and heat stress. *Plant Soil Environ.* 61 (11), 507–514.
- Hodges, H.F., Reddy, K.R., McKinnon, J.M., Reddy, V.R., 1993. Temperature Effects on Cotton. Mississippi Agri. & Forestry Exp.Sta. Mississippi State University, Miss.
- Hu, L., Bi, A., Hu, Z., Amombo, E., Li, H., Fu, J., 2018. Antioxidant metabolism, photosystem II, and fatty acid composition of two tall fescue genotypes with different heat tolerance under high temperature stress. *Front. Plant Sci.* 9, 1242.
- Jedemowski, C., Brüggemann, W., 2015. Imaging of fast chlorophyll fluorescence induction curve (OJIP) parameters, applied in a screening study with wild barley

- (*Hordeum spontaneum*) genotypes under heat stress. *J. Photochem. Photobiol. B Biol.* 151, 153–160.
- Jiang, Y., Li, C., Robertson, J.S., Sun, S., Xu, R., Paterson, A.H., 2018. GPhenoVision: a ground mobile system with multi-modal imaging for field-based high throughput phenotyping of cotton. *Sci. Rep.* 8 (1), 1–15.
- Kalaji, H.M., Schansker, G., Ladle, R.J., Goltsev, V., Bosa, K., Allakhverdiev, S.I., et al., 2014. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynth. Res.* 122, 121–158.
- Khan, N., Essemine, J., Hamdani, S., Qu, M., Lyu, M.J.A., Perveen, S., et al., 2021. Natural variation in the fast phase of chlorophyll a fluorescence induction curve (OJIP) in a global rice minicore panel. *Photosynth. Res.* 150, 137–158.
- Knight, C.A., Ackerly, D.D., 2002. An ecological and evolutionary analysis of photosynthetic thermotolerance using the temperature-dependent increase in fluorescence. *Oecologia* 130 (4), 505–514.
- Koller, S., Holland, V., Brüggemann, W., 2020. Seasonal monitoring of PSII functionality and relative chlorophyll content on a field site in two consecutive years: a case study of different oak species. *Photosynthetica* 58, 379–390.
- Ladjal, M., Epron, D., Ducrey, M., 2000. Effects of drought preconditioning on thermotolerance of photosystem II and susceptibility of photosynthesis to heat stress in cedar seedlings. *Tree Physiol.* 20 (18), 1235–1241.
- Law, R.D., Crafts-Brandner, S.J., 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1, 5-bisphosphate carboxylase/oxygenase. *Plant Physiol.* 120 (1), 173–182.
- Lázár, D., Ilík, P., 1997. High-temperature induced chlorophyll fluorescence changes in barley leaves: comparison of the critical temperatures determined from fluorescence induction and from fluorescence temperature curve. *Plant Sci.* 124 (2), 159–164.
- Lewis, H., May, L., Bourland, F., 2000. Cotton yield components and yield stability. In: *In2000 Proceedings Beltwide Cotton Conferences*, vol. 1. National Cotton Council, San Antonio, USA, pp. 532–536, 4–8 January, 2000.
- Liu, Z., Yuan, Y.L., Liu, S.Q., Yu, X.N., Rao, L.Q., 2006. Screening for high-temperature tolerant cotton cultivars by testing in vitro pollen germination, pollen tube growth and boll retention. *J. Integr. Plant Biol.* 48 (6), 706–714.
- Loka, D.A., Oosterhuis, D.M., 2010. Effect of high night temperatures on cotton respiration, ATP levels and carbohydrate content. *Environ. Exp. Bot.* 68 (3), 258–263.
- Mathur, S., Agrawal, D., Jajoo, A., 2014. Photosynthesis: response to high temperature stress. *J. Photochem. Photobiol. B Biol.* 137, 116–126.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51 (345), 659–668.
- Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305 (5686), 994–997.
- Mishra, K.B., Mishra, A., Novotná, K., Rapantová, B., Hodaňová, P., Urban, O., Klem, K., 2016. Chlorophyll a fluorescence, under half of the adaptive growth-irradiance, for high-throughput sensing of leaf-water deficit in *Arabidopsis thaliana* accessions. *Plant Methods* 12 (1), 1–17.
- Moffatt, J.M., Sears, R.G., Paulsen, G.M., 1990. Wheat high temperature tolerance during reproductive growth. I. Evaluation by chlorophyll fluorescence. *Crop Sci.* 30 (4), 881–885.
- Murata, N., Takahashi, S., Nishiyama, Y., Allakhverdiev, S.I., 2007. Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta, Bioenerg.* 1767 (6), 414–421.
- Nabi, G., Mullins, C.E., 2008. Soil temperature dependent growth of cotton seedlings before emergence. *Pedosphere* 18 (1), 54–59.
- Oosterhuis, D.M., 2002. Day or night high temperatures: a major cause of yield variability. *Cotton Grow.* 46, 8–9.
- Oukarroum, A., Schansker, G., Strasser, R.J., 2009. Drought stress effects on photosystem I content and photosystem II thermotolerance analyzed using Chl a fluorescence kinetics in barley varieties differing in their drought tolerance. *Physiol. Plantarum* 137 (2), 188–199.
- Paterson, A.H., Boman, R.K., Brown, S.M., Chee, P.W., Gannaway, J.R., Gingle, A.R., et al., 2004. Reducing the genetic vulnerability of cotton. *Crop Sci.* 44 (6), 1900–1901.
- Pettigrew, W.T., 2008. The effect of higher temperatures on cotton lint yield production and fiber quality. *Crop Sci.* 48 (1), 278–285.
- Reddy, K.R., Davidonis, G.H., Johnson, A.S., Vinyard, B.T., 1999. Temperature regime and carbon dioxide enrichment alter cotton boll development and fiber properties. *Agron. J.* 91 (5), 851–858.
- Reddy, K.R., Hodges, H.F., McKinion, J.M., 1995. Carbon dioxide and temperature effects on pima cotton development. *Agron. J.* 87 (5), 820–826.
- Reddy, K.R., Hodges, H.F., Reddy, V.R., 1992a. Temperature effects on cotton fruit retention. *Agron. J.* 84 (1), 26–30.
- Reddy, K.R., Reddy, V.R., Hodges, H.F., 1992b. Temperature effects on early season cotton growth and development. *Agron. J.* 84 (2), 229–237.
- Salvucci, M.E., Crafts-Brandner, S.J., 2004. Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. *Plant Physiol.* 134 (4), 1460–1470.
- Schrader, S.M., Wise, R.R., Wacholtz, W.F., Ort, D.R., Sharkey, T.D., 2004. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. *Plant Cell Environ.* 27 (6), 725–735.
- Singh, R.P., Prasad, P.V., Sunita, K., Giri, S.N., Reddy, K.R., 2007. Influence of high temperature and breeding for heat tolerance in cotton: a review. *Adv. Agron.* 93, 313–385.
- Snider, J.L., Chastain, D.R., Collins, G.D., 2015a. Field-grown cotton exhibits seasonal variation in photosynthetic heat tolerance without exposure to heat-stress or water-deficit conditions. *J. Agron. Crop Sci.* 201 (4), 312–320.
- Snider, J.L., Chastain, D.R., Collins, G.D., Grey, T.L., Sorensen, R.B., 2015b. Do genotypic differences in thermotolerance plasticity correspond with water-induced differences in yield and photosynthetic stability for field-grown upland cotton? *Environ. Exp. Bot.* 118, 49–55.
- Snider, J.L., Choinski, J.S., Slaton, W., 2010a. Juvenile leaves of *Rhus glabra* have higher photosynthetic thermal tolerance than mature leaves. *Botany* 88 (3), 286–289.
- Snider, J.L., Oosterhuis, D.M., 2011. How does timing, duration, and severity of heat stress influence pollen-pistil interactions in angiosperms? *Plant Signal. Behav.* 6, 930–933.
- Snider, J.L., Oosterhuis, D.M., Kawakami, E.M., 2010b. Genotypic differences in thermotolerance are dependent upon prestress capacity for antioxidant protection of the photosynthetic apparatus in *Gossypium hirsutum*. *Physiol. Plantarum* 138 (3), 268–277.
- Snider, J.L., Oosterhuis, D.M., Collins, G.D., Pilon, C., FitzSimons, T.R., 2013. Field-acclimated *Gossypium hirsutum* cultivars exhibit genotypic and seasonal differences in photosystem II thermostability. *J. Plant Physiol.* 170 (5), 489–496.
- Snider, J.L., Oosterhuis, D.M., Skulman, B.W., Kawakami, E.M., 2009. Heat stress-limitations to reproductive success in *Gossypium hirsutum*. *Physiol. Plantarum* 137 (2), 125–138.
- Snider, J.L., Thangthong, N., Rossi, C., Pilon, C., 2022. Root system growth and anatomy of cotton seedlings under suboptimal temperature. *J. Agron. Crop Sci.* 208, 372–383.
- Strasser, R.J., Tsimilli-Michael, M., Qiang, S., Goltsev, V., 2010. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. *Biochim. Biophys. Acta, Bioenerg.* 1797 (6–7), 1313–1326.
- Strauss, A.J., Krüger, G.H.J., Strasser, R.J., Van Heerden, P.D.R., 2006. Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient OJIP. *Environ. Exp. Bot.* 56 (2), 147–157.
- Ton, P., 2011. Cotton and Climate Change: Impacts and Options to Mitigate and Adapt. International Trade Centre, pp. 1–17.
- Van der Westhuizen, M.M., Oosterhuis, D.M., Berner, J.M., Boogaers, N., 2020. Chlorophyll a fluorescence as an indicator of heat stress in cotton (*Gossypium hirsutum* L.). *S. Afr. J. Plant Soil* 37 (2), 116–119.
- Van Esbroeck, G.A., Bowman, D.T., Calhoun, D.S., May, O.L., 1998. Changes in the genetic diversity of cotton in the USA from 1970 to 1995. *Crop Sci.* 38 (1), 33–37.
- Virk, G., Snider, J.L., Chee, P., Jespersen, D., Pilon, C., Rains, G., et al., 2021. Extreme temperatures affect seedling growth and photosynthetic performance of advanced cotton genotypes. *Ind. Crop. Prod.* 172, 114025.
- Whitaker, J., Culpepper, S., Freeman, M., Harris, G., Kemera, B., Perry, C., Porter, W., Roberts, P., Liu, Y., Smith, A., 2019. Georgia Cotton Production Guide. The University of Georgia, Athens, GA, p. 146.
- Wise, R.R., Olson, A.J., Schrader, S.M., Sharkey, T.D., 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant Cell Environ.* 27 (6), 717–724.
- Wu, T., Weaver, D.B., Locy, R.D., McElroy, S., van Santen, E., 2014. Identification of vegetative heat-tolerant upland cotton (*Gossypium hirsutum* L.) germplasm utilizing chlorophyll fluorescence measurement during heat stress. *Plant Breed.* 133 (2), 250–255.
- Zushi, K., Kajiura, S., Matsuzoe, N., 2012. Chlorophyll a fluorescence OJIP transient as a tool to characterize and evaluate response to heat and chilling stress in tomato leaf and fruit. *Sci. Hortic.* 148, 39–46.