Identification of heat tolerant upland cotton (*Gossypium hirsutum* L.) germplasm utilizing chlorophyll fluorescence measurement

by

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Abstract

Heat stress adversely affects upland cotton (*Gossypium hirsutum* L.) production in the U.S.. A low level of genetic variability in domesticated upland cotton is one of the major constraints for cotton germplasm improvement. Utilization of exotic accessions through introgression could have potential to improve the heat tolerance of upland cotton. Heat tolerance is difficult to determine, especially when yield and fiber quality are the parameters measured in photoperiodic, non-adapted genotypes. Chlorophyll fluorescence is a widely used technique in abiotic stress studies. My objectives were: (1) to evaluate wild (mostly photoperiodic, non-adapted) genotypes for heat tolerance using a chlorophyll fluorescence assay; (2) to determine the heritability of heat tolerance identified by chlorophyll fluorescence in the segregating generations and select elite lines among advanced populations based on this measurement; (3) and to identify if heat tolerance as measured by chlorophyll fluorescence translates to increased growth and vigor under high temperatures. Forty-four wild accessions of upland cotton were selected as a resource of heat tolerant germplasm for a growth chamber test and a field evaluation. In the growth chamber, six-week old cotton plants were subjected to heat stress at 45^{o}C and 80% relative humidity for 24 hrs. Chlorophyll fluorescence was measured before and after heat treatment. In the field, chlorophyll fluorescence (Φ_{PSII}) was measured between 1200 h and 1400 h on the two youngest fully developed leaves of each plant during the first square stage to the open boll stage. Based on the chlorophyll fluorescence measured in the growth chamber and the field test, three wild accessions (TX 2287, TX 2285 and TX 761) were identified as

being heat tolerant. Crosses were made between two elite wild accessions and DP 90. $F_{2:3}$ lines in these two segregating populations were also evaluated in a growth chamber test and a field evaluation which were in the same manner as wild accessions. The broad-sense heritability of chlorophyll fluorescence on $F_{2:3}$ lines in the growth chamber was markedly different from that in the field indicating this measurement is sensitive to environmental conditions. Most fiber quality traits of $F_{2:3}$ lines were comparable to that of adapted cultivars whereas most seed traits of $F_{2:3}$ were slightly lower than that of adapted cultivars. Furthermore, correlations were found between chlorophyll fluorescence measurement and seed traits as well as between chlorophyll fluorescence measurement and fiber quality traits indicating chlorophyll fluorescence can be used as a selection tool for simultaneous improvement of heat tolerance, seed trait and fiber quality in these introgressed lines.

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List of Abbreviation

Elo	Fiber Elongation
F_0	Minimal chlorophyll fluorescence (dark adapted)
F_m	Maximal chlorophyll fluorescence (dark adapted)
F_v	Variable chlorophyll fluorescence (dark adapted) $F_v = F_m - F_0$
F_v / F_m	Maximum quantum efficiency
F_s	Variable chlorophyll fluorescence (light adapted)
F_{ms}	Maximal chlorophyll fluorescence (light adapted)
Φ_{PSII}	Operating quantum efficiency (light adapted) $\Phi_{PSII} = (F_{ms} - F_s)/ F_{ms}$
HVI	High Volume Instrument
LSMEANS	Least Square Means
Mic.	Micronaire
PSII	Photosystem II
QS1	Q Score 1
QS2	Q Score 2
SFC	Short Fiber Content
SE	Standard Error
Str	Fiber Strength
UHM	Upper Half Mean fiber length
UI	Fiber Uniformity Index

1 Introduction

Cotton (*Gossypium sp.*) is a very important textile fiber crop which is produced in 76 countries covering more than 32 million ha worldwide (Singh et al., 2007). Cotton is also an important source of oil and livestock feed, even as low-protein food for humans in some third-world countries. The cotton processing industry promotes the development of a global economy (FAO, 2005). In 2007, the total cotton production of the US was 18.9 million bales with a value of $4.9 billion (USDA, National Agricultural Statistics Service, 2012). The cotton production area in the US is mainly in 17 southern states and the top five cotton production state were Texas (42.4%), Georgia (14.0%), Arkansas (6.4%), North Carolina (6.3%) and Mississippi (5.1%) in 2012 (USDA, National Agricultural Statistics Service).

Cotton belongs to the Malvaceae family. This family contains more than 200 genera with around 2,300 species. The famous flowering plant species in this family include cotton, okra (*Abelmoschus esculentus* L.) and cacao (*Theobroma cacao* L.). Cotton is predominantly a self-pollinated plant, but out-crossing occurs in nature. There are 50 species of *Gossypium* which are native to Africa, Australia, Central and South America and Asia (Fryxell, 1992).

Different cotton species have different numbers of chromosome sets. Among them, four domesticated cotton species are widespread. *Gossypium arboreum* L. and *Gossypium*

herbaceum L. are diploid (2n=26) and belong to the Old World species. *Gossypium barbadense* L., and *Gossypium hirsutum* L. are tetraploid (2n=52) and belong to the New World species. *Gossypium hirsutum* L. is called upland cotton and is native to Mexico and Central America (McCarty, 2004). It accounts for 90% of total cotton production worldwide (Lu et al., 1997). In the US, both upland cotton (*Gossypium hirsutum* L.) and extra-long staple cotton (*Gossypium barbadense* L.) (ELS, also called American Pima Cotton) are grown, but ELS only takes up 4% of total cotton production (Womach, 2004).

Various environmental stresses adversely affect cotton production worldwide. Heat stress is one of the major factors that inhibit plant growth, development, as well as yield. Global warming caused by the release of greenhouse gases may have an influential effect on the world climate, and has caused the global temperature to increase by 0.4-0.8 °C during the 20th century (IPCC, 2007). Ecologists have predicted that through the year 2028, global temperature will increase by a total of 0.29 °C (Viola et al., 2010). A loss of US $4.2 billion in agriculture was due to heat and drought stress in August 2000 (Mittler, 2006). The most effective way to alleviate the negative effect of heat stress is to identify heat tolerant germplasm and breed cultivars with heat tolerance.

This literature review begins with the introduction of heat stress response and heat tolerance mechanisms. It is followed by an overview of heat tolerance breeding and the utilization of exotic germplasm lines. Introductions of chlorophyll fluorescence technique and its application are also included in this review. Finally, the objectives of the current study are presented.

Heat stress response

Plants are unable to survive being exposed to a temperature above their threshold level for a long time (weeks). Heat stress can cause irreversible damage to plant development and growth such as leaf wiltness, flower and fruit shed. The influence of heat stress depends on the increase rate of temperature, the growth stage of the plant, and the duration of heat stress (Wahid et al., 2007). With increasing yield loss due to climate change, heat stress causes growing concerns. The harmful effects of heat stress can lead to morphological, physiological and molecular changes in plants.

At the morphological level, high temperatures cause root system redistribution, dark spots in leaves, flaccidness, leaf senescence and abscission, fruit discoloration and abortion (Guilioni et al., 1997; Ismail and Hall, 1999). High temperatures also inhibit plant developments such as seed germination, seed development, internode growth (Ebrahim et al., 1998; Hall, 1992), pollen germination and pollen tube growth, fertilization and post-fertilization processes (Foolad, 2005). Overall, seedling establishment and the floral development stages are the two stages most sensitive to high temperature stress (Howarth and Ougham, 1993).

On the other hand, high temperatures can modify the physiological functions of some structures. The modification of cell membranes by heat stress is either by denaturation of proteins or by transformation of saturated fatty acid to unsaturated fatty acid in membrane lipids (Larkindale and Huang, 2004). High temperatures loosen the chemical bonds (covalent or non-covalent bonds) between molecules within the membrane and enhance the mobility of molecules across membranes. Increase in membrane fluidity can lead to the leakage of organic and inorganic solutes and loss of physiological functions of membranes. Membrane stability under temperature stress is very important for processes such as photosynthesis and respiration. The

structure of the thylakoid membrane can be altered by high temperatures including swelling and loss of grana stacking which ultimately reduces photosynthesis (Wahid et al., 2007).

Under high temperatures, most photosynthetic C_3 and C_4 plants rely on transpiration to lower leaf temperature. However, water loss in the transpiration process causes decreased water potential in plants. Water deficit in the soil or high relative humidity in the air can reduce the transpiration rate. There are a lot of other factors influencing the transpiration rate including leaf area, leaf orientation, leaf shape and size, leaf thickness, distribution of stomata as well as leaf surface characteristics (Mahan et al., 1995). Cultivars with a higher transpiration rate would adapt to a heat stress environment better (Jackson et al., 1981). Canopy temperature depression (CTD), the difference between air and foliage temperature, is an important indicator of heat tolerance.

High temperatures also influence photosynthesis. The deactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) can decrease the CO_2-exchange rate under high temperatures. High temperatures can also denature and aggregate the components of integral membrane complexes for electron transport and ultimately influence the efficiency of the light reaction. Meanwhile, high temperatures increase the photorespiration rate (Ogren, 1984). Photosynthetic CO_2 fixation and photorespiratory oxygenation are competing reactions (Taiz and Zeiger, 2002). The increase in photorespiration causes carbon starvation because carbon assimilation cannot meet the needs of carbon consumption for the increased respiration. Although both photosynthesis and respiration are influenced by high temperatures, the decrease of the photosynthetic rate is prior to the decrease of the respiratory rate.

Like the other stresses, high temperatures can induce oxidative damage. Reactive oxygen species (ROS) including oxygen singlet ($^1O_2$), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and

hydroxyl radical (OH^-) are formed in aerobic organisms (Liu and Huang, 2000). These oxidative products can peroxidize membrane lipids and thus alter membrane permeability and structure (Xu et al., 2006). Moreover, ROS can modify protein, DNA and other import molecules in the plant metabolism and growth processes (Sairam and Tyagi, 2004).

Heat stress in cotton

65% of the cotton production regions are located in the North Temperate Zone (from 30°N to 37°N) and 25% of them are distributed from the northern tropics to 30° N (Singh et al., 2007). Though cotton is a tropical or sub-tropical crop, the optimum day/night temperature for cotton growth and photosynthesis was found to be 30/20°C (Reddy et al., 1991b). This optimum temperature for growth may vary from cultivar to cultivar. Temperatures higher than 35 °C lead to inhibition of plant growth and increased photorespiration (Bibi et al., 2008). However, average maximum daily temperatures exceeding 35 °C are common in the US cotton belt during the period of cotton reproductive growth and development.

High temperatures can affect every stage of cotton growth. The optimum temperature for cotton seedling development is 28℃-30℃. High temperature (40/32℃ day/night) can influence root development and thus affect water and nutrient uptake (Reddy et al., 1997). Compared with the shoot, the root has a lower optimum growth temperature and is more sensitive to temperature extremes (Nielsen, 1974). The optimum day/night temperature for leaf area expansion, main stem elongation, and biomass accumulation is 30/22℃ (Singh et al., 2007). The fruiting branch is more sensitive to temperature at 40/32℃ than the vegetative branch (Reddy et al., 1992a; Reddy et al., 1992b). Cotton reproductive growth is especially vulnerable to high temperatures and pollen germination is more sensitive than other reproductive organs. The appearance of flowers and squares as well as boll retention decreased in response to high temperatures (Oosterhuis and

Snider, 2011). When day temperatures are above 40℃, all the squares and flowers are aborted for some upland cotton cultivars (Reddy et al., 1991a). Even though some upland cotton cultivars can produce squares and flowers at hostile temperature environments, boll development is not as successful (Reddy et al., 1992a). Heat stress can potentially cause substantial losses in yield. Previous research has shown that there is a negative association between high temperatures and cotton yields. In Arkansas, for every degree of maximum daily temperature over 30 ℃ in July, lint yield decreased by nearly 50 kg/ha (Oosterhuis, 1999). High temperatures also decrease fiber length, fiber strength, fiber per seed, and increase micronarie (Haigler et al., 2005; Pettigrew, 1996).

Mechanism of heat tolerance

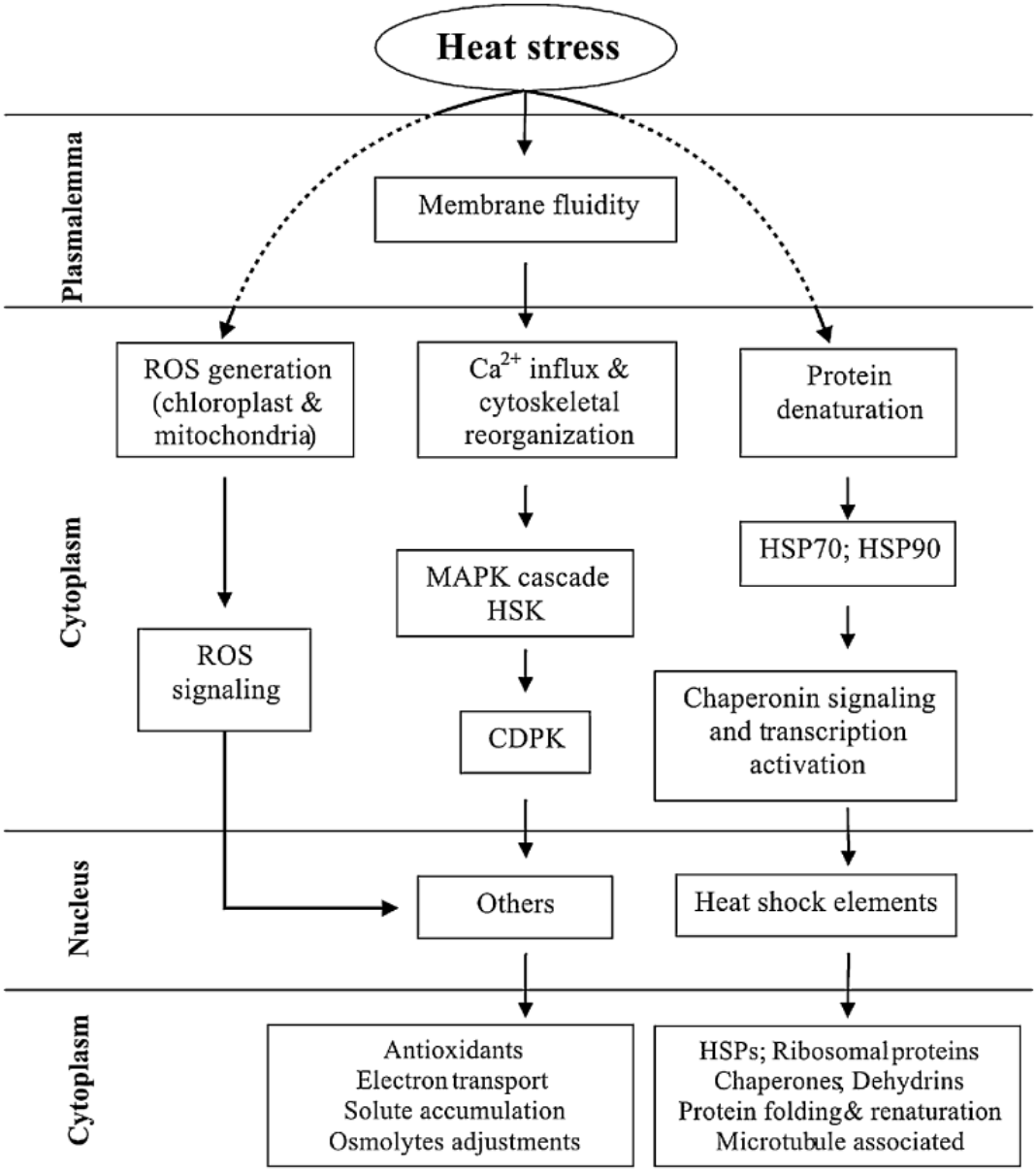
In response to heat stress, plants have a variety of mechanisms including heat tolerance, heat avoidance, and heat escape. Heat tolerance is the ability of plants to grow and produce yields under heat stress while heat avoidance is the avoidance of damage of high temperature in a stressed period. Heat escape is when a plant completes its life cycle before the onset of heat stress. Plants can also induce an acquired thermotolerance system when temperature is above the optimum level (Burke, 2001). This induced acclimation is obtained by exposing the plants to periods of sublethal heat stress prior to lethal heat stress.

Heat avoidance mechanisms include leaf rolling and changing leaf orientation, reflective leaf hairs and leaf waxes, and small leaf areas as well as an increase in evaporative cooling. Heat tolerance mechanisms can either be long-term or short-term in regards to morphological, physiological, biochemical and molecular strategies (Howarth and Ougham, 1993).

Wahid et al (2007) proposed heat tolerance mechanisms with three pathways (Fig. 1). Fluctuation of membrane fluidity is an important sensor or signal for turning on the adaptive responses. The first pathway is through the ROS (reactive oxygen system) generation in the chloroplast and mitochondria. The superoxide dismutase (SOD) can remove O^{2-} and produce H_2O_2 which can finally be scavenged by acerbate peroxidase (APX) or catalase (CAT). These antioxidants can effectively protect the plant from oxidative damage and thus enhance the ability of plants to resist heat stress (Maestri et al., 2002). The second pathway is the Ca^{+} influx and cytoskeletal remodeling in response to altered membrane fluidity. Under heat stress, the cytosolic Ca^{+} concentration increases. This leads to the increased expression of mitogen activated protein kinases (MAPK) and calcium dependent protein kinase (CDPK). These signals activate the production of antioxidants and compatible osmolytes. Compatible solutes are organic compounds which do not interact with enzyme function. There are several kinds of compatible solutes including the amino acid proline, sugar alcohols (e.g. sorbitol and mannitol) and a quaternary amine called glycine betaine. Heat stress always comes with reduced water potentials in plants. Commonly compatible solutes are accumulated in the cytosol to maintain water potential equilibrium within the cell. The accumulation of compatible solutes can maintain the integrity of membrane structure as well as stabilize the activity of proteins and complexes under osmotic stress (Bohnert and Shen, 1998; Bohnert and Sheveleva, 1998). Some of the solutes can serve as an osmoprotectant function, in that they can protect plants from damage of toxic byproducts and ROS during osmotic and ion stresses (Hasegawa et al., 2000). Previous research showed that the accumulation of compatible solutes can reduce the loss of K^{+}. Ion leakage is due to the change in membrane permeability (Cuin and Shabala, 2005). The third pathway is regarding the induction of heat shock proteins (HSP). HSP can be found in all the studied species

under high temperature stress (Howarth and Ougham, 1993). A change of fluidity of thylakoid membranes relates with an altered expression level of heat shock genes. Heat shock proteins improve heat tolerance of a cell by acting as molecular chaperones. There are considerable variations in HSP among species and even among genotypes within the species (Wood et al., 1998). HSP can be generally categorized into three classes based on their molecular weights: HSP90, HSP70 and low molecular weight proteins (LMW HSP) with 15-30 kDa. The functionality of HSP in thermotolerance includes degradation of misfolded proteins, inhibition of protein aggregation and reactivation of denatured proteins as well as assisting in the refolding of unfolded proteins (Parsell and Lindquist, 1993; Zhang et al., 2005). Among them, HSP 90 and HSP 70 play an essential role in stimulating thermotolerance under stress conditions. HSP 90 can prevent protein misfolding whereas HSP 70 can fold the HSP 90-released protein to the equilibrate state (Freeman and Morimoto, 1996; Jakob et al., 1995). LMW HSP plays a pivotal role in protecting PSII from heat stress by maintaining the electron transportation in the thylakoid membrane. HSP also interacts with other heat tolerance mechanisms. HSP can lower the concentration of ROS at the intercellular level (Arrigo, 1998). Previous research demonstrated that HSP can enhance defense ability against oxidative stress by increasing the expression level of the antioxidant APX (Panchuk et al., 2002). By controlling the stability of protein, HSP can maintain the intracellular concentration of osmolyte products (Diamant et al., 2001). HSP can interact with signaling molecules, such as hormone receptors, cell regulators and specific kinases in the signal transduction pathway, and regulate growth and development (Nollen and Morimoto, 2002). In addition to HSP, other stress proteins including ubiquitin (Sun and Callis, 2002), dehydrins (Wahid and Close, 2007) and late embryogenesis abundant (LEA)(Goyal et al., 2005) proteins can protect plants from the adverse effects of high

temperatures.



Heat tolerance in plants: an overview. Environmental and experimental botany 61(2007): 199-223 (Copied) .

Hormones are growth regulators. Abscisic acid (ABA), ethylene (C_2H_4) and salicylic acid (SA) are stress hormones which can help plants to resist different types of abiotic stresses including heat stress (Wahid et al., 2007). ABA is involved in many pathways of thermotolerance mechanisms. ABA can promote stomata closure to reduce water loss during heat and water stress. ABA can also modulate the up or down-regulation of genes and induce HSP under heat stress (Pareek et al., 1998; Xiong et al., 2002). SA promotes HSP transcription factors binding on the promoter region of the HSP genes (Jurivich et al., 1992). Ethylene acts as a signal molecule and is found to protect *Arabidopsis* from oxidative damage induced by high temperature stress (Larkindale and Knight, 2002). Other secondary metabolites also protect plants from heat stress such as carotenoids (Havaux, 1998; Wahid and Ghazanfar, 2006), phenolics (Chalker-Scott, 2002) and isoprenoids (Loreto et al., 1998).

Heat tolerance breeding in cotton

Heat tolerance is a complex quantitative trait. Quantitative traits have continuous values in a genetic population. They are always controlled by more than one gene and are influenced by the environment. Therefore the phenotype of a trait is affected by both environment and genotype : P = G + E + G×E where P is phenotype of the trait, G is genotype, E is environment, and G×E is the interaction of genotype and environment. The heritability of a quantitative trait in the broad sense (h^2_{BS}) is the proportion of phenotypic variation which is contributed by the genotypic term (V_G/V_P, V_G is genotypic variance; V_P is phenotypic variance). The heritability depends on the population, because all of the variance components (V_G and V_P) are population specific (Visscher et al., 2008). Traits with high heritability are less influenced by environmental conditions than those with low heritability. Therefore, genetic improvement is more efficient on high heritability traits than low heritability traits. Furthermore, the genotype term can be

classified into additive (A), dominance (D) and epistasis (I) effects. The narrow sense heritability (h^2_{NS}) is the ratio of additive to phenotypic variation (V_A/V_P). Heritability can be estimated either by variance components analysis, or by parent-offspring regression analysis (Ali and Wynne, 1994).

In a hybrid breeding program, heterosis is another interest of plant breeders besides heritability. Heterosis or hybrid vigor is the improved performance of hybrid progeny over their parents for certain traits. Heterosis increases with increased genetic heterogeneity of parents over a wide diversity. However, extremely diverse parents may cause gene incompatibility in F_1 hybrids (Moll et al., 1965). The identification of transgressive segregation in a hybridization population is very important for crop improvement. There are two types of heterosis: mid-parent heterosis (the outperformance of offspring over the average parent) and high-parent heterosis (the outperformance of offspring over the better parent).

Large yield losses attributed to the high temperatures is causing increased attention and effort on heat tolerance breeding in upland and Pima cotton in the US, especially in western US states, such as Arizona (Ulloa et al., 2009). Heat tolerance breeding in cotton is challenging in many facets. A low level of genetic variability in domesticated upland cotton is one of the major constraints for cotton germplasm improvement (Abdalla et al., 2001; Dongre and Kharbikar, 2004). Lost genetic variability in domesticated upland cotton underlies the necessity of exploiting genetic variation in undomesticated germplasm. Utilization of exotic accessions through introgression could have potential to improve the heat tolerance of upland cotton. Thus there is ample incentive for the discovery and utilization of cotton genotypes that are tolerant of high temperatures beyond the documented effects on yield and fiber quality.

Heat stress in the field is uncontrollable and has a wide variation. A consistent environment is required for selecting lines with heat tolerance, as such many studies in this field have been conducted in the greenhouse. Although the capacity for heat tolerance varies with growth stage, and the reproductive stage is more sensitive to heat stress than the vegetative stage, selection during the early growth stage is recommended in the greenhouse (Wahid et al., 2007). This is recommended because insects and pests will stress the plant in the later growth stage, and there is limited room for large plant growing in the greenhouse.

The lack of a reliable screening tool for heat tolerance is another limitation for the development of cotton heat tolerance cultivars (Singh et al., 2007). In previous studies, a variety of screening methods were applied to select heat tolerant genotypes including heat index (the ratio of the increase in coleoptile length after finite exposure to heat stress) (Young et al., 2001), fruit set, pollen viability, seed set (Berry and Rafique-Uddin, 1988), and in vitro pollen tube growth combined with pollen germination and boll retention (Liu et al., 2006). Various photosynthesis measurement techniques were also used in identifying heat tolerance including gas exchange, dry matter accumulation, the manometric method, the electronic sensor method and the carbon dioxide isotope method. Meanwhile, other physiological methods have been used as selection criteria of heat tolerance including measurement of chlorophyll fluorescence (Ranney and Ruter, 1997) and membrane leakage (Howarth et al., 1997; Kumar et al., 1999) as well as measurement of activity of antioxidant enzymes (Kumar et al.,1999), total soluble proteins and sugar alcohol levels (Ashraf et al., 1994). However, most of these techniques have their disadvantages. In terms of gas exchange measurement, the setup of and calibration of instruments is time consuming. The measurement of gas exchange, the electrochemical sensor and the activity of antioxidant enzymes as well as manometrics are influenced by environmental

factors, such as air flow, air temperature and air relative humidity as well as ambient oxygen concentration (Millan-Almaraz et al., 2009). Regarding the growth stage, measurements in the reproductive stage cannot reflect the growth vigor of the vegetative stage. Some measurements are destructive, such as dry matter accumulation and carbon dioxide isotopes. Some of them cannot provide instantaneous results such as dry matter accumulation, and some of the applications are limited to the lab such as the measurements of activity of antioxidant enzymes, total soluble proteins and sugar alcohol levels. Bibi et al., (2003) concluded chlorophyll fluorescence and membrane leakage are among the most applicable and sensitive methods compared with measurements of activity of antioxidant enzymes, total soluble proteins and sugar alcohol levels. In general, chlorophyll fluorescence has been a more reliable and stable technique than membrane leakage.

In the future, the determination of specific molecular markers linked with heat tolerance and utilization of biotechnology through functional genomic analysis to identify the specific genes controlling this trait is imperative. The gene transformation method is also an important alternative in breeding for heat tolerance in cotton. The combination of biotechnology tools with conventional breeding will dramatically improve the development of heat tolerance cultivars.

Utilization of wild genetic resource

A high level of genetic uniformity within a crop species is vulnerable to the outbreak of diseases and pests, and can cause yield losses. Genetically diverse germplasm, especially exotic lines are desirable genetic materials for plant breeding. In the later part of the 20th century, about 2,500 primitive cotton accessions were collected and stored in the U.S cotton germplasm collection (Percival, 1987). They are maintained at Texas A&M University in College Station,

Texas. Most of the accessions have been assigned a plant inventory (PI) number in the collection.

Although primitive lines can broaden the genetic base, they are underutilized because most of the accessions are photoperiodic. They can only flower and reproduce under the photoperiod of their countries of origin (Liu et al., 2000). Introgression of a day-neutral gene into these race stock accessions by a backcross method can alleviate this problem. McCarty and Jenkins (2001) successfully developed 16 day-neutral *Gossypium hirsutum* L. germplasm lines as converted stock races for use in the cotton breeding program. Another 97 primitive accessions were introgressed with day-neutral genes and were extensively used as genetic resources for pest and disease resistance (Jenkins et al., 1979).

Wild cotton accessions potentially possess genes for elite agronomic traits as well as tolerance to environmental stresses (drought, heat, salinity). Zeng et al. (2010) released four exotic germplasm lines by introgressing the genome of *Gossypium barbadense* L. into a *Gossypium hirsutum* L. background. These germplasm lines have elite fiber quality and high lint yield. Lafoe et al. (2005) extracted nematodes by the Baermann funnel technique and found that seven of eight *Gossypium arboreum* accessions have reniform nematode resistance. Converted Race Stock (CRS) accessions of *Gossypium hirsutum* L. (M-9044-0031-R) were identified with a large root system and low excised leaf water loss under drought stress condition and have a potential to be genetic materials for drought tolerance (Basal et al., 2005). Hemphill et al. (2006) found that exotic race stock accessions had the capability to survive under increased salt levels from the germination stage to the early seedling stage and can be used as a genetic source for salinity tolerance. Wild cotton species can survive extremely high temperatures such as 42℃ (Singh et al., 2007). Utilization of exotic accessions through introgression could also have the

potential to improve the heat tolerance of upland cotton. In my study, I aim to select heat tolerant lines from a group of wild upland cotton accessions.

Wild cotton accessions have many promising features. One of the specific characteristics of the wild cotton accessions in our study is okra leaf (palmate lobing). Okra leaf is varied from superokra (highly cleft) and okra to subokra (slightly cleft). Okra leaf has smaller leaf area and greater leaf chlorophyll content compared with normal broad leaf (Pettigrew, 2004). Canopy architecture is important for heat tolerance because the effect of heat stress is associated with the leaf area exposed under radiation. The small leaf area could decrease the mutual shading of plants. Research found that plants with okra leaf trait have greater CO_2 exchange rates, photosynthesis per unit leaf area and water use efficiency than normal leaf lines (Pettigrew et al., 1993). Okra leaf germplasm lines were used to improve fiber and agronomic traits in Pakistan (Hafeez-ur-Rahman et al., 2005). Pettigrew (2004) found that although okra leaf types have the similar level of dark adapted F_v/F_m as normal leaf types, the okra leaf lines associate with higher light-adapted PSII (Photosystem II) efficiency and photosynthetic electron transport rate than the normal leaf type. Research in Australia found that okra leaf cotton has an increased yield (Thomoson, 1995). One of my studies was to determine if heat tolerance identified by chlorophyll fluorescence is associated with okra leaf shape.

Chlorophyll fluorescence

Chlorophyll fluorescence is a widely used technique in physiological and biological studies. It can be applied in the lab, greenhouse and field. Due to its easy, quick, highly sensitive and non-invasive properties, there have been a lot of studies (over 3500 papers) using chlorophyll fluorescence (Baker and Rosenqvist, 2004). Another reason for using chlorophyll fluorescence in this research is that most of the wild accessions of upland cotton are short-day photoperiodic

plants and have delayed flowering and boll development because of photoperiodism. Therefore the evaluation of yield and fiber quality of these wild upland cotton accessions in the field, under local conditions (longer days during growing season) and high temperatures is not possible.

The principle behind the use of chlorophyll fluorescence as an indicator of plant stress is relatively straightforward. As photosynthetic pigments absorb light energy, there are three ways of dissipating that energy: the major portion of the energy is for driving photosynthesis. The second way is chlorophyll fluorescence emission which may account for up to 3% of the absorbed energy, and the third way is heat dissipation (Maxwell and Johnson, 2000). Each of these is complementary with the others, so a reduction in the chlorophyll fluorescence yield may reflect an increase in the photochemical reaction and heat dissipation.

During the light reaction of photosynthesis, the absorbed photons excite the chlorophyll in the reaction centers and emit electrons. Electrons travel through the protein complexes in the "Z scheme" electron transport chain (PSII, the cytochrome b_6f complex, PSI (photosystem I) and ATP synthase) to finally produce ATP (Adenosine Triphosphate). QuinoneA (Q_A) is the primary quinone electron acceptor bounded to the reaction center (Okamura et al., 2000). Q_A transfers two electrons to Q_B, another quinone, and is reduced to PQ_A^{2-}. Chlorophyll fluorescence yield is closely related with the redox state of the primary quinone electron acceptor Q_A.

When a dark-adapted plant or organism is exposed to continuous light, its chlorophyll fluorescence changes (Govindjee, 1995). This transient is called Kautsky effect and was first discovered by Kautsky and his colleagues in 1960 (Kautsky et al., 1960). This process can last from seconds to minutes. During this process, the yield of chlorophyll fluorescence will first increase then decrease. The rise in chlorophyll fluorescence is because the electron acceptor Q_A

is occupied by electrons and Q_A is fully reduced. At this time, the reaction center of PSII is closed. This gives rise to a decrease in photochemistry and an increase in the chlorophyll fluorescence yield. Later on, chlorophyll fluorescence starts to fall which is called quenching. It can be categorized into photochemical quenching (due to increased electrons transporting away from PSII) and non-photochemical quenching (due to heat dissipation) (Maxwell and Johnson, 2000).

A modulated chlorophyll fluorometer can measure and calculate both the chlorophyll fluorescence ratio (F_v/F_m) in the dark and the quantum efficiency of PSII (Φ_{PSII}) under the light (Schreiber et al., 1986). When a dark-adapted plant is exposed to light, the fluorescence level is minimal, termed (F_0). At this time, all the PSII reaction centers are open. A maximum chlorophyll fluorescence (F_m) is obtained when a dark-adapted plant is illuminated by a burst of short, intensive and saturated light. At this time all the photochemical reaction centers are assumed to be closed (Baker and Rosenqvist, 2004). The dark-adapted chlorophyll fluorescence is the maximum chlorophyll fluorescence which is calculated as $F_v/F_m=(F_m-F_o)/F_m$, whereas F_v is the variable fluorescence between two specific states in the dark (Genty et al., 1989). It is an indicator of the potential level of the chlorophyll fluorescence. F_v/F_m is a stable parameter which is about 0.83 under normal conditions (Björkman and Demmig, 1987). Various factors including abiotic stresses such as heat, cold or drought decrease F_v/Fm by essentially altering photochemical and non-photochemical quenching. This decrease in F_v/Fm provides the evidence of photoinhibition.

The quantum efficiency of a light-adapted test is calculated as $\Phi_{PSII}=(F_{ms}-F_s) / F_{ms}$. A light-adapted plant does not require dark adaption and takes at most a few minutes to reach the steady state of fluorescence value. The maximum chlorophyll fluorescence (F_{ms}) from the

illuminated plant is measured under saturated light while F_s is the fluorescence emission under the steady light state. Due to the non-photochemical quenching, the F_{ms} level in the light-adapted plant is less than the F_m level for the dark-adapted plants by the same saturated pulse. The quantum efficiency can give a measurement of the operating photochemical efficiency under a certain light condition. Thus it can reflect the overall performance of photosynthesis. Genty et al., (1989) developed a function to calculate the linear electron transport rate (*J*) based on the quantum efficiency (Φ_{PSII}). Previous researchers illustrated that the quantum efficiency is associated with CO_2 assimilation (Genty et al., 1990; Harbinson et al., 1990; Krall and Edwards, 1990, 1991). Moreover, many other parameters are derived from dark-adapted and light-adapted chlorophyll fluorescence including NPQ (Non-photochemical quenching), and F_v'/F_m' (PSII efficiency factor). They are all indicative of the metabolic and photosynthetic activity of plants under any given conditions.

Application of chlorophyll fluoresence

Chlorophyll fluorescence is widely used in abiotic stress studies. A research study used the decreased rate of F_R (the maximal rate of induced rise in chlorophyll fluorescence) as an *in vivo* measurement to identify effects of chilling, ice cover, frost, heat and high light stress (Smillie and Hetherington, 1983). They measured across a wide variety of crops and horticultural species including cucumber, wheat, snow bean, millet, peanut and papaya indicating that chlorophyll fluorescence is a broad-based technique in physiological studies.

Chlorophyll fluorescence was used as one of the means to screen the salt tolerance of rice cultivars (*Oryza sativa L.).* Under the salt stress, Φ_{PSII} of the salt tolerant cultivars (IR651 and IR 632) is stable whereas that for the salt sensitive cultivar IR29 is reduced by 15% in the

reproductive stage. However, non-photochemical quenching increases and the electron transport rate decreases in both of the salt-tolerant and sensitive cultivars at the vegetative and reproductive stages (Moradi and Ismail, 2007). Salt tolerance identified by chlorophyll fluorescence was confirmed by other screening methods such as photosynthetic CO_2 fixation, stomatal conductance and antioxidant enzyme activity. In another research, two naked oat genotypes (*Avena sativa L.*) were exposed to different levels of salt stress (0, 50, 100, 150, 200, and 250 mM NaCl). The investigation of the quantum efficiency for the light-adapted leaf (F_v'/F_m'), and photochemical quenching (*qP*) demonstrated that both of these two parameters decrease sharply at NaCl levels above 200 mM (Zhao et al., 2007). The authors concluded that there are variations in salinity tolerance among naked oat genotypes. The photosynthetic capacity under the salt stress identified by chlorophyll fluorescence is one of the most reliable tools for salinity tolerance studies.

Burke (2001) developed a new stress bioassay with chlorophyll fluorescence to detect the water-deficit stress response. The decline in F_v/F_m (chlorophyll fluorescence) in the dark over time was used as the criterion for the identification of drought stress response. The delayed decrease in F_v/F_m is a sensitive indicator of the water deficit tolerance in cotton. Chlorophyll fluorescence (F_v/F_m) was also used as a drought tolerance screening method in sugarcane seedlings (Zhu et al., 2010). Bajji et al., (2004) used chlorophyll fluorescence for screening drought resistance in durum wheat on tissue-culture derived lines. The selection based on the F_v/F_m value is sufficient to distinguish between drought resistant and susceptible lines *in vitro*.

In a chill damage study, the chlorophyll fluorescence (F_v/F_m) and the quantum efficiency (Φ_{PSII}) decreased dramatically in non-acclimated rice plants (*Oryza sativa L.).* However, acclimated plants have slowly decreasing or almost stable chlorophyll fluorescence and quantum

efficiency under chilling stress. This result presented chill acclimation is as an important way for chill-sensitive rice to obtain chill tolerance (Kuk et al., 2003).

Utilizing chlorophyll fluorescence, Oosterhuis et al. (2008) selected two adapted cotton genotypes with heat tolerance from 134 entries. Bibi et al. (2003) used chlorophyll fluorescence to detect heat tolerance response among obsolete and modern cotton cultivars and found that modern cultivars are less tolerant to heat stress than old cultivars at temperatures higher than 30.5 °C. Karademir et al., (2012) used chlorophyll fluorescence with other agronomic and physiological techniques including cell membrane thermostability, chlorophyll content, photosynthetic yield, photosynthetically active radiation (PAR) and other yield components for the evaluation of heat tolerance. They found there were significant differences among the fifteen upland cotton genotypes for these parameters. The results also showed that chlorophyll fluorescence can be one of the practical tools for screening for heat tolerance in field trials. Another study used the temperature corresponding to 50% of the maximal chlorophyll fluorescence as a parameter to identify the heat tolerance of potato cultivars (Havaux, 1995).

Moreover, chlorophyll fluorescence is also applied in other areas. Herbicides are often combined with certain kinds of additives to increase penetration. Habash et al., (1985) employed chlorophyll fluorescence to evaluate the most effective additives on diuron penetration in wheat leaves. Due to its non-destructive and sensitive properties, chlorophyll fluorescence can study the properties of photosynthetically active herbicides *in vivo* on plant tissues. Another herbicide, Propanil, is extensively applied for controlling grass weeds in rice and it is a PSII inhibitor. In a research study, chlorophyll fluorescence was used to differentiate resistance and sensitivity of barnyardgrass to propanil. And the performance of chlorophyll fluorescence in barnyardgrass can

be a basis for optimizing the application method under appropriate conditions (Norsworthy et al., 1998).

Furthermore, chlorophyll fluorescence is used in detecting environmental pollution. A study in India investigated chlorophyll fluorescence on maize plants with induced cadmium (Cd) stress and identified heavy metal tolerance in maize. They concluded chlorophyll fluorescence is a non-destructive and quick method to reflect photosynthetic activity under Cd stress (Dangre et al., 2006). Juneau et al., (2001) found that the presence of hydrophobic components in sewage can induce significantly modified fluorescence in *C. ehrenbergii* and algae. This change in chlorophyll fluorescence is indicative of the toxicity of the pollutants in the wastewater.

The objectives of my project are (1) to evaluate a broad range of cotton germplasm using chlorophyll fluorescence as an indicator of photosynthetic efficiency as affected by heat stress and establish chlorophyll fluorescence as an useful tool for heat tolerance evaluation; (2) to determine the heritability of heat tolerance identified by chlorophyll fluorescence in the segregating generations and select elite lines among the advanced populations based on this measurement; (3) and to identify if heat tolerance as measured by chlorophyll fluorescence translates to increased growth and vigor under high temperatures.

2 Materials and Methods

2.1 Experimental materials

Forty-four selected upland cotton accessions (*Gossypium hirsutum* L.) were included in this study. These were selected from 1,762 wild accessions of upland cotton based on a primary heat tolerance screening using the leaf sample chlorophyll fluorescence assay. Agronomic traits, fiber quality, and yield of these wild accessions are shown in Table 1 and Table 2. These tables were downloaded from the Germplasm Resources Information Network (GRIN). Only 5 out of the 44 accessions originated in the US and the regions of adaptability for other accessions are from a variety of countries including Mexico, France, Brazil, Venezuela and Columbia. The pubescence, leaf color, petal color and lint color of the accessions show large variation. Many of the accessions have (super) okra or lobed leaf shape. The cleft leaf has smaller leaf area than normal leaf and this leaf shape is associated with higher photosynthesis per unit leaf area as presented in previous research (Pettigrew et al., 1993). However, they also have some characteristics of unadaptation and wildness. Plant height ranges from 0.9 to 2.7 m (most are above 1.5 m) and they are taller than the adapted cotton cultivars and germplasm lines in the US. With respect to maturity, many of them are late-maturing types with a maturity score varying from 3 to 5 (3 - mostly green bolls, 4 - no bolls open, 5 - no flowers). The tall plant height and late development of flowers and bolls suggests that the reproductive growth stage of these accessions is delayed. Moreover, some of the accessions even cannot produce lint yield in the US (the productivity score is 5). The poor performance in yield production of these wild accessions is mainly due to photoperiodism. Fiber quality is moderate to good. In spite of the limitations

listed above, these wild accessions have the potential to increase the genetic diversification of the adapted cotton germplasm pool.

Another 44 *Gossypium hirsutum* L. accessions were randomly chosen from the wild accessions holding in the USDA cotton collection for comparison purposes. Four cultivar checks (Deltapine 90 (DP 90), Fibermax 966 (FM 966), SureGrow 747 (SG 747) and Acala Maxxa) were also included in this study. Seeds of 44 selected wild accessions of *G. hirsutum* and 44 random accessions were obtained from the USDA National Plant Germplasm System cotton germplasm collection in College Station, Texas. Seeds of commercial cultivars were provided by the Department of Agronomy and Soils, Auburn University.

2.2 Hybridization and population advance

Crosses were made between TX2287 (PI No.501467) and DP 90 (cross A), and TX2454 (PI No. 607759) and DP 90 (cross B) by hand pollination at the Auburn University Plant Science Research Center (PSRC). Both wild accessions are photoperiodic and cannot flower under local conditions. Therefore crosses were made in the greenhouse where growth conditions can be adjusted and controlled. In order to increase the successful rate of crossing, both wild accessions and DP 90 were planted at one week interval for two months. Wild accessions TX2287 and TX2454 were used as the pistillate parents. Flowers of pistillate parents were emasculated before anthesis and freshly collected pollen from the staminate parent (DP 90) was attached to the stigma of the pistillate parents. F_1 seeds of each crossing were bulk harvested and sent to the Winter Nursery in Mexico for generating F_2 seeds.

F_2 seeds were planted at the Plant Breeding Unit, E.V. Smith Research Center in Tallassee, Alabama for population advance in 2011. Due to photoperiodism, many of the plants grew as tall

as their exotic parents and had delayed square and flower development. At the end of November, $F_{2:3}$ seeds were harvested separately by individual F_2 plant. The remaining F_2 plants (396 plants of population A and 285 plants of population B), which did not produce seeds, were transplanted to produce $F_{2:3}$ seeds in the greenhouse. The plants were dug up and shoots were cut to 40cm. These plants were treated with a short-day photoperiod (day/night period 9h/15h) and were illuminated by nine 1000 watt metal halide lamps in the greenhouse. Meanwhile, the remaining F_2 seeds were sent to the winter nursery for producing $F_{2:3}$ population seeds in Tecoman Experimental Station, Mexico.

2.3 Heat tolerance screening with wild accessions

2.3.1 Whole-plant growth chamber test

A growth chamber test was conducted to evaluate the selected heat tolerant accessions. It was in a completely randomized design with three replications. The forty–four selected accessions with the greatest retention of F_v/F_m in the leaf sample screening, forty–four random accessions (randomly chosen from the wild accessions holding in the USDA cotton collection for a comparison purpose) and two checks (DP 90 and SG 747) were included in the growth chamber test, denoted as the selected group, the random group, and checks, respectively. Six-week-old plants were transferred to a growth chamber (Conviron®, Adaptis, A 1000, Leicestershire, UK) with 80% relative humidity and 14 hrs/10 hrs day/night photoperiod. Light intensity was 500 μmoles m^{-2} s^{-1} during the day. Plants were first acclimated to the growth chamber at 25°C for 24 hrs, and then they were heated at 45°C for 24 hrs followed by recovery at 25°C for 24 hrs. During the heat stress, plants were watered saturately.

The operating quantum efficiency of PSII ($\Phi_{PSII} = (F_{ms}-F_s)/F_{ms}$, where F_{ms} and F_s are maximum and variable fluorescence from a light-adapted leaf, respectively) is a light-adapted

parameter, and it represents the operating efficiency of PSII under a given light condition. A decrease in Φ_{PSII} reflects the reduced efficiency of the absorbed light energy used for the photochemical reaction (Baker and Rosenqvist, 2004). Φ_{PSII} was measured on the youngest fully developed leaf using a modulated fluorometer (OS1-FL) at 0 hr and 24 hr of heat treatment (Opti-Science, Hudson, NH) (two readings per leaf). Ten accessions in the selected group with the highest Φ_{PSII} after heat stress were selected for the field test.

2.3.2 Field screening

The top ten selected accessions (based on performance in the whole plant growth chamber test) and four checks (DP90, SG 747, FM 966 and Acala Maxxa) were grown in the field to evaluate their level of tolerance to field heat stress. Lines were grown in a randomized complete block design with four replications at the Plant Breeding Unit, E.V. Smith Research Center in Tallassee, Alabama. Cotton seeds were sown at a density of 10 seeds m^{-1} on 6 May, 2011. Each plot consisted of one 3 m long row, with 1 m spacing between rows. Based on the soil fertility condition, nitrogen fertilizer was applied at the rate of 3.36 g N /m^2 on Apr, 6^{th} and 6.73 g N/ m^2 on June, 14^{th}. Irrigation was applied as needed. The operating quantum efficiency of PSII (Φ_{PSII}) was measured on eleven different days between 1200 h and 1400 h on the two youngest fully developed leaves of each plant (two readings per leaf) during the interval from the 48^{th} DAP (days after planting) (first square stage) to the 113^{th} DAP (the open boll stage). Two plants of each plot were randomly chosen for measurements each sampling day. Temperature and humidity under the canopy were collected with an ACURITE® hygrothermometer (Chaney Instrument Co., Lake Geneva, WI) during the period (Table 3).

2.4 Heat tolerance screening with two segregating populations

2.4.1 Single leaf sample test of F_2 plants

396 transplants of population A and 177 of population B were included in the leaf sample test. This experiment was conducted in March, 2012 when most of the plants developed new branches and leaves after the transplanting. Two newest developed leaves were removed from each F_2 plant and put in a plastic sandwich bag (Glad® Storage) (Fig.1) and equilibrated in the dark for half an hour. Then the leaf samples were transferred into the dark growth chamber (Conviron®, Adaptis, A 1000, Leicestershire, UK) with a temperature of 45 ℃ and relative humidity of 80% for 90 min. Dark-adapted chlorophyll fluorescence (F_v/F_m) was measured by a modulated chlorophyll fluorometer OS1-FL (Opti-Science, Hudson, NH) in the dark at 0 min, 60 min, 90 min of the heat treatment, respectively. Mid-parent heterosis (%) ((MP-F_2)/MP; where MP is the average F_v/F_m of parents, F_2 is the F_v/F_m of F_2 progenies) was calculated on the chlorophyll fluorescence of the two populations at 0 min, 60 min and 90 min of heat treatment, respectively.

In population A, the wild parent TX2287 has an okra leaf shape (Fig.2). There was segregation of leaf shape trait in the F_2 population. An intermediate leaf shape which is phenotypically between the okra leaf (highly cleft) and normal leaf (not cleft) was observed in some plants. The number of plants of okra leaf shape, intermediate leaf shape and normal leaf shape were recorded. The reduction in percentage of F_v/F_m value after 60 min and 90 min of heat treatment was calculated as $R_1(\%)=(C_0- C_1)/C_0$ and $R_2(\%)=(C_0– C_2)/C_0$, respectively, where C_0, C_1 and C_2 were F_v/F_m after 0 min, 60 min and 90 min of heat treatment, respectively. A model was conducted to find the relationship between leaf shape and the reduction of chlorophyll fluorescence of leaf samples.

2.4.2 Whole plant growth chamber test of $F_{2:3}$ plants

76 $F_{2:3}$ families of population A (TX 2287× DP 90) and 64 $F_{2:3}$ families of population B (TX 2454 × DP 90) as well as four check cultivars (DP90, SG 747, FM 966 and Acala Maxxa) were included in the whole plant growth chamber test. It was followed a completely randomized design with two replications due to the limitation of seed availability. Growth conditions and heat treatment were the same as that for the wild accessions. Quantum efficiency (Φ_{PSII}) was measured with a modulated chlorophyll fluorometer OS1-FL (Opti-Science, Hudson, NH). Pictures were taken every 24 hrs of the heat treatment (after the pretreatment, heat treatment and recovery of heat treatment). The health and overall appearance of plants were recorded to monitor the morphological changes with the heat treatment. Analysis of variance was conducted to analyze the variation of chlorophyll fluorescence measurement. Variance components as well as standard errors of each variance component were calculated based on the formula (Hallauer, 1970):

$$SE(\sigma_i^2) = \sqrt{\frac{2}{C^2}\sum \frac{M_i^2}{df_i + 2}}$$

where c is the coefficient of the mean square, M_i and df_i are the appropriate mean squares and degrees of freedom used in the calculation of the variance component (σ_i^2). The broad–sense heritability ($H^2 = \sigma_G^2 / \sigma_P^2$, where genotypic variance (σ_G^2) = (progeny mean square- error mean square) /r and phenotypic variance (σ_P^2) = error mean square/r + σ_G^2) (Ali and Wynne, 1994) was determined on the Φ_{PSII} before and after the heat treatment. Standard errors (SE) of heritability were estimated by the formula:

$$\text{SE}(H^2) = \text{SE}(\sigma_G^2) / \sigma_P^2$$

Selection based on the D_1 (%) (the reduction of quantum efficiency, $D_1(\%)=(Q_0-Q_1)/Q_0$ where Q_0 is Φ_{PSII} after 24 hrs of pre-treatment at 25°C; Q_1 is Φ_{PSII} after 24 hrs of heat treatment at 45°C) were made of 20 highest accessions (with the smallest reduction percentage) and 20 lowest accessions (with the largest reduction percentage) in each population. A secondary whole plant growth chamber screening with the same heat treatment was conducted on the selected top 20 and bottom 20 $F_{2:3}$ families in each population.

2.4.3 Field test of $F_{2:3}$ plants

89 $F_{2:3}$ families of population A and 86 $F_{2:3}$families of population B as well as four check cultivars (DP90, SG 747, FM 966 and Acala Maxxa), including the same $F_{2:3}$ families in the whole plant growth chamber test as well as a few others, were grown in a randomized complete block design with four replications at the Plant Breeding Unit, E.V. Smith Research Center in Tallassee, Alabama. Herbicides Reflex®, Cotoran® and Pendant® were applied before planting. Cotton seeds were sown at a density of 10 seeds m^{-1} on 23 May 2012. Each plot consisted of one 3 m long row, with 1 m spacing between rows. Based on the soil fertility information, the nutrient levels of K and P were medium and N was insufficient. Fertilizer 0-20-20, 33-0-0-S and 28-0-0-S were applied at the rate of 22.4 g/m^2 on Apr, 26th, 10.2 g/m^2 on May, 23rd and 24.0 g/m^2 on July, 2nd, respectively. Irrigation was applied as needed. Stand count of each plot was determined 15 days after planting. Plant height was measured as the average height of plants in each plot on 48 and 77 days after planting, respectively. The quantum efficiency of PSII (Φ_{PSII}) was measured on the newest fully developed leaf from 37 days after planting (first square stage) to 84 days after planting (the open boll stage) (two readings per leaf) between 1200 h and 1400 h. Two plants of each plot were randomly chosen for measurements in each day. Measurements were conducted on ten different days. Temperature and humidity under the canopy were

collected with an ACURITE® hygrothermometer (Chaney Instrument Co., Lake Geneva, WI) (Table 4). $F_{2:4}$ seeds were bulk harvested from each $F_{2:3}$ family row. Compared with the F_2 population, $F_{2:3}$ population was more acclimated to the environment and more lines flowered and produced bolls and seeds. However, it was still not possible to conduct yield comparisons because of late flowering and boll formation of these lines.

2.5 Seed traits and fiber quality test on $F_{2:3}$ populations

25 bolls were harvested from high, middle, and low positions of fruiting branches in each $F_{2:3}$ progeny row. Bolls were ginned on a laboratory gin to determine gin turnout. Lint percent (%), seed index (100 seeds weight, g), lint index (100 seeds lint weight, g), seed number per boll and boll size (boll weight, g) were determined based on the 25 boll weight, lint weight per 25 bolls and 50 seed weight. After ginning the cotton, 206 samples (10 gram lint per sample) were sent to the High Volume Instrument (HVI) measurements at the Cotton Incorporated Textile Services Laboratory in Cary, NC. Data for determination of upper half mean fiber length in inch (UHM), fiber bundle strength in grams per tex (Str.), fineness in micronaire units (Mic.), percent fiber uniformity index (UI) defined as the ratio of the average length of all fibers to the average length of the longest 50% of fibers in the sample, elongation before break (Elo.), and percent short fiber content (SFC). The correlations between fiber quality parameters and chlorophyll fluorescence were analyzed.

2. 6 Statistical methods

Chlorophyll fluorescence data were analyzed using mixed models procedures as implemented in PROC GLIMMIX procedure (SAS, 2010). The student panel showed that the residuals followed the normality assumption. In the growth chamber test for wild accessions, the fixed effects were treatment (0 hr and 24 hr of the heat treatment), accession and their interaction. Day,

day × treatment (error term for the treatment effect) and day × accession (error term for the accession effect) were random effects. In the growth chamber test for $F_{2:3}$ lines, the fixed effects were treatment, population ($F_{2:3}$ progeny, wild parent and DP 90) and their interaction. Day, day × treatment (error term for the treatment effect), and day × population (error term for the population effect) were random effects. In the field test, 35 °C was considered to be the critical temperature for cotton growth. Therefore, field test days were divided into two temperature ranges: days with high temperatures (T > 35 °C) and days with mild temperatures (T < 35 °C). In the field test for wild accessions, temperature, accession and their interaction were analyzed as fixed effects and day (temperature), block, block × accession (error term for accession effect) and block × temperature (error term for temperature effect) were random effects. Lsmeans (Least Square Means) of each group (selected group, random group and check group in the growth chamber test and the top ten selected group and check group in the field test) were computed and compared by linear contrasts of accession means in the LSESTIMATE statement. In the field test for $F_{2:3}$ lines, temperature, population ($F_{2:3}$ progeny, wild parent and DP 90) and their interaction were analyzed as fixed effects and day (temperature), line (population), block, block × population (error term for the population effect), block × temperature (error term for the temperature effect) and block × temperature × population (error term for temperature × population effect) were random effects. All statistical comparisons were made at the 0.05 level of probability. The LSMEANS were shown in Lsmeans $\pm$ standard error.

Table 1 Morphological characteristics of 44 selected wild upland cotton accessions in this study

Texas No.	PI No.	Country of origin	Maturity [†]	Plantht (m)	Pubescence [‡]	Petal color	Pollen Color	Lint Color	Leaf Color	Leaf Shape
1528	530159	Mexico	5	1.8	2	Cream		White	Green	Crenate
884	529858	Dominican Rep.	3	2.1	3	Lt Yellow		White	Green	Lobed
2030	530661	France (Fwi)	5	---[§]		Cream	Yellow	Tan	Green	Normal
2044	530674	France (Fwi)	5	---	3	Lt Yellow	Cream	White Ns	Green	Lobed
9	153998	Mexico	3	1.5	1	Yellow	----------	Tan	Green	Super Okra
1037	232874	Venezuela	4	2.0	1	Lt Yellow	----------	White	Green	Normal
462	154014	Mexico	3	1.5	1	----------	----------	----------	----------	----------
190	165241	Mexico	3	2.0	4	Lt Yellow	----------	White	Green	Lobed
786	265175	Belize	4	1.8	2	----------	----------	White	----------	----------
188	163732	Guatemala	2	0.9	3	Lt Yellow	----------	White	Green	Normal
337	165350	Mexico	-	---	-	----------	----------	White	Green	Okra
338	165361	Mexico	-	---	-	Cream	----------	Tan/Brown	Green	Normal
1308	381948	Argentina	4	2.7	2	----------	----------	White	Green	Normal
1351	530079	Mexico	-	---	1	White	----------	White	Green	Crenate
761	201597	Mexico	-	---	2	----------	----------	Tan	Green	Super Okra
1453	530133	Mexico	4	1.2	1	----------	----------	White	Green	Normal
2285	501465	United States	5	1.8	-	----------	----------	White	Green	Normal
1046	341876	Mexico	-	---	3	Yellow	----------	Brown	Green	Normal
2050	530680	France (Fwi)	-	---	3	----------	----------	White	Green	Normal

2454	607759	Brazil	5	2.1	2	Cream	Cream	Off White	Green	Normal
1429	420827	Nicaragua	3	1.2	2	Cream	----------	White	Green	Normal
1197	376030	Brazil	3	1.9	3	Cream	Cream	White	Green	Normal
1704	530335	Colombia	5	---	2	----------	----------	White	Red	Normal
259	165267	Mexico	-	1.2	-	Cream	Yellow	White	----------	----------
2028	530659	France (Fwi)	4	---	1	Yellow Lt	Yellow	Tan	Green	Normal
960	529878	Mexico	3	---	1	Yellow	Cream	White	Green	Normal
804	529818	Bahamas	4	1.9	3	Cream Lt	Cream	White	Green	Normal
1299	530043	Spain	5	2.5	2	Yellow	----------	White	Green	Normal
2	153982	Mexico	2	1.2	1	Cream	Cream	White	Green	Cup+Broad
2326	607652	Mexico	1	1.6	1	Yellow	Cream	White Ns	Green	Normal
1401	476946	Brazil	4	---	3	Cream	Yellow	White Ns	Green	Normal
2047	530677	France (Fwi)	-	---	3	Cream	----------	White	Green	Normal
783	265172	Belize	5	1.5	3	Cream	----------	White	Green	Normal
256	165245	Mexico	3	2.1	2	Yellow	Yellow	White	----------	Normal
325	165393	Mexico	-	---	1	----------	----------	White	Green	Super Okra
1427	530118	Mexico	4	2.7	-	----------	----------	----------	----------	----------
2287	501467	United States	4	1.8	-	----------	----------	White	Green	Okra
1950	530581	Colombia	5	1.5	3	----------	----------	White	Green	Normal
1366	530094	Mexico	-	---	2	Yellow	----------	Brown	Green	Okra
816	529830	United States	5	1.5	2	----------	----------	White	----------	----------
2288	501468	United States	4	1.5	-	Cream	----------	White	Green	Super Okra
2279	501459	United States	4	1.5	-	----------	----------	White	Green	Lobed

1042	529905	Mexico	-	---	-	----------	----------	White	----------	----------
852	529843	Venezuela	3	2.7	1	Cream	----------	White	Green	Lobed

[†]Maturity: Relative maturity of entries when earliest cotton has all bolls open. 1-all bolls open; 2-1/2 bolls open; 3-mostly green bolls; 4-no bolls open; 5-no flowers

[‡]Pubescence: hairiness of plant in general. 1-no plant hairs; 2- few plant hairs; 3-hairy plant; 4-very hairy plant

[§]------- denotes information is not available

Table 2 Fiber quality and yield of 44 selected wild upland cotton accessions in this study

PI No.	UHM†	Micronaire†	Strength†	Length†	Elongation†	Seed Index†	Lint PCT†	Lint Index†	Productivity†
530159	----‡	----	----	----	----	8.2	--	----	-
529858	----	----	----	----	----	6.3	--	----	3
530661	----	----	----	----	----	4.6	--	----	4
530674	----	----	----	----	----	7.8	--	----	3
153998	0.76	5.65	32.3	0.64	8.7	6.6	30	2.9	2
232874	----	----	----	----	----	4.0	13	----	2
154014	1.06	4.10	32.7	0.82	6.0	9.0	--	----	4
165241	1.02	3.63	36.4	0.84	9.5	8.4	--	----	4
265175	1.09	2.51	44.1	7.08	50.7	8.7	3	4.1	2
163732	0.91	4.28	35.2	0.77	8.9	9.0	31	4.0	2
165350	0.87	4.85	39.2	0.73	8.5	6.5	--	----	-
165361	1.17	3.82	38.9	0.98	8.4	11.8	--	----	-
381948	----	----	----	----	----	11.4	--	----	2
530079	----	----	----	----	----	6.7	--	----	-
201597	0.67	----	31.5	0.55	----	5.3	--	----	-
530133	----	----	----	----	----	6.0	--	----	3
501465	----	----	----	----	----	6.8	--	----	-
341876	----	----	----	----	----	6.2	--	----	-
530680	----	----	----	----	----	7.3	--	----	-
607759	----	----	----	----	----	----	--	----	1
420827	----	----	----	----	----	12.0	--	----	-
376030	----	----	----	----	----	9.5	--	----	3
530335	----	----	----	----	----	5.9	--	----	5
165267	0.90	3.23	36.2	0.74	8.9	8.0	--	----	-
530659	----	----	----	----	----	4.6	--	----	2
529878	----	----	----	----	----	6.7	--	----	3
529818	1.01	5.79	43.8	0.89	7.7	7.6	21	2.4	4

530043	----	----	----	----	----	6.4	22	----	5
153982	0.91	4.60	35.3	0.80	8.2	13.6	28	5.3	2
607652	----	----	----	----	----	----	--	----	1
476946	----	----	----	----	----	8.0	--	----	3
530677	----	----	----	----	----	6.8	--	----	-
265172	0.98	4.99	33.7	0.79	8.4	8.8	18	2.3	4
165245	0.91	5.15	43.6	0.77	6.4	10.8	--	----	4
165393	0.92	4.38	39.6	0.71	8.3	8.2	29	3.3	-
530118	----	----	----	----	----	8.9	--	----	-
501467	----	----	----	----	----	9.2	--	----	-
530581	----	----	----	----	----	7.1	--	----	5
530094	----	----	----	----	----	5.9	--	----	-
529830	1.07	4.08	42.8	0.86	5.2	7.6	17	1.9	5
501468	----	----	----	----	----	8.6	--	----	-
501459	----	----	----	----	----	8.1	--	----	-
529905	----	----	----	----	----	6.2	--	----	-
529843	----	----	----	----	----	5.9	--	----	4

†UHM : The length in inches of the half of the fibers by weight that contains the longer fibers. UHM approximates classer's staple and 2.5 percent span length; Micronaire : the fineness of the sample taken from the ginned lint measured by the Micronaire and expressed in standard curvilinear micronaire ; Strength: the fiber strength of a bundle of fibers measured on a Stelometer with the jaws holding the fiber bundle tightly appressed. Measured in grams force per tex; Length: the average length in inches of all fibers longer than 1/4 inch; Elongation : the percentage elongation at break of the center 1/8 inch of the fiber bundle measured for T1 strength on the Stelometer; Seed Index: The weight of 100 seed in grams; Lint Index: The weight of lint from 100 seed in grams; Productivity: relative productiveness. 1-Most productive; 2-Good production ; 3 –Fair production ; 4-Poor production; 5-No production.

‡------- denotes information are not available

Table 3 Temperature and humidity of measurement days in the field in 2011 at Tallassee, AL.

Days after planting	Temperature	Humidity
	℃	%
47	27.8	47
62	33.3	50
64	29.4	45
69	36.4	43
74	37.5	41
85	28.9	50
87	33.3	60
97	36.9	48
104	35.9	41
109	35.4	42
113	36.4	30

Temperatures in red color are high temperatures (> 35 ℃)
Temperatures in black color are mild temperatures (< 35 ℃)

Table 4 Temperature and humidity of measurement days in the field in 2012 at Tallassee, AL.

Days after planting	Temperature	Humidity
	(℃)	(%)
37	36.1	71
40	32.0	41
47	33.3	68
55	35.0	55
63	35.6	45
68	36.1	48
70	35.0	81
77	33.9	57
79	25.0	82
84	30.0	82

Temperatures in red color are high temperatures (> 35 ℃)
Temperatures in black color are mild temperatures (< 35 ℃)

Figure 1 The newest developed leaves from each F_2 plant in a plastic sandwich bags

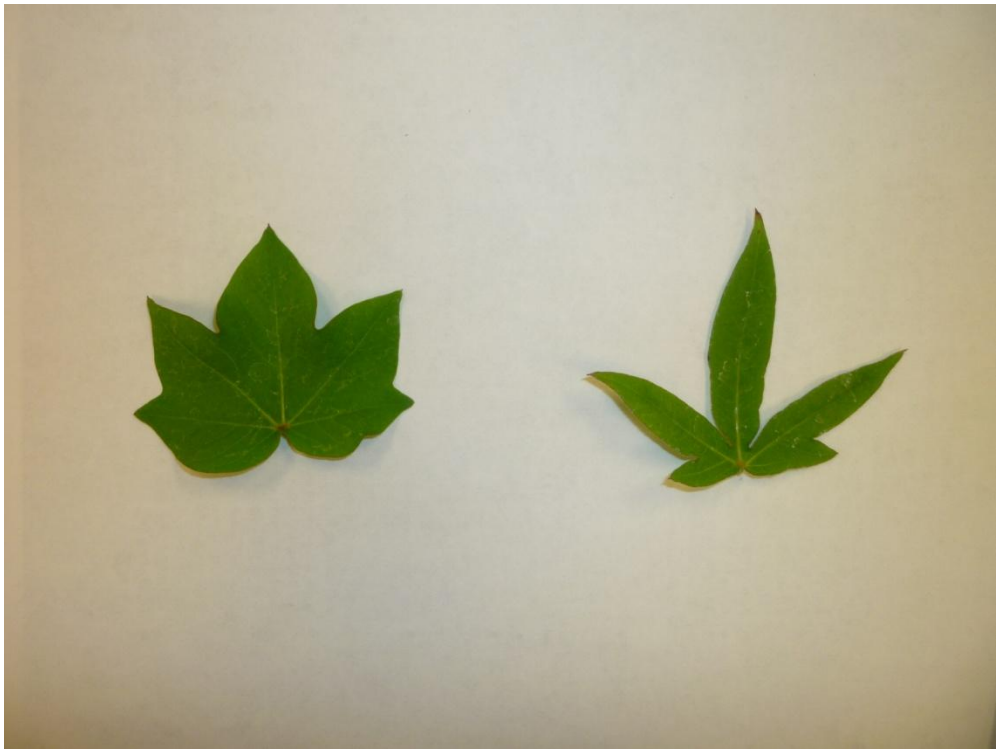


Figure 2 Normal and okra leaf shape of upland cotton

Normal leaf (left)
Okra leaf (right)

3 Results and Discussion

3.1 Weather condition in 2011 and 2012 summer

The daily maximum and minimum air and soil temperatures in the growth season (April to October) of 2011 and 2012 are presented in Fig.3 and 4. The monthly maximum and minimum air temperatures in 2011 and 2012 did not show much deviation from the long-term average of maximum and minimum air temperature (from 2003 to 2012) in Tallassee, AL (Fig.5). The monthly cumulative precipitation of the growing season in 2011 was 559 mm which was 35 mm lower than the long-term average rainfall received during the past ten years; whereas the growing season rainfall in 2012 was 629 mm, which was 35 mm higher than the long-term average rainfall (Fig.6).

3.2 Identification of heat tolerant germplasm among wild accessions using chlorophyll Fluorescence

3.2.1 Whole plant growth chamber screening

Due to the issue of seed viability which is the rate of seed will grow, forty-one selected accessions (of the 44 selected accessions above), forty-one random chosen accessions, and 2 checks were included in this test. An analysis of variance revealed that the effect of interaction

between treatment and accession was not significant (p=0.56) (Table 5). The treatment effect was the main source of variation of Φ_{PSII} (P<0.01). The lsmeans of Φ_{PSII} for the selected group, random group and checks were comparable under the unstressed temperature (p>0.05). After 24 hrs exposed to 45 ℃, the lsmeans for the selected group, random group and checks dropped to 0.53 ±0.02, 0.47 ± 0.02 and 0.24 ± 0.07, respectively. The differences among them became markedly significant (Table 6). The interaction effect between group and treatment was manifested that the Φ_{PSII} of selected accessions were noticeably higher than that of random accessions and checks after the heat treatment. This result indicated that the quantum efficiency of the selected group was less influenced than the random group and checks under the stressed temperature.

Based on the Φ_{PSII} after heat stress, all accessions were classified into four groups. The ranges of Φ_{PSII} for each group were 0.17 to 0.29 (bottom group), 0.30 to 0.42 (mid-low group), 0.43 to 0.55 (mid-high group), and 0.56 to 0.67 (top group). Seventeen of the forty-one selected accessions were included in the top group with the highest Φ_{PSII} after heat stress and were considered to be the most tolerant to heat stress, while seven random accessions and none of the checks appeared in this group. A different pattern was observed in the bottom group (group with the smallest Φ_{PSII} after heat stress and considered to be the least heat tolerant) that included two random accessions, two checks and no selected accessions (Table 5). There were 16 selected accessions and 22 random accessions in the mid-high group and eight selected accessions and ten random accessions in the mid-low group. These results indicated that when subjected to heat stress, a larger proportion of the random group and checks had greater reduction of quantum efficiency than the selected group of accessions. In other words, the operating quantum efficiency of selected accessions was more tolerant to heat stress than random accessions and

checks. The top ten accessions in the selected group with the highest Φ_{PSII} after heat stress were selected for the field test.

Plants can induce different mechanisms of heat tolerance. Since photosynthesis is one of the most heat-sensitive processes, the influences of high temperature on photosynthesis include the disruption of the integrity of thylakoid membrane structure, the reduction of the activity of photosynthetic apparatus and the occurrence of photoinhibition under the stressed conditions (Xu et al., 1995; Camejo et al., 2005). A reduction in F_v/F_m and $\Phi_{PSIIopen}$ can reflect the damage effects of heat stress on the structure and function of photosynthetic apparatus (Murkowski, 2001). Cui et al. (2006) concluded the tall fescue (*Festuca arundinacea* Schreb.) cultivar with higher F_v/F_m and Φ_{PSII} under the high temperature stress had less heat susceptible photosynthetic apparatus. Therefore, in the present study, the elite selected accessions can maintain high levels of photosynthetic efficiency during the heat stress and therefore showed photosynthetic heat tolerance.

In the growth chamber, although Φ_{PSII} decreased with increased temperature for all the genotypes, the sensitivity of the genotypes appeared to be highly varied among the groups. The selected accessions had the smallest reduction of Φ_{PSII} compared with random and check groups when exposed to the heat treatment. Consequently, it can be concluded that simple and reliable chlorophyll fluorescence determination can be a useful phenotyping tool for identifying large quantities of useful stress tolerant resources under the controlled condition.

3.2.2 Field Screening

Most of wild accessions grew much taller than commercial cultivars at the harvest season suggesting that these wild accessions were unadapted to the local environment (long day length)

(Fig.7). One of the top ten accessions (TX 2454) had a poor stand and was removed from testing. Analysis of variance indicated that all the fixed effects, temperature, accession and temperature × accession, were significant for Φ_{PSII} (Table 5). Under high temperatures (temperature of 35 ℃ and above), the lsmeans of Φ_{PSII} for the top nine accessions in the selected group and checks were 0.50 ± 0.02 and 0.47 ± 0.02, respectively. The difference between the two groups were significant ($P<0.01$). In contrast, the lsmeans of the top nine accessions in the selected group (0.63 ± 0.02) was not noticeably higher than that of the checks (0.63 ± 0.02) ($P=0.77$) under mild temperatures (temperature below 35 ℃) (Table 6). It appeared a smaller reduction of Φ_{PSII} for the top nine selected accessions than checks with the impact of the stressed temperature. This result confirmed that heat stress had a smaller influence on the top nine accessions than on checks under these conditions.

The Φ_{PSII} of the top ten selected accessions and check cultivars in the field screening is in Table 6. Under the mild temperature, the top nine wild accessions had a similar level of Φ_{PSII} as checks. Four of the top nine wild accessions were superior to check cultivars and three of them (TX 2287, TX2285 and TX761) were significantly different from checks at high temperatures (above 35 ℃). Three phases of screenings were conducted under different conditions of heat stress and measured at different growth stages. Based on these results, the elite selected accessions (TX 2287, TX2285 and TX 761) identified by the three-phased process have the potential to broaden the genetic variability of heat tolerance in upland cotton.

Note that the trait of heat tolerance can be very broadly defined, and a major issue involves other environmental factors that impact heat tolerance at various stages of plant development. Heat stress is usually accompanied by a reduction in water availability to the plants (Simoes-Araujo et al. 2003). Heat stress can increase transpiration, depending on relative humidity, and

thus increase water loss (Wahid et al. 2007). Under high humidity, heat stress could cause more severe damage to the plant than under low humidity. In the field, environmental conditions can vary widely from day to day, especially humidity. In our experiment, the relative humidity ranged from 30 to 60% (Table 1). Φ_{PSII} appeared to have variations in the top accessions and not all of the nine selected accessions had higher Φ_{PSII} than commercial cultivars when subjected to high field temperatures. The reason could be attributed to the environmental variations affecting the field assay. Moffatt et al. (1990) also found that the ranking of wheat (*Triticum aestivum* L.) genotypes differed under controlled heat stress environments and field stress conditions as measured by chlorophyll fluorescence. This indicates that chlorophyll fluorescence screening is sensitive to environmental conditions. Field tests in multiple environments might be desirable to validate the results. Nevertheless, it appears clear from the supporting results that most of the selected elite genotypes demonstrated a useful heat tolerant chlorophyll fluorescence phenotype at multiple levels of screening and at varying levels of plant growth suggest that the chlorophyll fluorescence is a broadly based method capable of assaying the physiological status of multiple aspects of heat tolerance, and as such may be a useful and valid screening tool for identifying the complex trait of heat tolerance.

Future studies will be aimed at demonstrating the heritability of the trait which should further help to establish the validity and limitations of this method for screening and as a tool of cotton improvement. This will hopefully facilitate transferring these important traits in these wild genotypes into commercial lines and therefore improve the heat tolerance of upland cotton.

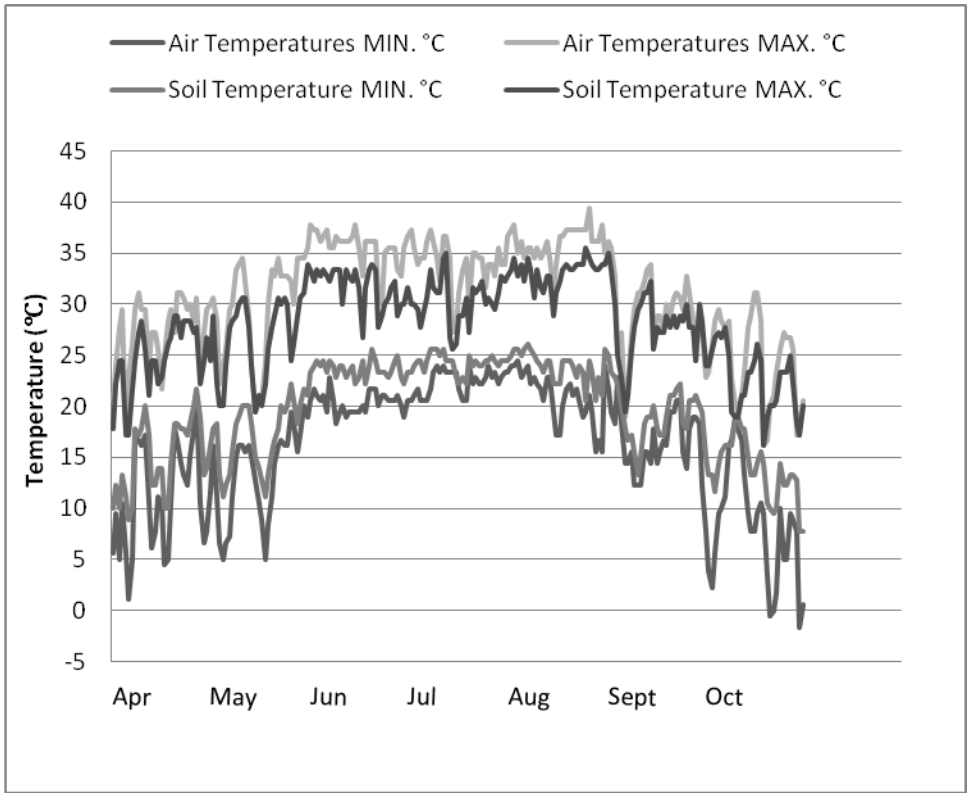


Figure 3 Daily maximum and minimum temperatures (air and soil) at E.V. Smith Plant Breeding Unit in 2011

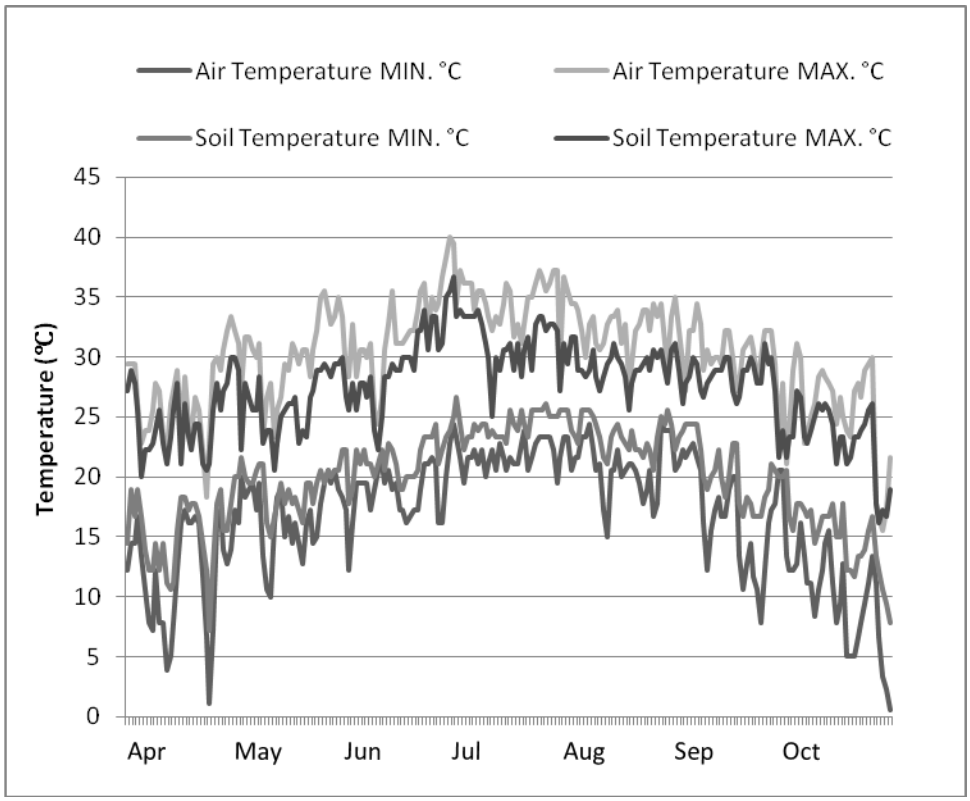


Figure 4 Daily maximum and minimum temperatures (air and soil) at E.V. Smith Plant Breeding Unit in 2012

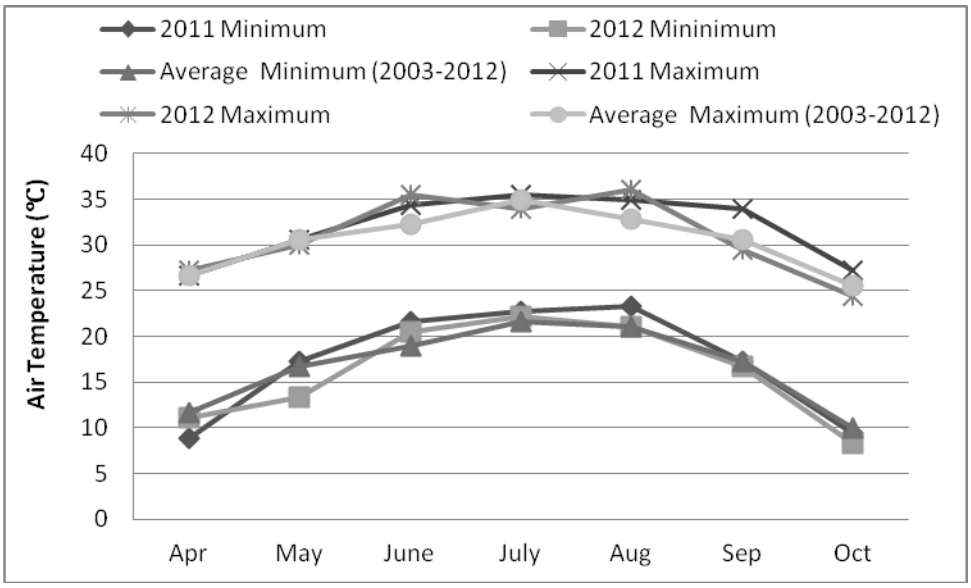


Figure 5 Monthly maximum and minimum air temperature during the growing season in 2011 and 2012

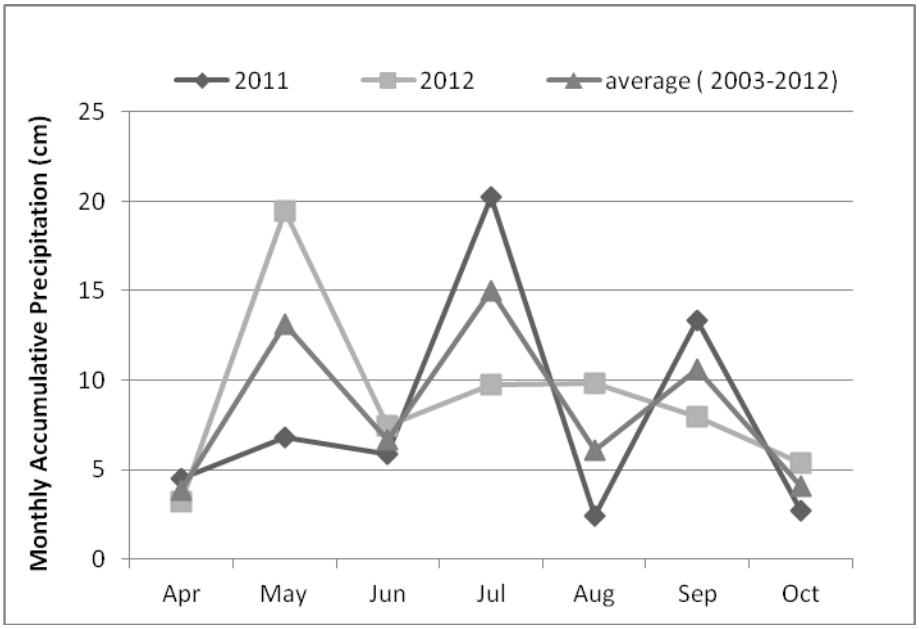


Figure 6 Monthly accumulative precipitation during the growing seasons in 2011 and 2012

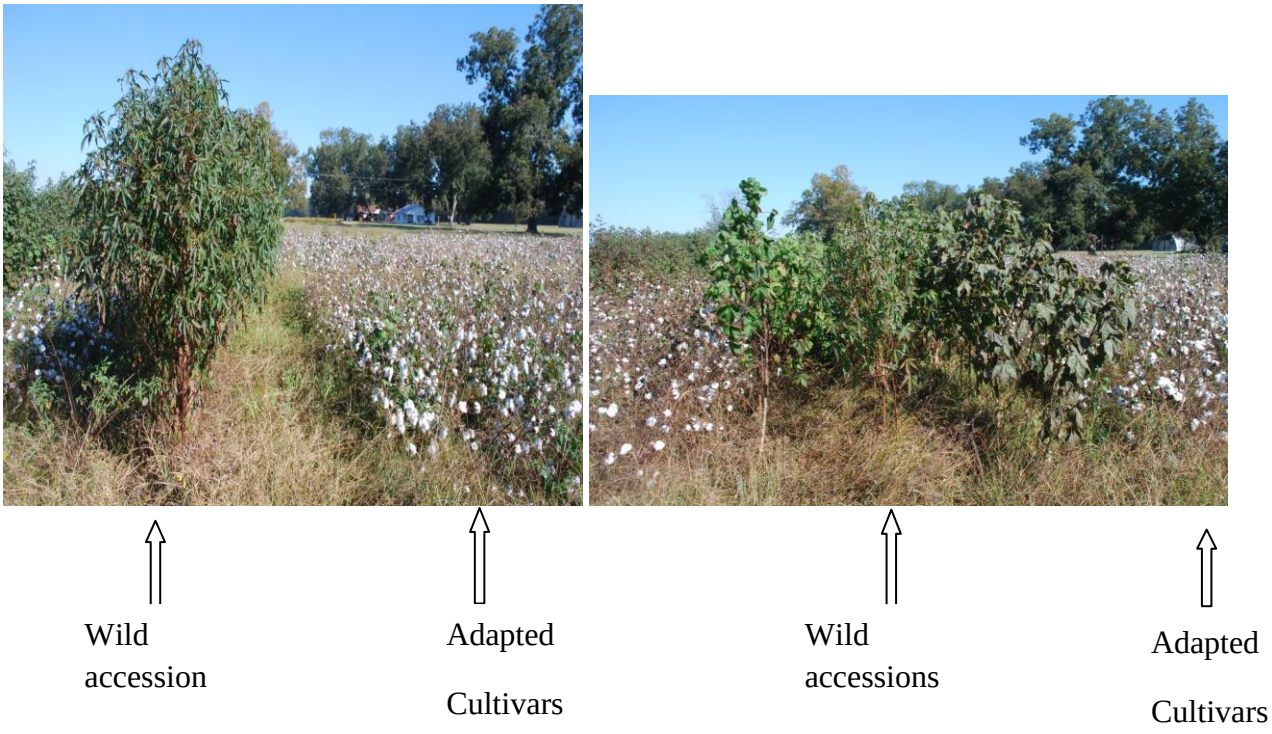


Figure 7 Selected wild accessions and adapted cultivars in the field on Oct, 26, 2011 (173 days after planting)

Table 5 Analysis of variance for Φ_{PSII} of selected accessions, random accessions and checks in the growth chamber screening and field screening

Source[†]	Num DF	Den DF	F value	P-value
Accession	83	99	1.05	0.4040
Treatment	1	2	260.93	0.0038
Accession × Treatment	83	99	0.97	0.5570
Source[‡]	Num DF	Den DF	F value	P-value
Accession	12	36	4.30	0.0003
Temperature	1	3	30.81	0.0115
Accession × Temperature	12	1475	3.10	0.0002

[†] Analysis of variance for Φ_{PSII} in the growth chamber screening
[‡] Analysis of variance for Φ_{PSII} in the field screening

Table 6 Φ_{PSII} of selected accessions, random accessions and checks in the growth chamber screening (0 hr and 24 hr) and the field screening (mild and high temperature)

Growth chamber screening	Lsmeans of Φ_{PSII}	
	0 hr	24 hrs
Selected accessions	0.70±0.02a†	0.53±0.02a
Random accessions	0.70±0.02a	0.47±0.02b
Checks	0.70±0.03a	0.24±0.07c
Field screening	Mild temperature	High temperature
Top nine selected accessions	0.63 ± 0.02a	0.50 ± 0.02a
Checks	0.63 ± 0.02a	0.47 ± 0.02b

Lsmeans within the same column with different letters differs (P< 0.05)

†Lsmeans ± Standard Error

Table 7 Frequency of selected accessions, random accessions and checks in four groups by Φ_{PSII} in the growth chamber screening

Group	>=0.17<=0.29	>=0.30<=0.42	>=0.43<=0.55	>=0.56<=0.67
selected group		8	16	17
random group	2	10	22	7
check group	2			

Table 8 Φ_{PSII} of the top ten selected accessions and checks in the field screening under high and mild temperature

Group	Accessions	Mild temperature (< 35 °C)	High temperature (>35 °C)
wild	TX1453	0.64±0.03bc†	0.49±0.02bc
wild	TX2050	0.62±0.03ab	
wild	TX 1308	0.65±0.02bc	0.50±0.02c
wild	TX2287	0.67±0.02c	0.57±0.02d
wild	TX2285	0.59±0.03a	0.56±0.03d
wild	TX1351	0.64±0.03ab	
wild	TX2454	NT‡	NT
wild	TX786	0.62± 0.03ab	0.44±0.03ab
wild	TX1950	0.62±0.03ab	0.42±0.03a
wild	TX761	0.62±0.03ab	0.56±0.03d
check	DP90	0.63±0.03ab	0.45±0.02ab
check	SG747	0.64±0.03ab	0.49±0.02bc
check	FM966	0.62±0.03ab	
check	Acala	0.61±0.03ab	

Lsmeans in the same column with different letters differ (P<0.05)

† Lsmeans ± Standard Error

‡. NT, not tested because of low stand rate of the accession TX 2454

3.3 Evaluation of heat tolerance and the heritability of chlorophyll fluorescence in two segregating populations

3.3.1 F_2 leaf sample test

Under the short-day photoperiod and high light intensity illumination in the greenhouse, transplants grew faster than that in the field. Most of them developed new branches and leaves after three months of transplantation. Some of them even produced flowers and bolls. However, due to the high plant density (four plants in a pot), plants developed diseases and some of new leaves were not healthy.

An analysis of variance of fixed effects for F_v/F_m in the leaf sample test is shown in Table 9. The effect of the heat treatment was significant for both population A and population B ($p<.0001$). However, effects of population and treatment × population interaction for F_v/F_m were not significant for the two populations ($p=0.41$ and $p=0.24$, respectively). These transplanted plants were different from six-week-old plants. Transplants were ten-months old and some of them had already been in the reproductive stage for several months. Growth stage may be an important factor influencing the chlorophyll fluorescence readings. Due to the limited space in the greenhouse, some of the transplants were infected with disease. Therefore chlorophyll fluorescence may not be measured accurately under the biotic stress condition. Compared with the whole plant, leaf sample does not have water source from the root and stem, and is more sensitive to high temperature than the whole plant.

In Population A, the lsmeans of F_v/F_m value for F_2 (DP 90 / TX 2287) was comparable to that of parents (DP90 and TX2287) at 0 min of the heat treatment. After 60 min, F_2 had a mean F_v/F_m (0.58 $\pm$ 0.01) intermediate between the high parent TX2287 (0.59 $\pm$ 0.08) and the low parent DP90 (0.50 $\pm$ 0.08). However, the mean F_v/F_m of F_2 (0.53 $\pm$ 0.01) was superior to both parents TX 2287 (0.41 $\pm$0.08) and DP 90 (0.45 $\pm$0.08) after 90 min of 45 ℃ heat treatment in the dark (Fig. 8). Mid-parent heterosis was 9.0 %, 6.0 % and 23.2 % for 0 min, 60 min, 90 min, respectively. This result suggests that the F_2 population outperformed their parents with respect to the chlorophyll fluorescence measurement under this controlled condition.

In population B, TX 2454 and DP 90 consistently had the high and low chlorophyll fluorescence values at each measurement time, respectively. However, the mean F_v/F_m of the F_2 population were inferior to both parents in each time point, (0.70 $\pm$ 0.01, 0.48 $\pm$ 0.01 and 0.42 $\pm$ 0.01 at 0 min, 60 min and 90 min of the heat treatment, respectively). F_2's tended to be lower than either of the parents in terms of F_v/F_m values regardless of the heat treatment. Based on this result, there was no hybrid vigor of F_2 progenies in population B (Table 10).This also indicated that parents contain different gene combinations in these two populations.

The exotic parent of population A (TX 2287) has an okra leaf shape. Observations of leaf shape of F_2 plants in population A were made in the greenhouse. Among the 396 plants, there were 231 plants with the okra leaf, 75 plants with the intermediate leaf and 88 plants with the normal leaf as well as two plants with no leaves. The reduction (R_1 (%)) for the normal, intermediate and okra leaf shape were 24.16 $\pm$ 1.15, 24.19 $\pm$ 1.24 and 24.15 $\pm$ 0.7 (%) after 60 min of the heat treatment, respectively. And after 90 min of heat treatment, the reduction (R_2 (%)) for the normal, intermediate and okra leaf shape increased to 29.90 $\pm$ 1.37, 31.06 $\pm$ 1.48 and 29.91 $\pm$ 0.84 (Fig. 9), respectively. There were no significant differences of the reduction

percentage among the different leaf shape categories both after 60 min and 90 min of the heat treatment (p=0.7802). There was apparently no relationship between leaf shape and response to heat treatment. Pettigrew (2004) also found that okra leaf types have a similar level of dark adapted F_v/F_m as normal leaf types in both the dryland and irrigated land conditions. Further study in the advanced population is required to validate this result.

3.3.2 Growth chamber test of $F_{2:3}$ populations

The growth chamber tests serves two objectives: first, the controlled environment is exclusive of extraneous environmental variation, allowing the differences among observations are due to different heat tolerance properties. Second, the environment can be reproduced whereas field conditions cannot.

According to the studentized residual plots, there was no pattern in the residual vs predictor plot and the percent vs residual histogram. This suggested that it followed the normal distribution. All fixed effects were not significant on Φ_{PSII} in two populations except for the interaction effect in population B (Table 11).

Parents were analyzed separately for mean comparison with $F_{2:3}$ progeny. Statistics of quantum efficiency (Φ_{PSII}) of the parents and $F_{2:3}$ progeny in the growth chamber test are summarized in Table 12. In population A, before the heat treatment, there were no significant differences in Φ_{PSII} among means of the high parent (TX2287) (0.70 ± 0.07), the low parent DP 90 (0.63 ± 0.14) and the $F_{2:3}$ progeny (0.66 ± 0.08). However, after exposure to heat stress for 24 hrs, the differences of Φ_{PSII} between the high parent (0.40 ± 0.20) and the low parent (0.30 ± 0.19), as well as between the high parent and $F_{2:3}$ progeny (0.31 ± 0.16) became significant. This

suggested that $F_{2:3}$ progenies were more similar to the low parent (DP 90) than high parent (TX 2287) in terms of fluorescence tolerance to heat stress.

In population B, the mean quantum efficiency of $F_{2:3}$ progenies (0.07 $\pm$0.05 and 0.37 $\pm$ 0.13 before and after the treatment, respectively) were closer to the range of the high parent (TX 2454) (0.70 $\pm$ 0.06 and 0.43 $\pm$ 0.22, before and after heat treatment) than the low parent (DP 90) (0.63 $\pm$ 0.14 and 0.3 $\pm$ 0.19 before and after heat treatment). Therefore, the difference between the $F_{2:3}$ progeny and TX 2454 (high parent) was not significant (Table 12). There was a significant difference between the mean Φ_{PSII} of the $F_{2:3}$ progeny and DP 90 (low parent) under the heat treatment. This indicates that $F_{2:3}$ individuals in population B harbor more of the exotic parent gene of chlorophyll fluorescence heat tolerance than population A. The reason of the varied performance of $F_{2:3}$ progenies in these two populations was attributed to different gene combinations among different parental genotypes. The genetic variance can be mainly split into dominant and additive variance. Dominant variance is due to the interaction between alleles and makes it greater or smaller than the sum of the alleles when they act alone. It is unpredictable and not heritable because only one allele is passed down from each parent to offspring. Additive variance is due to adding the effect of individual allele which is the real inherited part of genetic variance. The genetic variance of different population is determined by different gene interactions.

Pictures of $F_{2:3}$ plants and checks during the heat treatment are shown in Fig. 10. In Fig.10 (a), all $F_{2:3}$ plants grown at 25℃ were all green and healthy before the heat treatment. After 24 hrs of heat treatment, most of plants were wilted and flaccid but showed no leaf discoloration, dark spots, leaf chlorosis or dryness (Fig. 10(b)). However, after a recovery period at 25 ℃ for 24 hrs plants did not show much recovered appearance and were not as turgid as healthy plants

(Fig. 10(c)). We did not observe different effects of the heat stress on $F_{2:3}$ progenies and parents as manifested by the chlorophyll fluorescence readings. Therefore the chlorophyll fluorescence technique can identify the influence of heat stress before plants get obvious symptoms of stress. Obenland and Neipp (2005) also found that chlorophyll fluorescence imaging can detect the hot water-induced rind injury in green lemons (*Citrus limon (*L.*) Burm*.) before visible symptoms.

The analysis of variance of Φ_{PSII} within each population is shown in Table 13. In population A, the effect of genotype was significant for Φ_{PSII} both before and after the heat treatment ($p=0.0009$ and $p<.0001$, respectively). In population B, the effect of genotype became significant after the heat treatment ($p=0.002$) indicating there was genetic variance of Φ_{PSII} among the $F_{2:3}$ progenies under the effect of the heat treatment. Overall, there were genetic variations for Φ_{PSII} among $F_{2:3}$ progenies in both populations which would increase the possibility of beneficial transgressive segregations. The effect of replicate was not significant for both populations.

Heritability estimates can demonstrate the genetic consequences of hybridizations or inbreedings (Ali and Wynne, 1994). In population A, the estimated genotypic variances were 0.0022 ± 0.0004 and 0.0126 ± 0.0010, respectively which were larger than variance estimates of replicate effect (the environmental effect) and that of the error (the genotype × environment interaction). The broad-sense heritabilities of Φ_{PSII} were 52.38 ± 9.52 % and 67.02 ± 5.32 % before and after the treatment, respectively (Table 14). This indicated that genotype contributed greatly to the overall phenotype, especially with the heat treatment.

In population B, the estimated variance of genotype were 0.0005 ± 0.0002, 0.0062 ± 0.0008 before and after the heat treatment, respectively. The broad-sense heritabilities of

Φ_{PSII} were 35.71 ± 14.29 % before the treatment and 52.99 ± 6.84 % after the treatment (Table 14). This relative high heritability suggested that over half of the observed phenotype was contributed by the genotype in population B under the stressed condition. Overall, these heritabilities indicated that it is possible to improve heat tolerance in upland cotton by breeding. However, this result was confined to the growth chamber study. Heritability would be different under different environmental conditions. Hague et al., (2011) found that broad-sense heritability estimates of fiber quality traits were higher at Weslaco than in College Station. Multiple tests in varied conditions are required to identify the dominant effects of phenotype.

Based on the reduction percentage of Φ_{PSII} (D_1 (%), function shown in Materials and Methods), 20 top $F_{2:3}$ lines with the least reduction percentage and 20 bottom $F_{2:3}$ lines with the largest reduction percentage were selected in each population. In population A, the mean reduction of Φ_{PSII} (D_1 (%)) for the 20 top lines was 0.25 ± 0.02 and that for the 20 bottom lines was 0.31 ± 0.02. The difference among them was significant (p=0.0090). This indicates that these top lines had more stable quantum efficiency value than bottom lines under the heat stress. In population B, the reduction percentage of quantum efficiency (D_1 (%)) for the 20 top lines and the 20 bottom lines were 0.17 ± 0.02 and 0.27 ± 0.01, respectively. The reduction of quantum efficiency of the top lines was significantly lower than that of the bottom lines (P<.0001) (Fig. 11). Therefore the top lines were less influenced by the heat treatment than the bottom lines. These top 20 $F_{2:3}$ lines in each population have the potential to become the upland cotton germplasm for further improvement of heat tolerance.

However, the growth chamber test was conducted under a controlled environment with consistent light intensity, temperature and relative humidity. This is different from field conditions. Therefore the results are restricted to interpretation within controlled environments.

3.3.3 Field test of $F_{2:3}$ population

Stand count A stand count was taken at 15 days after planting. The average survival rate (%) of population A and population B were 64.84 ± 24.44 and 48.94 ± 26.49 (%), respectively. Some plots had 100% stand whereas other plots had no germinated seeds. The survival rate (%) for TX 2287 and TX 2454 were 34.17 ± 8.33 and 39.17 ± 7.19, respectively. Seeds of DP 90 had a very low survival rate (Table 15). Therefore DP 90 was replanted later to make up for the initial poor stand.

Plant height Cotton plants grow indeterminately which means they will continue vegetative growth when plants start to flower and develop bolls. However, the vegetative growth should decrease after the first flower due to competition for resources between the reproductive growth and the vegetative growth. On the other hand, heavy vegetative growth can induce a high rate of boll rot and boll abortion (Ritchie et al., 2004).

In Population A, plant heights of TX2287, DP90 and $F_{2:3}$ progenies were 78 ± 7, 74 ± 6 and 86 ± 9 cm, respectively at 48 days after planting. At 77 days after planting, plant height increased to 152 ± 6, 103 ± 2 and 149 ± 14 cm for TX 2287, DP90 and $F_{2:3}$ progenies. Both TX2287 and $F_{2:3}$ progeny were taller than DP 90 regardless of the developmental stage. In population B, plant heights of TX2454, DP 90 and $F_{2:3}$ progenies were 48 ± 11, 74 ± 6 and 66 ± 11 cm, respectively 48 days after planting. At 77 days after planting heights were 107 ± 3, 103 ± 2 and 134 ± 14cm for TX2454, DP 90 and $F_{2:3}$ progenies, respectively (Fig. 12). Different from TX2287, TX2454 did not grow much taller than DP90. $F_{2:3}$ progenies grew taller than TX 2454 and DP 90 on the second measurement day. Overall, progeny lines were taller than DP 90 in both populations indicating that they were sensitive to the local photoperiod and had delayed reproductive growth. A backcross method is proper to incorporate the improved chlorophyll fluorescence heat

tolerance of these elite lines into commercial lines. This method would also increase their adaptation under the local environment.

Field test of $F_{2:3}$ population

Neither the fixed effects (temperature, population) nor their interaction (temperature × population) was significant for Φ_{PSII} in Population A or B ($P>0.05$) (Table 16). The insignificant effect of temperature was partially because the monthly cumulative precipitation in the growing season of 2012 was 35 mm more than a long-term average and 70 mm more than that of 2011 in the station. Moreover, the stressed temperatures on the measurement days ranged from 35.0 ℃ to 36.1 ℃ which was apparently not sufficient to induce a heat stress response.

In population A, the lsmeans of Φ_{PSII} for the high parent (TX2287) was 0.63 ± 0.04 which was significantly higher than that of the low parent (DP 90) (0.49 ± 0.03) ($P=0.0346$) under the high temperature. The lsmeans of the $F_{2:3}$ progeny was intermediate between values of the high parent and the low parent and did not show a significant difference from parents. However, under the mild temperature, there were no significant variations between the high parent and the low parent as well as among parents and the progeny (Table 17).

In population B, although the lsmeans of Φ_{PSII} of $F_{2:3}$ progeny did not differ significantly from parents, it was numerically greater than the high parent regardless of temperature (Table 17). The reason could probably be due to the residual heterosis left in these randomly chosen $F_{2:3}$ lines. Field trials in multiple years and locations are required to identify the interaction effect between genotype and environment.

A further investigation of variance components of Φ_{PSII} was conducted among lines within each $F_{2:3}$ population. In population A, the dominant source of variation of Φ_{PSII} was contributed

by day under each temperature regime. This influence was followed by the effect of block (Table 18). There was no significant differentiation of Φ_{PSII} among the $F_{2:3}$ lines under either temperature regime. The mean squares for line were 0.0078 and 0.0064 under the high temperature and the mild temperature, respectively. These were negligible in comparison to the effect of day, especially under the high temperature. None of the interaction effects was significant. Similarly, in population B, day exerted the most dramatic effect on Φ_{PSII}. The difference of Φ_{PSII} among the $F_{2:3}$ lines was significant under the high temperature (P=0.0002) and was not significant under the mild temperature (p=0.3328).

Based on these field results, the variation for Φ_{PSII} among $F_{2:3}$ lines within each population, (phenotypic variation), was mainly attributed to effects of environment, e.g. the air temperature, humidity and light intensity conditions across days and the different soil conditions, water availability and light interception condition among blocks. Genotypic variation ascribed to the lines was not comparable to the environmental variation. Therefore, the broad-sense heritability of Φ_{PSII} for these $F_{2:3}$ lines was estimated to be zero in this field test. This is consistent with another study with durum wheat (*Triticum durum* Desf.), that also found that the broad-sense heritability of F_v/F_m was low (h^2=0.143) in a drought tolerance field test in Mediterranean region (Northwest Syria) (Araus et al., 1998). The broad-sense heritability of chlorophyll fluorescence was 0.28 in a heat tolerance study with maize (*Zea maize* L.) recombinant inbred line population suggesting selection of heat tolerance genotypes is required to be replicated in multiple environments (Bai, 2003).

Compared with the relatively moderate to high broad-sense heritability in the growth chamber test, the field test showed a marked difference. This may be because in the growth chamber, the

environmental condition was controllable and kept constant. The proportion of phenotypic variation attributed to genotype was relatively large in the growth chamber. This was not the case of the field test. The temperature and relative humidity varied widely from day to day (Table 4). Especially the relative humidity varied from 41% to 82% in the measurement days. Chlorophyll heat tolerance is a quantitative trait which is highly influenced by environmental conditions. Therefore environment is the primary contributing factor of the observed phenotype in the field. Longenberger et al., (2009) found that the chlorophyll fluorescence procedure could not be used to select plants for drought tolerance of upland cotton in the field.

A regression relationship of Φ_{PSII} between the field measurements and growth chamber measurements was modeled. The relationship between the two measurements was not significant (R^2=0.0052) (Fig. 13).The reason may be due to different heat stress conditions. The figure shows that the Φ_{PSII} of the $F_{2:3}$ lines had large fluctuations under the heat treatment in the growth chamber test. In contrast, the Φ_{PSII} did not show as much variation among lines in the field test under the high temperature. The reason could be that the temperature and humidity in the growth chamber were higher than that of measurement days in the field. Air temperature and relative humidity together determine the vulnerability to heat stress (College of Agriculture and Life Sciences, 2008). Therefore, stress conditions in the field were not sufficient to distinguish among the $F_{2:3}$ lines for phenotypic heat tolerance, as demonstrated by chlorophyll fluorescence. More extreme field temperatures are required to reflect the capability of heat tolerance of these $F_{2:3}$ lines. This also indicated that chlorophyll fluorescence screening is sensitive to environmental conditions.

Table 9 Analysis of variance of fixed effects for F_v/F_m measured in F_2 populations in the leaf sample test

Population A				
Source of variation	Num DF	Den DF	F value	P value
Treatment	2	786	24.54	<.0001
Population vs parent†	2	393	0.91	0.4050
Treatment*Population vs parent	4	786	1.38	0.2394
Population B				
Source of variation	Num DF	Den DF	F value	P value
Treatment	2	286	8.81	<.0002
Population vs Parent	2	176	0.16	0.8495
Treatment*Population vs Parent	4	286	0.09	0.9845

† F_2 population, wild parent and control parent

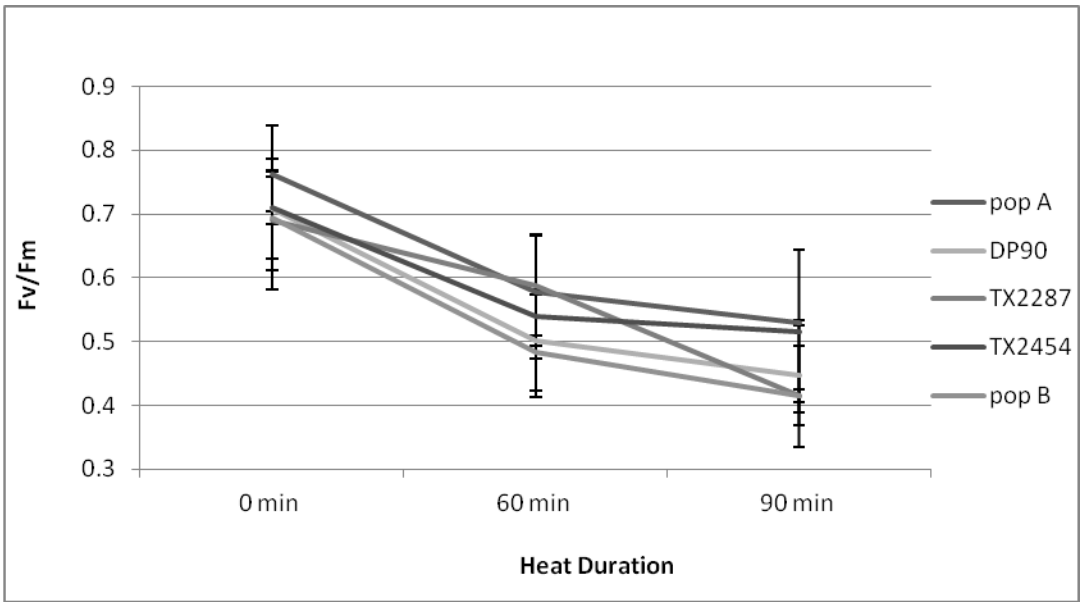


Figure 8 F_v/F_m of F_2 populations and parents measured under the heat stress (45 ℃ and 80% humidity) for 60 min and 90 min in the dark growth chamber

Lsmeans ± Standard error

Table 10 Mid-parent heterosis estimations of population A and population B (F_2) progeny based on leaf sample chlorophyll fluorescence measurements

Treatment	Population A	Population B
	%	
0 min	9.0	0
60 min	6.0	0
90 min	23.2	0

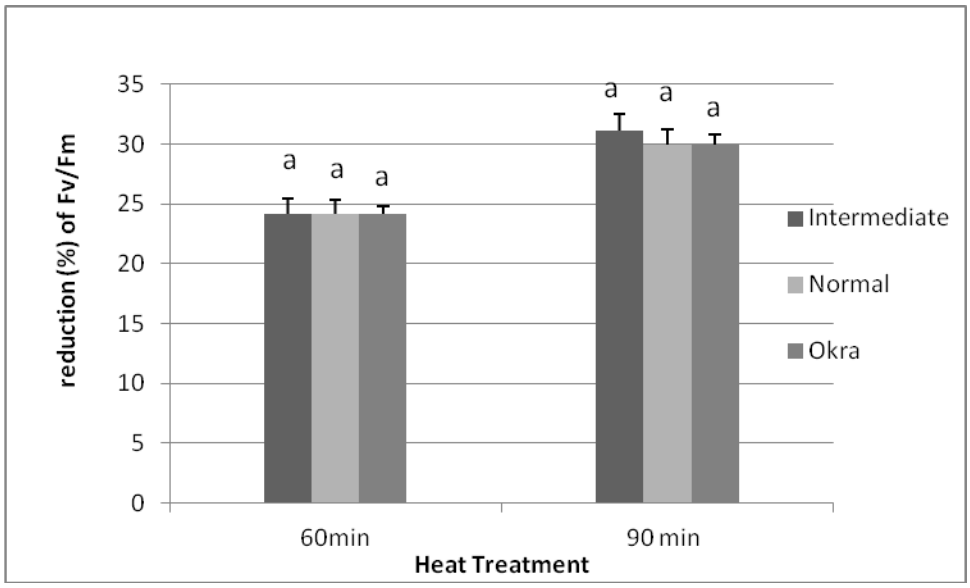


Figure 9 Reduction of F_v/F_m (%) for different leaf shape categories after 60 min and 90 min of heat treatment at 45℃ and 80% humidity in the dark growth chamber

Lsmeans within each treatment with different letters differ ($p<0.05$)

Table 11 Analysis of variance of Φ_{PSII} for $F_{2:3}$ populations in the growth chamber test

Population A				
Source of variation	Num DF	Den DF	F value	P value
Treatment	1	1	59.86	0.0818
Population vs parent	2	3	0.84	0.5449
Treatment ×Population vs parent	2	296	2.46	0.0871
Population B				
Source of variation	Num DF	Den DF	F value	P value
Treatment	1	1	37.22	0.1034
Population vs parent	2	2	2.90	0.2565
Treatment ×Population vs parent	2	244	3.07	0.0482

Table 12 Statistics of Φ_{PSII} of parents and progeny in each $F_{2:3}$ population in the growth chamber test

	Before treatment				After treatment			
	Max	Min	Mean	STD†	Max	Min	Mean	STD
Population A								
DP 90	0.80	0.26	0.63a	0.14	0.69	0	0.30a	0.19
TX 2287	0.79	0.58	0.70a	0.07	0.68	0.05	0.40b	0.20
$F_{2:3}$ progeny	0.80	0.45	0.66a	0.08	0.63	0.02	0.31a	0.16
Population B								
DP 90	0.8	0.26	0.63a	0.14	0.69	0	0.30a	0.19
TX 2454	0.78	0.61	0.70b	0.06	0.67	0	0.43b	0.22
$F_{2:3}$ progeny	0.82	0.53	0.70b	0.05	0.65	0.03	0.37b	0.13

Means within each column with different letters differ ($p<0.05$)
†STD: Standard Deviation

Table 13 Analysis of variance of Φ_{PSII} of $F_{2:3}$ lines within each population in the growth chamber test

Source	df	Expected mean square	Mean square		p-value	
			before treatment	after treatment	before treatment	after treatment
Population A						
Replicate	1	$\sigma^2_e+76\sigma^2_b$	0.0122	0.0311	0.0857	0.1178
Genotype	75	$\sigma^2_e+2\sigma^2_g$	0.0084	0.0375	0.0009	<.0001
error	72	σ^2_e	0.004	0.0124		
Population B						
Replicate	1	$\sigma^2_e+64\sigma^2_b$	0.0062	0.0028	0.0296	0.6144
Genotype	63	$\sigma^2_e+2\sigma^2_g$	0.0027	0.0234	0.0711	0.002
error	58	σ^2_e	0.0018	0.011		

Table 14 Estimated variance components and broad heritability of Φ_{PSII} for the $F_{2:3}$ lines in the growth chamber

Source	Variance estimate			
	Population A		Population B	
	Before treatment	After treatment	Before treatment	After treatment
Replicate	0.0001 ± 0.0032†	0.0003 ± 0.0042	0.0001± 0.0020	0
Genotype	0.0022 ± 0.0004	0.0126 ± 0.0010	0.0005 ± 0.0002	0.0062 ± 0.0008
error	0.004 ± 0.0002	0.0124 ± 0.0003	0.0018 ± 0.0001	0.011± 0.0003
Heritability	52.38 ± 9.52 %	67.02 ± 5.32 %	35.71 ± 14.29 %	52.99 ± 6.84 %

† Variance estimate ± Standard error

(a) After 24 hr of the pre-treatment

(b) After 24 hrs of the heat treatment

(c) After 24 hrs of the recovery

Figure 10 Pictures of $F_{2:3}$ lines in the growth chamber test

From the left to the right, the first row is TX 2454, the second row are DP 90 (the front two plants) and FM 966 (the back two plants), the third row are SG 747 (the front two plants) and Maxxa Acala (the back two plants) and the rest are the $F_{2:3}$ progeny (each line or accession has two plants).

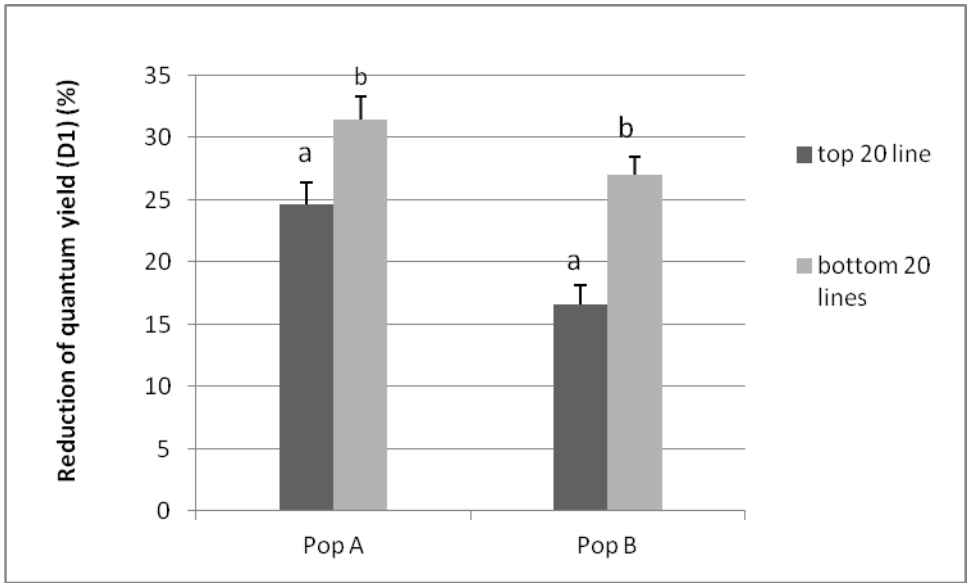


Figure 11 D_1 (%) (Reduction percentage of quantum efficiency Φ_{PSII}) of 20 top lines and 20 bottom lines in each $F_{2:3}$ population in the growth chamber test

LSMEANS within each population with different letters differ ($p<0.05$)

Table 15 Stand (%) of $F_{2:3}$ progenies and parents in the field, 2012

	Max (%)	Min (%)	Mean (%)	STD (%)
Population A	100.00	0.00	64.84	24.44
Population B	100.00	0.00	48.94	26.49
DP90	13.33	0.00	5.83	4.27
TX2287	43.33	23.33	34.17	8.33
TX2454	46.67	30.00	39.17	7.39

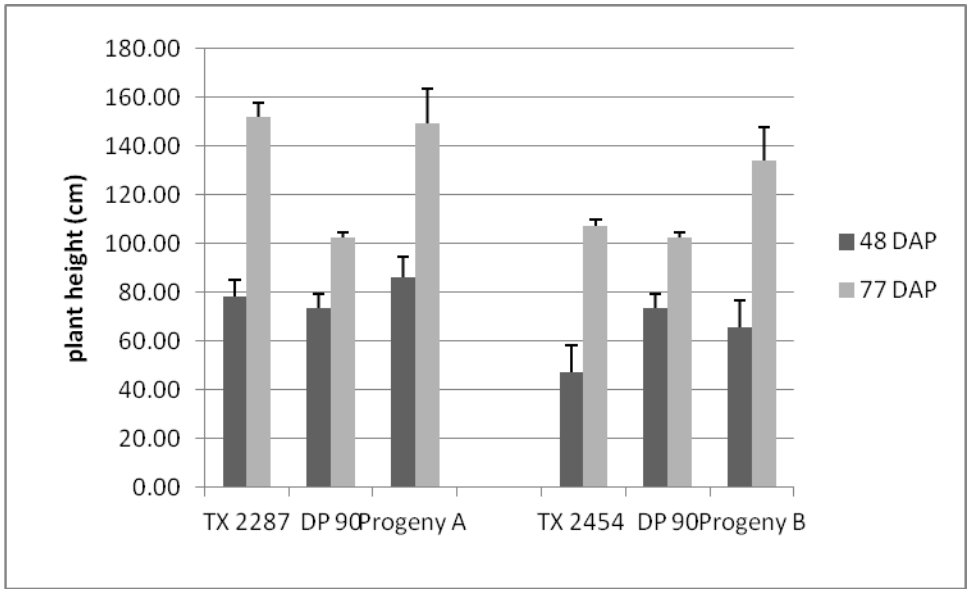


Figure 12 Plant height of parents and $F_{2:3}$ progenies in the field test on 48 and 77 days after planting

Table 16 Tests of fixed effects for Φ_{PSII} of $F_{2:3}$ populations in the field test

Population A				
Effect	Num DF	Den DF	F Value	P value
Temperature	1	3	1.40	0.3222
Population vs parent	2	4	2.49	0.1981
Temperature×Population vs parent	2	4	3.64	0.1258
Population B				
Effect	Num DF	Den DF	F Value	P value
Temperature	1	3	2.25	0.2304
Population vs parent	2	5	1.46	0.3162
Temperature×Population vs parent	2	5	0.39	0.6969

Table 17 Φ_{PSII} of $F_{2:3}$ populations and parents in the field test

Population A		
	high temperature	mild temperature
TX 2287	0.63 ±0.04a†	0.60± 0.03a
DP90	0.50 ± 0.04b	0.58± 0.04a
$F_{2:3}$ population	0.55 ± 0.02ab	0.60 ± 0.02a
Population B		
	high temperature	mild temperature
TX 2454	0.53 ± 0.04a	0.58 ± 0.04a
DP90	0.50± 0.04a	0.58± 0.04a
$F_{2:3}$ population	0.56± 0.03a	0.60 ± 0.03a

Lsmeans within the same column with different letters differ (P <0.05)

†Lsmeans ± Standard Error

Table 18 Analysis of variance for Φ_{PSII} of $F_{2:3}$ lines in the field test

Population A				
	mean square		P value	
	High temperature	Mild temperature	High temperature	Mild temperature
Block	0.0171	0.0472	0.0605	<.0001
Day	0.3063	0.0853	<.0001	<.0001
Genotype	0.0078	0.0064	0.0868	0.0596
Block*Genotype	0.0077	0.0058	0.0909	0.1511
Day*Genotype	0.0051	0.0046	0.8632	0.7684
Block*Day	0.0131	0.0102	0.0729	0.0489
Error	0.0059	0.0050		
Population B				
	mean square		P value	
	High temperature	Mild temperature	High temperature	Mild temperature
Block	0.0139	0.0327	0.0769	<.0001
Day	0.4305	0.2438	<.0001	<.0001
Genotype	0.0115	0.0039	0.0002	0.3328
Block*Genotype	0.0068	0.0048	0.2193	0.0684
Day*Genotype	0.0062	0.0038	0.4127	0.3945
Block*Day	0.0086	0.0017	0.1933	0.7605
Error	0.0060	0.0036		

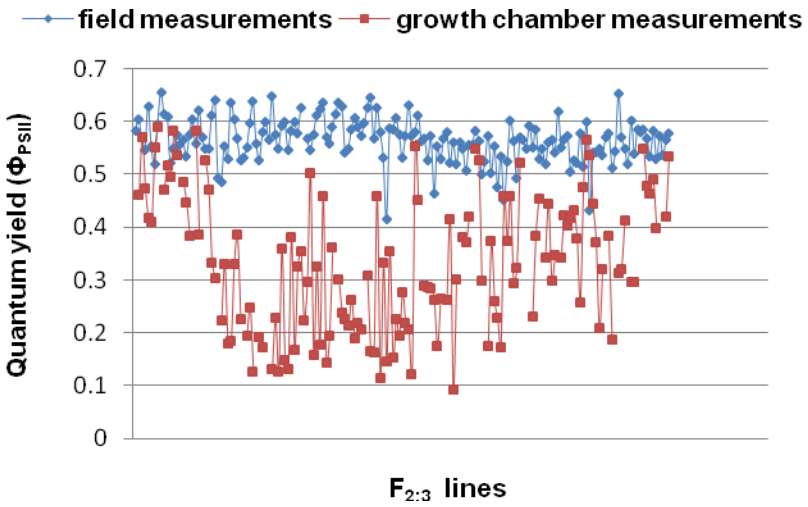


Figure 13 Correlations of Φ_{PSII} between field measurements and growth chamber measurements of $F_{2:3}$ lines (population A and population B)

Field measurements included measurements on high temperature days

Growth chamber measurements included measurements after the heat treatment

3.4 Determination of correlations between chlorophyll fluorescence and seed and fiber-related traits

3.4.1 Seed trait test of $F_{2:3}$ populations

Due to their photoperiodic sensitivity, wild parents (TX2287 and TX 2454) and some of the $F_{2:3}$ lines did not produce bolls at the harvest season. Therefore this studies seed and fiber tests only included the lines with bolls (53 $F_{2:3}$ lines of population A and 29 $F_{2:3}$ lines of population B). Tests for fixed effects showed there was significant difference among populations (population A, population B and checks) with lint index and lint percent ($p< 0.05$) (Table 19). Analysis of variance within each population showed that the variation of each seed trait was mainly from the effect of line factor rather than the block factor. The effect of line was significant for most of seed traits except for boll size and seed per boll in population A. This effect was significant for all seed traits in population B. However, it was only significant in seed index for checks (Table 20)

The lsmeans of seed traits were compared between $F_{2:3}$ populations (population A and population B) and checks (DP 90, FM966, Acala Maxxa and SG 747) (Table 21). With respect to boll size (g), means of $F_{2:3}$ populations were lower than checks numerically. However, the difference was not significant. Seed per boll exhibited the same rank as boll size. Population A had the highest seed index (100 seeds weight, g) of all and it was different from population B. The seed index (g) of the parents intermediated between that of population A and population B. The mean lint index (100 seeds lint weight, g) of population A and population B were noticeably lower than checks. The mean lint percent (%) of checks also showed apparent outperformance over population A and population B. Overall, $F_{2:3}$ populations have lower means than commercial lines for most seed traits. However, $F_{2:3}$ progenies were better than exotic parents

(Although seed traits of the two wild parental accessions were not available due to their photoperiodic nature, average seed trait of 44 wild accessions is noticeably lower than adapted cultivars according to the data from the Germplasm Resources Information Network (GRIN) (http://www.ars-grin.gov/npgs/college.html) shown in Table 1).

3.4.2 Fiber quality test of $F_{2:3}$ populations

Fiber quality is important to the technological value of cotton fiber. Azhar et al., (2009) found that heat tolerant accessions has more stable yield and better fiber quality than heat susceptible accessions. Analysis of variance showed that there were significant variations among populations (population A, population B and checks (DP 90, FM966, Acala Maxxa and SG 747)) with regard to fiber length, fiber strength and elongation ($p<0.05$) (Table 22). Analysis of variance within each population showed that the variation of each fiber quality trait was mainly stemmed from the effect of line factor whereas the effect of the block factor contributed little to the total variation. The effect of line was significant for all fiber quality traits in population A and population B except for UHM. In contrast, the effect of line factor was not significant for checks in terms of all fiber quality traits (Table 23).

Fiber quality traits of population A, population B and checks are summarized in Table 24. Fiber length is defined as the average length of the longer one-half of the fibers (upper half mean length, UHML) and it influences yarn strength and thus affects the efficiency of the spinning and weaving processes. Both $F_{2:3}$ population means were higher than commercial lines, but only population B was significantly different from checks.

Fiber uniformity is the ratio between the mean length and the UHML of cotton fiber. Improvement of uniformity of fiber length would increase the spinning efficiency and reduce

wastage of fibers during processing. Average fiber uniformity of population A and of population B was classified as average uniformity (80-82) whereas that for checks was ranked as high uniformity (83-85) (USDA,2005). However, the differences among $F_{2:3}$ populations and checks were not significant.

Fiber strength expresses the force required to break a bundle of fibers one tex unit in size and is an important factor determining the value and the price of cotton fiber. Both means for $F_{2:3}$ populations and checks were identified as very strong fiber (above 31) (USDA, 2005). Population B produced stronger fiber than checks whereas the fiber strength of population A was lower than checks.

Micronaire measures the fineness of cotton fiber. Micronaire and fiber maturity are closely correlated within a cotton genotype. High micronaire values (above 5.0) are suggestive of coarse fiber whereas low (below 3.5) values are suggestive of immature fiber. Checks had higher fiber micronaire than population A and population B, but the difference was not significant statistically.

Fiber elongation and short fiber content (SCF) are traits for which plant breeders normally do not directly select. Fiber elongation is elongation before break. Fiber elongation is influenced by environmental conditions, such as temperature, light intensity, mineral nutrition and water (Bradow and Davidonis, 2000). The mean fiber elongation of $F_{2:3}$ populations was inferior to check cultivars. This indicated $F_{2:3}$ populations had a “brittle” fiber that was more prone to damage during ginning. The short fiber content (SFC) (resulting from fiber damage) is an indirect measure of fiber uniformity. Checks exhibited the lowest SCF (%). Overall, fiber traits of $F_{2:3}$ lines did not show much deviation from adapted cultivars. However, there is difference between population A and population B. Hague et al., (2011) found that F_2 populations had

intermediate fiber micronaire, length and strength between the high parent (TAM 182-39ELS, unreleased line) and the low parent (DP 50). To increase the genetic diversity of upland cotton, day-neutral lines converted from race accessions have been used to improve fiber quality traits and yield (McCarty et al., 2003, 2004). In my study, I conclude that it should be possible to select in later generations of backcrossing to improve both fiber quality and heat tolerance in upland cotton.

Numerical indexes have been developed by incorporating weighted values of fiber quality properties, including Q-score 1 (QS1) and Q-score 2 (QS2). They are used to evaluate the overall value of cotton fiber and facilitate comparisons in cultivar testing and selection in breeding trials. To calculate Q-scores, fiber quality properties are first normalized to 0 to 1 and then they are combined with quality-weighting factors (Bourland et al., 2010). However, weighted values of fiber quality properties are different for quality scores. QS1 put high weight on fiber length (50%) and fiber micronaire (25%) while QS2 is more related to fiber strength (50%) and fiber uniformity (30%) (Table 25). In terms of QS1, population A and population B were slightly higher than checks indicating the $F_{2:3}$ populations had longer fiber length and better fiber fineness than commercial lines. In contrast, checks had a slightly higher QS2 value than population A and population B. This suggests that fiber strength and fiber uniformity of $F_{2:3}$ populations did not outperform checks (Fig.14). Bourland et al., (2010) discarded individual plants with Q-score 1 lower than 50 in breeding programs.

Overall, in this environment with moderate heat stress, $F_{2:3}$ populations had fiber quality comparable with commercial lines. However, these lines only represented the limited number of lines which were able to produce mature bolls.

3.4.3 Correlations between chlorophyll fluorescence and seed and fiber-related traits

Pearson correlation coefficients among seed traits, fiber quality traits and chlorophyll fluorescence are presented in Table 26. The field chlorophyll fluorescence measurement (under high temperatures) of $F_{2:3}$ lines was positively correlated with most of the seed traits (boll size, seed per boll, lint index and seed index), some of fiber quality traits (uniformity, elongation, UHM) and fiber quality indexes (QS1 and QS2), and negatively correlated with plant height. Among them, chlorophyll fluorescence was highly associated with boll size, seed index and plant height ($p<0.1$). This indicated that under field heat stress, lines with higher chlorophyll fluorescence had higher capability of photosynthetic tolerance to heat stress and thus were able to produce larger bolls, larger seeds and grew taller. This indicates that heat tolerance in the vegetative growth stage can contribute to stress tolerance during the reproductive growth stage. Thus heat stress, although it was moderate during 2012, had less damaging effects on the boll development and fiber production in lines with higher chlorophyll fluorescence values than heat susceptible genotypes. However, this relationship may be affected by plant age. The correlation coefficients between wheat yield and biomass with F_m and F_v was markedly higher when measurements were taken within 10 days after heat stress than when plant senesced (Moffatt *et al.*, 1990). These relationships between chlorophyll fluorescence and fiber qualities were also influenced by environmental conditions. Longenberger et al., (2009) conducted a two-year and two-location field test to evaluate the drought tolerance of twenty upland cotton genotypes using chlorophyll fluorescence technique. They found that the pearson correlation coefficients for fiber qualities or lint yield versus chlorophyll fluorescence (F_v'/F_m') were not consistent across years, locations, growth stages and treatments.

Long-term effect of high temperature is different from acute effect of high temperature because long-term high temperature impacts crop development. Kropff *et al*. (1993) found that rice yields decrease 9% with every degree increase in seasonal average temperature. Heat stress is one of the factors that influence cotton lint yields in Arizona. Lint yields in mild heat stress years have been 11.20-28.47 g/m^2 higher than in high heat stress years (College of Agriculture and Life Sciences, 2008). Moffatt et al., (1990) found that there is positive correlation between grain yield and F_v for six wheat genotypes differing in adaption regions in a field test. It is remains to be demonstrated whether heat tolerant introgressed lines identified by chlorophyll fluorescence can demonstrate improved lint yield under the stressed condition.

Plant height is a trait indicative of the adaptation of these $F_{2:3}$ lines. Exotic types generally have greater plant height. It was negatively associated with seed trait related to yield and most of the fiber quality related traits. The reason could be attributed to their photoperiod sensitivity. A taller plant has a stronger vegetative growth under the local environmental condition. This result was consistent with another heat tolerance study of upland cotton accessions in Pakistan. They found that plant height was significantly negatively associated with boll weight and gin turnout (Azhar *et al.*, 2009).

Based on the above relationships and the general lack of negative relationships between chlorophyll and seed traits as well as fiber quality traits, six $F_{2:3}$ lines in population A and four in population B were selected as the elite $F_{2:3}$ lines with excellent fiber quality, and seed traits as well as heat tolerance measured by photosynthetic efficiency (Table 27).

Table 19 Tests of fixed effects for seed traits of $F_{2:3}$ lines

	Num DF	Den DF	F value (P-value)				
			boll size	seed per boll	seed index	lint index	lint percent
Population	2	6	0.88 (0.46)	0.54 (0.61)	3.63 (0.09)	5.68* (0.04)	15.56* (0.0042)

*represents F value significant at the 0.05 significance level

P-values are in parentheses

Table 20 Analysis of variance for seed traits of $F_{2:3}$ lines within each population in the field

Population A						
	DF	F value				
		Boll size	seed per boll	seed index	lint index	lint percent
block	3	0.94	0.31	1.87	5.29*	2.76*
line	52	1.34	1.08	1.88*	3.25*	3.29*
error	60					
Population B						
	DF	F value				
		Boll size	seed per boll	seed index	lint index	lint percent
block	3	1.24	0.98	1.67	0.98	0.07
line	28	5.48*	2.79*	5.12*	5.90*	6.70*
error	31					
Checks						
	DF	F value				
		Boll size	seed per boll	seed index	lint index	lint percent
block	3	1.8	2.77	0.96	0.13	0.45
line	3	0.14	1.6	10.59*	4.04	0.75
error	6					

*represents F value is significant at the 0.05 significance level

Table 21 Lsmeans of seed traits of $F_{2:3}$ populations and checks

	boll size (g)	seed per boll	seed index(g)	lint index (g)	lint percent (%)
Population A	3.67±0.12a†	22.70±0.49a	10.40±0.15a	5.63±0.17a	34.60±0.46a
Population B	3.61±0.15a	22.78±0.67a	9.76±0.19b	5.81±0.20a	37.13±0.58b
Checks	4.15±0.37a	24.50±1.66a	9.98±0.51ab	7.30±0.47b	42.03±1.43c

Lsmeans within each column with different letters differ (P< 0.05)

† Lsmeans ± Standard Error

Table 22 Tests of fixed effects for fiber quality traits for $F_{2:3}$ lines

	Num DF	Den DF	F value (p-value)					
			MIC	UHM	UI	STR	Elo	SFC
Population	2	6	1.27 (0.35)	11.92* (0.0081)	1.72 (0.26)	9.79* (0.01)	9.87* (0.01)	0.01 (0.99)

*represents F value is significant at the 0.05 significance level

P-values are in parentheses

Elo: elongation before break；

SCF:percent short fiber content in percent；

MIC: micronarie；

UHM: upper half meanfiberlength in inch；

UI: percent fiber uniformity index；

STR:fiber bundle strength in grams per tex；

Table 23 Analysis of variance for fiber quality traits of $F_{2:3}$ lines within each population in the field

Population A							
	DF	F value					
		MIC	UHM	UI	STR	Elo	SFC
block	3	3.55*	0.84	1.85	0.88	0.95	1.98
line	52	2.30*	2.91*	2.13*	1.97*	2.53*	1.92*
error	60						
Population B							
	DF	F value					
		MIC	UHM	UI	STR	Elo	SFC
block	3	1.00	0.22	1.06	0.09	1.33	1.32
line	28	3.14*	1.79	2.34*	2.09*	2.69*	3.68*
error	31						
Checks							
	DF	F value					
		MIC	UHM	UI	STR	Elo	SFC
block	3	1.34	0.23	3.02	0.22	0.74	1.47
line	3	1.1	1.79	1.08	1.52	1.22	0.33
error	6						

*represents F value is significant at the 0.05 significance level
Elo: elongation before break
SCF: percent short fiber content in percent
MIC: micronarie
UHM: upper half meanfiberlength in inch
UI: percent fiber uniformity index
STR:fiber bundle strength in grams per tex

Table 24 Cotton fiber quality traits of the $F_{2:3}$ populations and checks

Population	Micronaire (units)	Upper Half Mean Length (inch)	Uniformity Index (%)
Population A	4.3±0.1a	1.15±0.01a	82.8±0.2a
Population B	4.0±0.1a	1.20±0.01b	82.3±0.2a
Checks	4.3±0.3a	1.12±0.02a	83.1±0.5a
	Fiber strength (Gram/tex)	Elongation (%)	Short Fiber Content (%)
Population A	31.3±0.2a	5.4±0.1ab	7.8±0.1a
Population B	32.9±0.3b	4.8±0.1b	7.8±0.1a
Checks	32.4±0.8ab	5.5±0.3a	7.8±0.3a

Lsmeans within each column with different letters differ ($P< 0.05$)
Lsmeans $\pm$ Standard Error

Table 25 Weighting values used for calculation of fiber quality index (QS1 and QS2)

	Weighting value	
Factor	QS1	QS2
Length	0.50	0.10
Micronaire	0.25	0.10
Uniformity	0.10	0.30
Strength	0.15	0.50
Short Fiber Index	0.00	0.00
Elongation	0.00	0.00

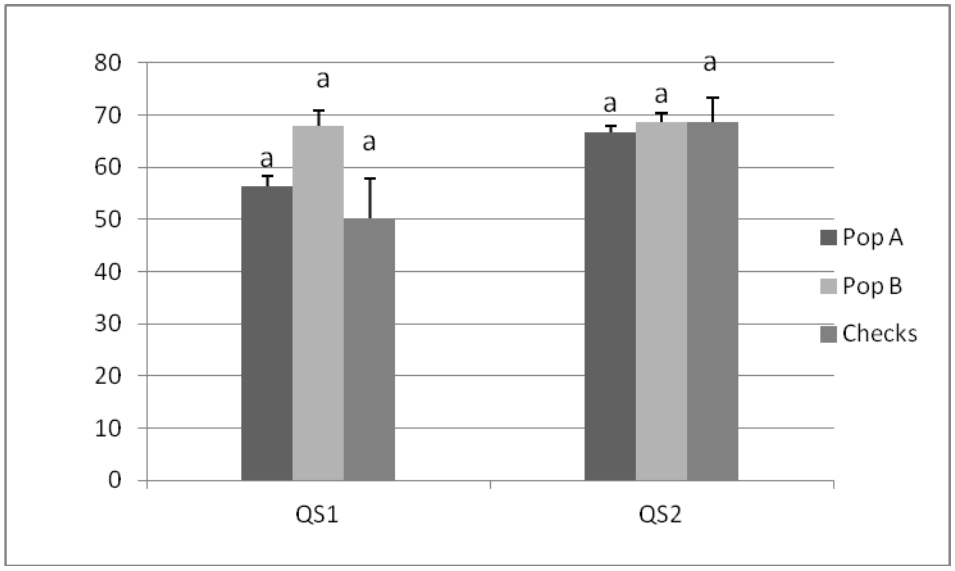


Figure 14 Fiber quality index (QS1 and QS2) for the $F_{2:3}$ populations and checks

Lsmeans within each fiber quality index with different letters differ (P< 0.05).

Table 26 Correlation among seed traits, fiber quality and chlorophyll fluorescence

	Seed traits				Fiber quality traits	
	Seed per boll	Seed index	Lint index	Lint percent	Micronaire	UHM
Boll size	0.9203**	0.6142**	0.6991**	0.3882**	0.4077**	0.2729*
Seed per boll		0.3212**	0.4552**	0.3128**	0.3878**	0.1578
Seed index			0.5221**	-0.0879	0.1387	0.3402**
Lint index				0.7966**	0.3596**	0.2815**
Lint percent					0.3174**	0.0775
Micronaire						-0.3706**
UHM						
Uniformity						
Strength						
Elongation						
SCF						
QS1						
QS2						
Chlorophyll (field)						

Table 26. Correlation among seed traits, fiber quality and chlorophyll fluorescence (Continued)

	Fiber quality				Fiber quality index			
	Uniformity	Strength	Elongation	SCF	QS1	QS2	Chlorophyll (field)	Plant height
Boll size	0.4343**	0.0744	0.1847	-0.3168**	0.4917**	0.4475**	0.2949**	-0.2573*
Seed per boll	0.2843**	-0.0185	0.1920	-0.2229	0.3670**	0.2922**	0.2308	-0.2180
Seed index	0.5744**	0.0972	0.1837	-0.4003**	0.4855**	0.5362**	0.3477*	-0.1243
Lint index	0.3088**	0.1907	0.0555	-0.1946	0.3723**	0.3500**	0.1692	-0.2171
Lint percent	-0.0454	0.1300	-0.0600	0.0814	0.0766	0.0191	-0.0361	-0.1678
Micronaire	0.1418	-0.2994**	0.4291**	-0.1868	-0.1112	0.0019	-0.0036	-0.0286
UHM	0.4394**	0.6267**	-0.5683**	-0.4041**	0.7913**	0.6002**	0.1504	-0.2179
Uniformity		0.3210**	0.0022	-0.8057**	0.7155**	0.9145**	0.2071	-0.0775
Strength			0.4326**	0.3974**	0.4934**	0.5288**	-0.0537	0.0319
Elongation				-0.0274	-0.3688**	-0.1686	0.0234	0.0829
SCF					-0.5692**	-0.7299**	-0.0099	0.1089
QS1						0.8791**	0.2020	-0.2756*
QS2							0.1887	-0.0965
Chlorophyll (field)								0.2359*

*Correlation coefficient is significant at α=0.1;

**Correlation coefficient is significant at α=0.05.

Chlorophyll (field) is the Φ_{PSII} under the high temperature condition in the field

Table 27 Selected $F_{2:3}$ lines with high chlorophyll fluorescence as well as good seed and fiber traits in two populations

population	line	bollsize	seedperboll	seedindex	lintindex	QS1	QS2	chlorophyll†
A	A146	4.34	25.51	10.84	6.12	93.00	87.00	0.47
A	A131	4.45	29.44	9.89	5.15	86.00	82.33	0.59
A	A219	4.08	24.26	11.04	5.58	66.50	74.50	0.58
A	A226	3.53	24.86	9.41	4.69	70.00	74.00	0.54
A	A638	3.92	25.26	9.68	5.85	47.00	70.00	0.50
A	A254	3.68	23.96	10.30	4.91	59.50	67.00	0.58
B	B326	3.90	23.17	11.34	5.37	83.50	81.00	0.57
B	B630	4.23	25.11	10.50	6.35	84.67	75.33	0.55
B	B742	4.19	26.96	9.63	5.66	56.25	61.75	0.49
B	B233	2.51	18.84	9.38	3.80	63.33	59.33	0.45

† Chlorophyll measured in the field under high temperatures

4 Conclusions

Based on selection by chlorophyll fluorescence measurements, it was clear that wild accessions in the selected group were more tolerant to heat stress than a set of random accessions and check cultivars in the growth chamber. In the field, three out of top nine accessions in the selected group significantly outperformed checks under the high temperature (>35 ℃). Consequently, chlorophyll fluorescence is a broadly based, high throughput method capable of assaying the physiological status of heat tolerance, and as such may be a useful screening tool for identifying useful stress tolerant resources. However, this phenotyping technique is sensitive to environmental conditions. Multiple locations and years of field tests are required for validation.

Further research of heat tolerance with two introgressed populations of wild accessions showed that there was variation for mid-parent heterosis of leaf sample chlorophyll fluorescence values. This suggested that different gene combinations may be involved in these two F_2 populations. No correlation was found between leaf shape and chlorophyll fluorescence measurement in one of populations that was segregating for leaf shape.

The broad-sense heritability of chlorophyll fluorescence measurement was above 50 % after exposure to high temperatures in the growth chamber for both $F_{2:3}$ populations. However, it was zero in the field for both $F_{2:3}$ populations. The marked difference in heritability could be attributed for two reasons. First, the heat treatment in the growth chamber was kept constant whereas the environmental conditions in the field varied widely from day to day. Therefore, there was much more environmental variation contributing to the phenotypic variation in the

field than in the growth chamber. Second, the precipitation during the growing season was high in 2012 in the field study and the temperature in the measurement days was milder than in the growth chamber (the maximum was 36.1 ℃). This stress condition was not sufficient to distinguish among the $F_{2:3}$ lines for phenotypic heat tolerance, as demonstrated by chlorophyll fluorescence. The treatment temperature in the growth chamber was 45 ℃, much higher than that in the field. Testing under extreme heat conditions may aid the ability to separate genotypes efficiently. Based on the above results, we conclude that it is possible to improve heat tolerance in upland cotton by selecting under controlled conditions. Selections in the field would be required to be replicated in multiple environments with more high temperature days.

Most seed traits for $F_{2:3}$ lines was lower than that of the adapted cultivars, whereas the most fiber quality traits for $F_{2:3}$ lines was comparable with adapted cultivars. A positive correlation was found between chlorophyll fluorescence measurement and seed traits as well as between chlorophyll fluorescence and fiber quality index in both $F_{2:3}$ populations. Since there were no negative correlations between chlorophyll fluorescence and most seed and fiber traits, simultaneous selection for heat tolerance and seed and fiber quality traits should be possible.

Although, chlorophyll fluorescence measurement varies with environmental conditions as well as populations, it still can be used as a quick and easy screening tool for detection of vegetative heat tolerance, especially in photoperiodic, non-adapted wild genotypes. Utilization of this technique should be cautious to the impact of the surrounding environment in the field. Wild accessions or introgressed lines identified that may have potential as genetic materials for development of adapted heat tolerant germplasm.

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