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Challenges and Perspectives on Improving Heat and Drought Stress Resilience in Cotton

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ABSTRACT

Global climate change in the form of rising temperatures and increasingly variable rainfall patterns, along with heightened competition for scarce natural resources, potentially threatens the sustainability of cotton cropping systems. Thus, future cotton production is likely to occur under an increased prevalence of multiple abiotic stresses, including extreme and prolonged high temperatures and water deficits. Therefore, it is of increasing relevance that the combined effects of heat and drought stresses on cotton productivity are more comprehensively examined under field conditions. This article reviews the separate influences of heat and drought stress on cotton, outlines known effects of the combination of high temperature and water deficit on cotton and model plant species, discusses the genetic dissection of heat or drought stress tolerance traits in cotton, investigates the potential of field-based phenotyping methods for evaluating the response of cotton plants to heat and drought stresses, and, finally, offers perspectives on the development of stress-resilient cotton germplasm. Importantly, the integration of approaches from several disciplines is needed to allow cotton breeders to efficiently develop superior cultivars for optimal stress resilience in a farmer's field.

Cotton (*Gossypium* spp.) is the most important fiber crop in the world, with more than 125 million bales produced in the 2011/2012 season. China was the top producer of cotton at 34 million bales, followed by India (29 million bales), the U.S. (15.6 million bales), Pakistan (10.6 million

bales), and Brazil (8.7 million bales) (USDA, 2013). However, climate change on a global scale in the form of rising temperatures, increasingly variable rainfall patterns, and scarcity of freshwater resources potentially threatens the long-term sustainability of cotton production.

In the past couple of years, Texas and the southeastern cotton-growing regions of the U.S., which together account for more than 60% of domestically grown cotton (NASS, 2012), experienced record high temperatures and sustained periods of drought, resulting in a concomitant reduction in cotton lint yield and continued depletion of groundwater reserves. It is estimated that the Ogallala Aquifer—one of the largest aquifers in the world and a major source of groundwater in Texas—dropped nearly 0.55 meters (median change of 26 wells) between 2010 and 2011 (Neffendorf and Hopkins, 2013). During the 2011 drought in Texas alone, a record 55% of planted cotton acres were abandoned with an estimated economic loss of approximately $2.2 billion U.S., exemplifying the economic devastation brought on by a severe weather episode (Anderson et al., 2012).

Cotton lint yield is dependent on several factors such as genotype, environment, and management. In addition to the individual effects of these factors, the interactions among them also affect crop production (Romagosa and Fox, 1993). Furthermore, crops often are exposed simultaneously to multiple abiotic stresses such as high temperature and limited water supply under field conditions. Even though cotton cultivars are typically well adapted to specific growing environments and selected for maximum genetic potential, the lack of available water and exposure to high temperature often can act as an insurmountable barrier for a cotton crop to reach its maximum yield potential. To help mitigate these environmental effects, it is common management practice to increase soil moisture holding capacity through the use of a cover crop or minimum tillage. When implemented, these management practices could help bolster yield performance of locally adapted, stress-tolerant cotton cultivars in dryland (rainfed) cropping systems.

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The phenotypic consequences of combined heat and drought stresses on cotton or even model plant species have not been dissected thoroughly at the physiological and genetic levels. Experiments exposing cotton plants to one or more abiotic stresses in highly controlled growth facilities undoubtedly will provide insights into altered cotton physiology and metabolism. However, to decisively close the yield gap for sustainable cotton production over the next few decades, cotton breeding lines will need to be evaluated rigorously in the presence of multiple abiotic stresses under field conditions. By testing cotton in the field, different types of abiotic stresses can be imposed on plants at the community level in the presence of natural soil and weather conditions that are impossible to replicate accurately in growth chambers and greenhouses.

In this review, we focus on the independent influence of heat and drought stress on cotton development and physiology. We highlight findings of previous works that studied the combined effect of heat and drought stresses in cotton and other plant species. Next, we review quantitative trait loci (QTL) mapping of traits related to heat or drought tolerance and discuss the potential application of field-based phenotyping tools for abiotic stress-tolerance traits. Finally, we offer perspectives on the genetic improvement of cotton for environments that are prone to high temperature and water-limited conditions. Interested readers can refer also to previous reviews on other aspects of abiotic stress in cotton (Loka and Oosterhuis, 2012; Loka et al., 2011; Lubbers et al., 2007; Oosterhuis and Snider, 2011; Saranga et al., 2009; Snider and Oosterhuis, 2012).

EFFECTS OF HEAT STRESS ON COTTON

The reproductive growth phase of cotton is most vulnerable to heat stress. This is of primary concern given that peak bloom can co-occur with periods of high temperature throughout many of the cotton-producing regions in the U.S. (Brown, 2008). There are several routes through which heat stress at peak bloom can negatively affect the reproductive performance and eventual lint yield of cotton (Brown and Zeiher, 1997; Hodges et al., 1993; Oosterhuis and Snider, 2011; Reddy et al., 1992b; Snider and Oosterhuis, 2012). Growth chamber, greenhouse, and field studies conducted by the University of Arizona's Cooperative Extension revealed that heat stress produces floral abnormalities approximately

15 d after the initial exposure to high temperature (Brown, 2001, 2008; Brown and Zeiher, 1997). These floral abnormalities include smaller inflorescences, shorter anther filaments with nondehiscent anthers, and asynchronous development of reproductive organs—all of which decrease cotton reproductive performance.

To understand the further effects of heat stress on cotton production, Reddy et al. (1992b) conducted a series of growth chamber experiments to examine square, flower, and boll retention in the presence of day temperatures ranging from 30° to 40° C, a temperature range up to 8° C higher than the predicted critical temperature threshold for cotton (Schlenker and Roberts, 2009). High day/night temperatures of 40°/30° C resulted in extremely low boll retention. Boll weight at this high temperature regime was only 0.8 g plant^{-1} compared to the more optimum day/night temperatures of 30°/20° C where boll weight was 143.3 g plant^{-1}. In a second, complementary study, Reddy et al. (1992a) showed that mainstem node addition rate was positively correlated with day/night temperatures up to a tested maximum of 40°/32° C, but as day temperatures increased above 30° C, there was a decrease and increase in the number of fruiting and vegetative branches, respectively, produced by cotton plants.

Pollen development, tube growth, and fertilization are postulated to be the most heat-sensitive stages of the reproductive growth phase in cotton (Zinn et al., 2010). In a study identifying proper methods for in vitro analysis of cotton pollen, Burke et al. (2004) found that pollen germination rates decreased as temperature increased to 40° C and that germination of pollen was almost completely arrested at 43° C. Through an in vivo analysis, high temperature was also shown to affect reproductive development by decreasing the number of fertilized ovules due to a lower rate of pollen tube growth (Snider et al., 2009, 2011). Importantly, such decreased fertilization efficiency could lead to higher boll abortion rates; thus, high fertilization efficiency should be considered a target of selection when breeding for heat tolerance in cotton.

The influence of heat stress on germinating seeds and developing seedlings has yet to be explored extensively in cotton. This is a potentially important area of research because some cotton production areas, such as India, experience high temperature at planting and seedling establishment. In one of the few studies in this research area, Ashraf et al. (1994)

showed that the germination percentage, shoot fresh and dry weights, leaf turgor potential, leaf soluble proteins, total amino acids, and epicuticular wax content of five cotton cultivars were affected under heat stress at germination and early seedling development. With direct application to genetic improvement, a tentative positive correlation was observed between heat tolerance at germination and in later growth stages, suggesting that selection for heat-tolerant cotton lines could be performed at the seedling stage.

In cotton, high temperature can reduce the production and movement of newly fixed carbon assimilates to growing organs. For example, Snider et al. (2009) showed a decline in carbohydrate allocation to flowers from subtending leaves in the presence of heat stress. This decline in carbon supply was in combination with subtending leaves having lower photosynthetic rates and photochemical efficiency of photosystem II (Φ_{PSII} and F_v/F_m). In a second, related study, Snider et al. (2010) further showed that the photosynthetic apparatus of subtending leaves of a thermotolerant cotton cultivar had enhanced pre-stress antioxidant enzyme activity relative to a thermosensitive cotton cultivar. High night temperature also can reduce carbohydrate (sucrose and hexose) content due to increased respiration (Loka and Oosterhuis, 2010) and, when coupled with the disruption of carbon assimilation, could result in carbon starvation at periods of peak energy demand such as flowering and boll filling.

Even though heat stress disrupts key cellular processes, biochemical and physiological mechanisms have evolved that allow cotton to tolerate or avoid heat stress. Carbon fixation at high temperature could be enhanced through induced expression of an isoform of Rubisco activase that is speculated to prevent the dissociation of activase subunits (Law et al., 2001). In response to high temperature and other abiotic stresses, heat-shock proteins (HSPs) are "molecular chaperones" that accumulate to prevent protein misfolding and aggregation in virtually all plants (Vierling, 1991). In cotton, HSPs were shown by Fender and O'Connell (1989) to be induced at 37° C, with expression peaking at 45° C. Given the importance of Rubisco activase and HSPs in the heat-stress response, the genes encoding these proteins should be considered for use in the development of transgenic thermotolerant cotton.

Heat avoidance is a physiological mechanism built on high stomatal conductance that allows Pima cotton (*Gossypium barbadense* L.) to attain high productivity under irrigation in hot, dry environments. In a seminal study, Radin et al. (1994) constructed a Pima cotton population by crossing an elite, heat-adapted Pima cultivar to a heat-sensitive, Sea Island-type landrace from the Caribbean and evaluated the resultant F_2 progeny under thermal stress in an irrigated, low-desert cotton production region of Central Arizona. In the F_2 population, stomatal conductance and leaf temperature were found to have a strong inverse relationship. The authors showed that directional selection for increased stomatal conductance, and thereby higher evaporative leaf cooling via transpiration (i.e., heat avoidance), allowed for more bolls per plant during the hottest periods of the growing season and thus higher yields. Given these findings and the expectation of sufficient genetic variation, continued selection for high stomatal conductance could help to further increase the yield of irrigated Pima cotton grown in high-temperature, semiarid environments (Lu et al., 1998).

EFFECTS OF DROUGHT STRESS ON COTTON

Upland cotton (*Gossypium hirsutum* L.), which comprises nearly 90% of the cotton grown worldwide, is believed to have been first domesticated in the semiarid regions of southern Mexico and northern Guatemala (Brubaker et al., 1999; Brubaker and Wendel, 1994). Even though cotton likely is adapted to periodic drought episodes, its optimum production for high lint yield requires between 2,158 and 3,906 m^3 of water each growing season, depending on local cultivation practices and meteorological patterns (McWilliams, 2003). Therefore, production of cotton is highly vulnerable to increasingly variable rainfall patterns and diminishing fresh water resources. The developmental stages for which adequate water supply is most important include stand establishment, pre-bloom, and post-boll set (McWilliams, 2003). The amount of moisture needed per stage depends greatly on plant transpiration and soil evaporation (Allen et al., 1998). Consequently, the timing, duration, and severity of water deficit throughout the life cycle of cotton dictate potential yield losses (Boman and Lemon, 2006; Edmisten et al., 2007; McWilliams, 2003).

When the demand for water by cotton exceeds the available supply in a period of insufficient rainfall or irrigation, drought stress can develop and manifest itself through a number of modified phenotypes. The

most apparent effect of drought stress on plants is a reduction in height relative to irrigated plants (Pace et. al., 1999; Pettigrew, 2004b). Additionally, in general, there are also decreases in leaf area, leaf dry weight, root diameter, and node number, whereas the ratio of root to shoot dry weight increases as roots elongate to seek additional water within the soil column (Ball et al., 1994; Eaton and Ergle, 1952; Pace et al., 1999; Pettigrew, 2004b; Shahenshah and Isoda, 2010; Wilson et al., 1987). Interestingly, Ball et al. (1994) showed that root elongation was less sensitive to drought stress than leaf expansion. Given their connection to productivity and resilience (Lynch and Brown, 2012), it would be worthwhile to evaluate the extent of natural variation for root architecture traits in addition to canopy architecture traits in diverse cotton germplasm grown under water deficit.

Not only is the reproductive growth phase of cotton sensitive to heat stress, but it is also sensitive to prolonged periods of drought stress. Drought-stressed cotton plants experience earlier cutout (i.e., final stage of cotton plant growth before boll opening) compared to irrigated cotton plants, thus having a prematurely shortened flowering period later in the growing season (Pettigrew, 2004a). The work of Guinn and Mauney (1984) revealed that severe water deficits limit the yield of cotton by reducing the number of bolls, in part by decreasing flowering and boll retention; however, high boll load also partly contributed to decreased retention of bolls. Both the vertical and horizontal distribution of bolls moving up the main stem nodes and out along sympodial branches, respectively, are altered by drought stress (Gerik et al., 1996; Pettigrew, 2004a). Such a response allows irrigated cotton plants to typically have a greater number of bolls from the increased number of higher vertical and more distal horizontal fruiting sites relative to drought-stressed cotton plants. In addition to the number of bolls produced per area, boll weight, seed formation, seed and lint indexes, as well as fiber length, uniformity, maturity, strength, and elongation are all negatively affected by drought stress (Eaton and Ergle, 1952; Gerik et al., 1996; Grimes et al., 1969; McMichael and Hesketh, 1982; Pettigrew, 2004b; Saranga et al., 1998b; Wen et al., 2013).

The closing of stomata from a reduction in turgor of guard cells, mediated via an abscisic acid signaling cascade, is one of the primary physiological responses that a cotton plant evokes in response to drought stress (Ackerson, 1980). The resultant decrease in stomatal conductance to minimize transpirational water loss also lowers the intercellular CO_2 concentration, potentially resulting in a stomatal limitation of photosynthesis (Chaves et al., 2003; Wise et al., 1992). This effort by the plant to conserve water also could lead to higher leaf temperature (Carmo-Silva et al., 2012; Pettigrew, 2004b; Shahenshah and Isoda, 2010), as the capacity for evaporative leaf cooling is diminished under high temperature (Radin et al., 1994). Under severe drought stress, the nonstomatal inhibition of photosynthesis from metabolic limitations such as decreased synthesis of ribulose-1,5-bisphosphate (RuBP) might become more prominent (Tezara et al., 1999). Similar to what was found for heat-stressed cotton plants by Snider et al. (2009), chlorophyll fluorescence measurements on drought-stressed cotton plants by Deeba et al. (2012) revealed that the operating (Φ_{PSII}) and maximum efficiency of photosystem II (F_v/F_m) were lower compared to control plants.

There are a tremendous number of molecular responses at the cellular level in drought-stressed cotton plants. One of the unavoidable penalties of drought stress is the heightened production of reactive oxygen species (ROS) from pathways such as the photosynthetic apparatus and photorespiration (Cruz de Carvalho, 2008). Even though ROS play an emerging important role in stress signaling, they are damaging to cellular membranes and components (Foyer and Noctor, 2009). However, these oxidative bursts are countered in part by the detoxifying ability of ROS scavenging enzymes. In cotton, the activities of the ROS scavenging enzymes superoxide dismutase, glutathione reductase, and ascorbate peroxidase were found to be elevated in a likely response to the drought-induced production of ROS (Deeba et al., 2012; Ratnayaka et al., 2003). Another way in which cotton plants seemingly attempt to cope with drought stress is through the active accumulation of inorganic and organic solutes such as alanine, citric acid, glycerol, myoinositol, potassium, and calcium to lower the osmotic potential in a process called osmotic adjustment that helps cells retain water and maintain turgor pressure (Levi et al., 2011; Zhang et al., 1999).

Scratching the surface of the transcriptional regulatory network in the drought response, Park et al. (2012) identified several ROS scavenging enzymes, along with other defense/stress genes that included HSPs, which were differentially expressed in the leaf and/or root tissue of drought-stressed cotton plants. Also in this study and that of Payton et al.

(2011), drought stress resulted in a higher number of genes associated with metabolism being expressed differentially in roots relative to leaves. Such a finding could result from the increased production of organic solutes to reduce the osmotic potential in roots, or from the root metabolome simply being more responsive to water deficit or dehydration. To further enhance this research area, newly developed computational tools for RNA-seq transcriptome profiling in tetraploid cotton (Bowman et al. 2013) should be used to construct a drought expression atlas that is built on several types of tissues collected from multiple developmental stages for cotton lines with complementary existing or forthcoming whole-genome resequencing data.

Water use efficiency (WUE) can be defined as the total yield harvested per unit of water used over the entire growing season (Condon et al., 2004). Given the difficulty of this measurement, leaf carbon isotope ratio ($^{13}C/^{12}C$ expressed as $\delta^{13}C$) or carbon isotope discrimination commonly is used to provide a time-integrated estimate of long-term WUE. Cotton, a C_3 plant, naturally discriminates against ^{13}C during photosynthesis in favor of ^{12}C until intercellular CO_2 decreases from a high carbon assimilation rate or low stomatal conductance (Farquhar et al., 1982). Genetic variability for $\delta^{13}C$ exists in cotton germplasm pools and mapping populations (Lu et al., 1996; Saranga et al., 2001). In addition, positive correlations between $\delta^{13}C$ and WUE values were detected for several cotton cultivars evaluated under well-watered and water-limited field conditions (Saranga et al., 1998a). In contrast to these promising findings, in other irrigated and dryland field trials, inconsistent (Leidi et al., 1999) and weak negative correlations (Stiller et al., 2005) were found between yield and $\delta^{13}C$ for cotton cultivars. Therefore, the utility of $\delta^{13}C$ to indirectly select high WUE for maximal productivity and harvest index (Saranga et al., 1998a) is still largely an unanswered question in cotton.

EFFECTS OF COMBINED HEAT AND DROUGHT STRESSES ON PLANTS

It is believed that all cropping systems are affected by multiple abiotic stresses (Barnabás et al., 2008). For many years, the influences of high temperature and water deficit on the development and physiology of crop plants have been predominantly tested independently in controlled environments, although both commonly co-occur in crop production environments. When cotton has been field evaluated under drought in high-temperature semiarid or humid summer environments, the influence of drought in combination with high temperature was not regularly considered experimentally or statistically modeled to separate heat- and drought-stress effects. Even though this concept has not been explored extensively in a highly deliberate manner for cotton, we can glean knowledge from the few recent studies in cotton and other plant species where it has been evaluated.

In a study that focused on the differential physiological responses of C_3 and C_4 cereals, Machado and Paulsen (2001) independently and simultaneously imposed high temperature and drought on spring wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* L.) plants in highly controlled growth chambers. Several measures of water relations in both wheat and sorghum were strongly affected by high temperature in the presence of drought, whereas water relations were generally constant at high temperature with the soil moisture at field capacity. Shah and Paulsen (2003) showed that when combined with drought, exposure of spring wheat to high temperature intensified reductions in photosynthetic rate, stomatal conductance, leaf area, yield of biomass and grain, and WUE that were initially observed from only heat or drought stress. In a study of 10 natural accessions of the model plant *Arabidopsis thaliana* (L.) Heynh. (hereafter Arabidopsis), Vile et al. (2012) showed that the phenotypic effects from coincident heat and drought stresses appeared to be additive, but that some of the evaluated traits such as root allocation and specific leaf area responded specifically to only one of the two stresses.

One of the first integrated physiological and gene expression studies to understand how a plant responds to concurrent high temperature and drought was conducted by Rizhsky et al. (2002) in tobacco (*Nicotiana tabacum* L.). The combination of heat and drought stresses simultaneously reduced photosynthetic activity and stomatal conductance, while increasing respiration rate and leaf temperature. Gene expression profiling with a filter cDNA array revealed that the expression patterns of HSPs and ROS scavenging enzymes were more responsive to combined heat and drought stresses relative to either stress alone, suggesting that plants have a distinct gene expression response to both applied stresses. Several other studies also have demonstrated an increase in the gene expression level of HSPs in wheat

and maize under the combination of heat and drought stresses (Grigorova et al., 2011a,b; Hu et al., 2010).

In a more extensive study of gene expression patterns with a DNA microarray in Arabidopsis, Rizhsky et al. (2004) found nearly 1,000 transcripts up-regulated and more than 700 transcripts down-regulated in the leaves of Arabidopsis plants that had enhanced respiration and reduced photosynthesis from the imposition of both high temperature and drought. Nearly 42% of the transcripts differentially expressed under combined stress did not overlap with those differentially expressed under only high temperature or drought. Included among the many differentially expressed genes were HSPs and ROS scavenging enzymes, which had elevated expression in the leaves of plants that were exposed to both stressors. Taken together, the findings of these few studies suggest that both conserved and distinct mechanisms are involved in response to high temperature, drought, or their combination. However, all of the preceding studies were conducted in highly controlled environments, which can provide only limited insights into the biological processes influenced by these stresses under field conditions.

The study of Carmo-Silva et al. (2012) is one of the first field experiments that separated limitations on the photosynthetic apparatus into specific drought- and heat-stress effects. Several Pima cotton cultivars were grown under managed well-watered and water-limited conditions in a semiarid environment, allowing for a comprehensive physiological assessment of drought stress in the presence of high temperature. Similar to the physiological results showed by Rizhsky et al. (2002, 2004) in Arabidopsis and tobacco, the enhancement of stomatal closure in the drought-stressed Pima cotton cultivars resulted in a stomatal limitation on photosynthesis compared to the same cultivars under well-watered conditions. The lower stomatal conductance of drought-stressed plants limited their capacity for evaporative leaf cooling via transpiration at high day temperatures, leading to a significant increase in leaf temperature. It was this increase in leaf temperature that produced a moderate heat-stress effect on photosynthetic performance, which manifested itself as a diminished capacity to maintain Rubisco in an active state.

In a field study that analyzed the separate and combined influences of heat and drought stresses, 300 maize testcross hybrids representing tropical and subtropical maize improvement programs were evaluated across five Latin American, African, and Asian countries (Cairns et al., 2013). Through multienvironment field trials under managed stress conditions at the reproductive stage, no significant genetic relationships between grain yield under the combination of heat and drought stresses and either stress alone were detected in the maize hybrid panel. This result suggests that the genetic basis of tolerance to combined heat and drought stresses is distinct from that of individual tolerance to heat or drought stress. However, further research is needed to determine if these findings are extendable to other maize germplasm pools as well as to future climate-oriented cotton breeding programs.

GENETIC DISSECTION OF HEAT AND DROUGHT TOLERANCE TRAITS IN COTTON

Experimental efforts to identify QTL that are responsible for natural phenotypic variation in model plant and crop species have relied on linkage mapping in family-based populations with known pedigrees (Myles et al. 2009; Zhu et al., 2008). The power of this family mapping approach to resolve complex traits, however, is limited to only the recombination events that occurred when constructing the mapping population and a mere fraction of the phenotypically relevant variation existing for a species. Traditionally, family mapping has been used for the genetic dissection of heat and drought tolerance traits in cotton, limiting the localization of QTL to large chromosomal regions that span millions of base pairs. Perhaps more importantly is that the results of these studies potentially generate opportunities for cotton breeders to select on favorable QTL alleles with linked genetic markers in marker-assisted selection (MAS) breeding programs. Progress towards elucidating the genetic basis of abiotic stress tolerance in cotton has been reviewed extensively elsewhere (Lubbers et al., 2007; Saranga et al., 2009), thus we will only highlight a few of these QTL studies.

There is extensive genetic variation for physiological traits associated with abiotic stress tolerance within and between the two cultivated tetraploid cotton species, *G. hirsutum* and *G. barbadense*. With this knowledge, Saranga et al. (2004) conducted a QTL analysis for 10 physiological and productivity traits evaluated in an interspecific cotton (*G. hirsutum* × *G. barbadense*) mapping population replicated under well-watered and water-limited treatments in an arid, desert environment. Of the 79 QTL identified, 33 were

associated with osmotic potential, $\delta^{13}C$, chlorophyll *a* and *b,* and canopy temperature. The remaining 46 QTL were associated with dry matter, seed cotton yield, harvest index, boll weight, and boll number. Indicative of sensitivity to the environment, 27 (34.2%) of the 79 QTL were detected for only one of the two irrigation regimes. Given the statistical concordance of QTL and strength of phenotypic correlations between the physiological and productivity traits, only lower osmotic adjustment was found to appreciably contribute to higher seed cotton yield under drought stress (i.e., partially shared genetic architecture).

In a translational extension of the findings from Saranga et al. (2004), several of the identified favorable QTL alleles for seed cotton yield and three physiological traits were targeted by Levi et al. (2009) to develop near-isogenic lines (NILs) via a marker-assisted backcross strategy. The performance of NILs and parental lines was measured under well-watered and water-limited conditions in a multienvironment field trial. Although a yield advantage for NILs relative to the recipient parent (*G. barbadense* or *G. hirsutum* cultivar) was seldom detected, many of the NILs were observed to have the expected phenotypic outcomes of lower osmotic potential, higher $\delta^{13}C$, or increased leaf chlorophyll content. Notably, several of the NILs had unintended changes in potentially drought-adaptive traits that included stomatal conductance, specific leaf weight, leaf size, stomatal density, and epidermal cell size. These results highlight the inherent difficulty in the application of marker-assisted backcrossing for the genetic improvement of polygenic traits such as yield and drought tolerance (Xu, 2010). As a possible alternative to marker-assisted backcrossing, genomic selection (genome-wide selection) is now being intensely explored as an approach to accelerate the breeding cycle and enhance genetic gain per unit time for heritable polygenic traits in plant breeding programs (Bernardo and Yu, 2007; Heffner et al., 2009; Heslot et al., 2012; Lorenz et al., 2011; Meuwissen et al., 2001).

Although multiple QTL studies have made significant strides towards elucidating the genetic architecture of traits associated with improved drought tolerance in cotton, none have attempted to dissect the genetic basis of tolerance to combined heat and drought stress and each stressor alone in a single population. In essentially the only QTL study that exclusively focused on the response of cotton to high temperature, Ulloa et al. (2000) identified two

QTL associated with stomatal conductance at high day temperatures in a cotton mapping population that was evaluated under irrigated conditions in the semiarid environment of Maricopa, AZ. This QTL analysis was complemented by the use of divergent phenotypic selection on families of the mapping population to construct two groups with either low or high stomatal conductance, followed by irrigated field trials in Maricopa and the lower temperature environment of Las Cruces, NM. Stomatal conductance and lint yield were positively associated and only significantly different between the two contrasting groups in Maricopa. This suggests that high evaporative cooling via transpiration resulting from a large vapor pressure gradient between the leaf and the low-humidity air provides more of an adaptive advantage in terms of productivity when grown under irrigation in hotter, drier environments.

CONNECTING GENOTYPE TO PHENOTYPE WITH EMERGING TECHNOLOGIES IN COTTON

In the last few years, there have been spectacular advances in the development of technologies that have reduced radically the cost of DNA sequencing. Even though affordable whole-genome resequencing is nearly within reach, it is not yet at a cost that would allow most plant breeders to catalog exhaustively millions of sequence polymorphisms for a considerable number of breeding lines every year. Given that the number of markers needed by breeders for most genetic analyses is two orders of magnitude lower, genotyping-by-sequencing (GBS) is a suitable lower-cost alternative approach to simultaneously detect and score thousands of genome-wide single nucleotide polymorphism (SNP) markers across multiple individuals from diverse populations (Elshire et al., 2011; Poland et al., 2012). Although GBS has been remarkably rapid and robust for a wide range of crop species (Poland and Rife, 2012), modification to the GBS protocol of Poland et al. (2012) likely is needed to maximize the cost-effective concurrent discovery and genotyping of SNPs within cotton populations that have relatively lower levels of nucleotide diversity (Gore et al., 2014).

In stark contrast, the development of rapid, robust methods for plant phenotyping has followed a substantially slower evolutionary trajectory. The large-scale collection of accurate phenotypic data from plant populations growing in the field has

been historically, and continues to be, a significant limiting factor in terms of cost, time, and labor requirements for plant breeding programs, especially when considering the plethora of traits ranging from the whole-plant to the cellular level that potentially are predictive of yield and abiotic stress tolerance (Fig. 1). In terms of complex trait dissection studies in plants, phenotyping limitations have become ever more relevant as large mapping populations are needed to provide sufficient statistical power for resolving natural phenotypic variation down to causative variants with small effect sizes (Myles et al., 2009; Zhu et al. 2008). Thus, there is a pressing need for field-based systems that can accurately and efficiently phenotype large plant populations, allowing for the scale of complex trait dissection, MAS, and genomic selection efforts to increase to sizes already achievable for genomics studies (Cabrera-Bosquest et al., 2012; Cobb et al., 2013; Montes et al., 2007; White et al., 2012).

There is great promise in the use of remote sensing technologies for the rapid, nondestructive measurement of canopy traits associated with the response of cotton to heat and drought stresses in the field. Remote sensing with aircraft and satellite-based systems have provided informative canopy thermal emittance and spectral reflectance data for monitoring the growth patterns and physiological responses of a few cotton cultivars grown in sizeable plots or fields to local environmental conditions (DeTar and Penner, 2007; DeTar et al. 2006; Yang et al., 2006). Although these systems are certainly indispensable at the field and regional levels, the meter-scale spatial resolution often only afforded

by these systems unquestionably is inadequate for plant phenotyping studies. With regards to plant phenotyping, close range or proximal sensing at a spatial scale of at least 0.5-m resolution is essential. However, it is possible that low-altitude unmanned aircraft systems (UAS) will be able to provide routinely such needed resolution for proximal sensing of field-grown plants in the not too distant future (Berni et al., 2009).

Proximal sensing in cotton has been achieved initially through the use of hand-held, noncontact sensors while traversing field plots on foot (Hatfield et al., 1987; Stamatiadis et al., 2010). However, proximal sensing at walking speed becomes prohibitively restrictive if several temporally sensitive phenotypes such as canopy temperature and reflectance must be phenotyped within a narrow two-to-three-hour time interval on large cotton populations. Therefore, proximal sensing of the crop canopy for thousands of small field plots in a matter of hours with multiple types of sensors mounted to one of several possible types of vehicles having global positioning system enabled centimeter-level positional accuracy is likely to be more rapid, flexible, and reliable. There have been several recently developed ground-based vehicle systems that have made great strides towards meeting these criteria (Andre-Sanchez et al., 2014; Busemeyer et al., 2013; Comar et al., 2012; Lan et al., 2009; McCarthy et al., 2010). The interested reader is referred to a review by White et al. (2012) for an extensive discussion on the strengths and weaknesses of a high-clearance tractor, crane-like vehicle, cable robot, aerostat, and UAS as vehicles for proximal sensing.
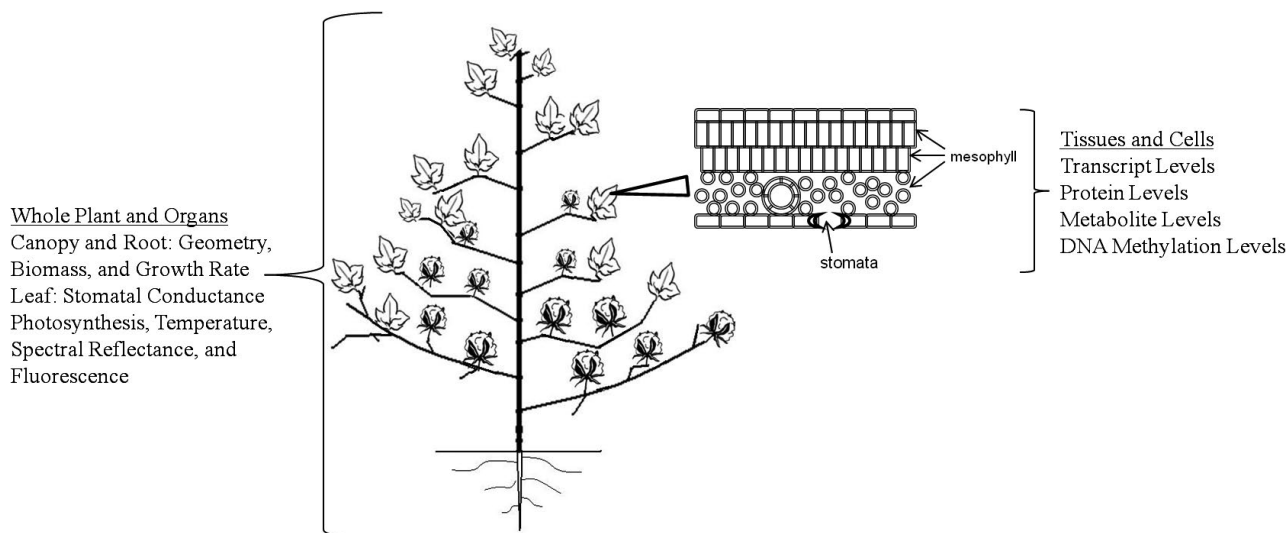

Figure 1. High-throughput phenotyping for the genetic improvement of cotton.

According to the Stefan-Boltzmann law, the emittance of radiation in the thermal infrared region of the electromagnetic spectrum by the crop canopy is proportional to its absolute surface temperature raised to the fourth power (Pinter et al., 2003). As discussed earlier, leaf temperature is influenced strongly by soil-plant-water relationships, with an increase in radiant leaf temperature when insufficient supply of water in the soil column limits transpiration and associated evaporative cooling effects. Importantly, the resultant higher temperature of leaves also can lead to heat-stress effects—for example, non-stomatal inhibition of photosynthesis (Carmo-Silva et al. 2012). There are a number of developed thermal plant water stress indices such as the crop water-stress index (CWSI) (Idso et al., 1981; Jackson et al., 1981) and stress degree day (Idso et al., 1977) that have been used to infer water status in cotton plants and other crops. Highlighting the prospective value of thermal indices, Pinter and Reginato (1982) and Pinter et al. (1983) calculated the CWSI from measurements of canopy temperature with a hand-held infrared thermometer (IRT) and showed the potential of the CWSI for scheduling irrigations and predicting yield under soil water deficit for cotton grown in semiarid environments.

In a study that demonstrated the possible usefulness of IRTs for screening cotton germplasm, Hatfield et al. (1987) used a hand-held IRT to measure canopy temperature for a diverse germplasm panel of cotton (*G. hirsutum*) accessions evaluated under irrigated and dryland field conditions in Lubbock, TX. The cotton accessions with a relatively higher canopy temperature under irrigation accumulated greater biomass production in the dryland environment, suggesting that the lower transpiration of certain cotton accessions helped them to better conserve soil moisture for later stage growth under dryland conditions. With four sets of IRTs mounted to a high-clearance tractor, Andrade-Sanchez et al. (2014) detected highly repeatable (broad-sense heritability) responses in canopy temperature at multiple times of day for a diverse panel of Pima cotton cultivars when grown under consistent and uniform irrigation conditions in a semiarid environment. As a complement to IRTs or perhaps an eventual replacement, thermal infrared imaging also has potential for screening the canopy temperature of breeding populations in the field (Jones et al., 2009).

Healthy green leaves strongly absorb light (i.e., low reflectance) in the photosynthetically active radiation (PAR) region (400-700 nm) of the electromagnetic spectrum, but weakly absorb light (i.e.,

high reflectance) in the near-infrared (NIR) region (700-1300 nm). It is this striking contrast between the reflectance properties of the PAR and NIR regions that produce a distinct transition or "red edge" for healthy green vegetation (Pinter et al., 2003). In contrast, there is generally higher and lower reflectance in the PAR and NIR regions, respectively, for senescent (non-green) or sparse vegetation and soil backgrounds. The measurement of crop canopy reflectance in the red and NIR wavelengths allow for the calculation of the normalized difference vegetation index [$NDVI = (NIR - Red)/(NIR + Red)$] (Tucker, 1979). It is important to emphasize that NDVI is not the measurement of a single agronomic parameter, but that it is a function of vegetation cover, biomass, leaf area index (LAI), and leaf chlorophyll concentration within the field of view of the sensor (Eitel et al., 2008). When implemented in cotton, time series NDVI data have been used to schedule the application of nitrogen fertilizer and predict lint yield, but for a perennial species with an indeterminate growth habit such as cotton, the prediction accuracy of nitrogen uptake and lint yield tend to be growth-stage dependent (Bronson et al., 2011; Plant et al., 2000). An important limitation of NDVI and the reason to explore other vegetation indices such as the near-infrared index (R_{810}/R_{560}) for prediction of lint yield is that NDVI can reach saturation (i.e., insensitive to changes in vegetation structure) at higher biomass and LAI (Guiterrez et al., 2012).

In contrast to the instantaneous nature of leaf temperature data, broad waveband vegetation indices such as NDVI have a delayed response to rapid onset drought stress of plants (Pinter et al., 2003). However, broad waveband vegetation indices are still somewhat useful for identifying drought-induced adaptive changes in canopy architecture from leaf wilting or rolling (Andrade-Sanchez et al., 2014; Jackson and Ezra, 1985). In a study that focused on the likely cumulative effects of reduced transpiration and photosynthesis on biomass and yield from a prolonged water deficit, Stamatiadis et al. (2010) detected a strong, positive correlation between seed cotton yield and canopy NDVI values at boll ripening for a single cotton cultivar. If there had been a striking imbalance between vegetative and reproductive growth that resulted in higher biomass and lower boll retention in this study, such as what can occur for cotton under high temperatures (Reddy et al., 1992a,b), then NDVI probably would have been less predictive of yield (Guiterrez et al., 2012). Given the aforementioned possibility and the ineffectiveness of NDVI

to identify specific types of abiotic stresses imposed on plants, narrower waveband indices such as the photochemical reflectance index (531 and 570 nm) for measuring photosynthetic radiation use efficiency (Gamon et al., 1997) also should be considered for proximal sensing. Importantly, narrower waveband indices respond more rapidly to drought stress because of their stronger association with plant physiological processes (Pinter et al., 2003). The interested reader is referred to a comprehensive review by Govender et al. (2009) that discusses the usefulness of many indices that incorporate the visible (leaf pigments), NIR (cell structure), and/or shortwave infrared (water content) regions for the measurement of plant-water stress.

Although foreseeably more laborious than non-destructive proximal sensing of the crop canopy, high-throughput phenotyping of cotton at the tissue and cellular levels could potentially provide deeper insights into the physiological and biochemical bases of heat and drought tolerance. In cotton, there has been exploration into the value of peptide mass fingerprinting, metabolite and mineral profiling, enzyme viability and activity, and cellular membrane thermostability for screening cotton germplasm for tolerance to heat or drought stresses (Azhar et al., 2009; Carmo-Silva et al., 2012; Cottee et al., 2010; Deeba et al., 2012; Levi et al., 2011; Rahman et al., 2004; Snider et al., 2010). As a prospective complement to the more traditional physiological and biochemical assays, the investigation of epigenetic modifications to DNA and chromatin could help to better resolve the genetic basis of adaptation to stress in cotton (Chinnusamy and Zhu, 2009). In cotton, differential cytosine DNA methylation patterns were detected between seedlings of two cultivars with either high or low tolerance to salt stress, with the salt-intolerant cultivar having higher levels of DNA hypermethylation (Zhao et al., 2010). Even though it is too early to draw definitive conclusions on the importance of DNA methylation in stress adaptation, it is tempting to speculate that this is yet another trait to consider when developing stress-tolerant cotton.

If field-based high-throughput phenotyping via proximal sensing is to become an integral component of modern public and commercial cotton breeding programs (Fig. 2), the collected data for traits will not only need to be highly heritable and genetically correlated with yield (Tuberosa, 2012), but also provide significantly valuable information for making future breeding decisions beyond what is already routinely provided by lint yield and fiber quality measurements.

It is important to recognize that yield is the product of multiple biological processes under the control of many genes having small effects that interact with the environment, and that the most advantageous combination of traits for one environment might not be transferable to a second environment (White et al., 2012). Therefore, it is highly improbable that a single trait would ever be sufficient to predict yield across a panel of diverse cotton cultivars evaluated in a multitude of environments. In that light, the selection of canopy traits to measure by proximal sensing for a particular cotton breeding population should be through collaboration between cotton breeders and physiologists in an effort to understand truly the phenotypic responses of cotton plants to heat and/or drought stresses in all of the target environments.

Which phenotypes should be targeted for selection in cotton breeding programs to enhance the development of stress-resilient cultivars? It is evident that high stomatal conductance (i.e., heat avoidance), which can be indirectly measured via canopy temperature with IRTs (Carmo-Silva et al. 2012), is strongly positively correlated with cotton yield under high irrigation in a dry, hot environment. Vegetation and narrower band indices derived from multi- or hyperspectral data hold promise for the accurate prediction of cotton yield under prolonged drought stress at specific developmental stages (Li et al., 2001; Stamatidas et al, 2010), but certainly any detected significant association between indices and yield of diverse cotton cultivars must be tested for stability across many target environments. In addition to these traits, there is supporting evidence in the literature that osmotic adjustment (Saranga et al. 2004), fertilization efficiency (Snider et al., 2009, 2011), root architecture (Ball et al., 1994), and boll retention (Reddy et al. 1992b) should be explored further to evaluate their heritabilities and correlations with yield and/or fiber quality traits under high temperature and water deficit field conditions. Most importantly, prior to embarking on a long-term phenotypic selection program for any combination of these suggested and possible additional traits, serious consideration should be given to initially evaluating the potential value of constitutive or stress-responsive traits (Blum, 2006) for tolerance to coincident drought and heat stresses in cotton simulation models for a wide range of production environments (Thorp et al., 2014). Such a simulation modeling approach was highly successful for estimating the yield benefit from the increased capture of water by drought-adaptive root architectural traits in wheat (Manschadi et al., 2006).
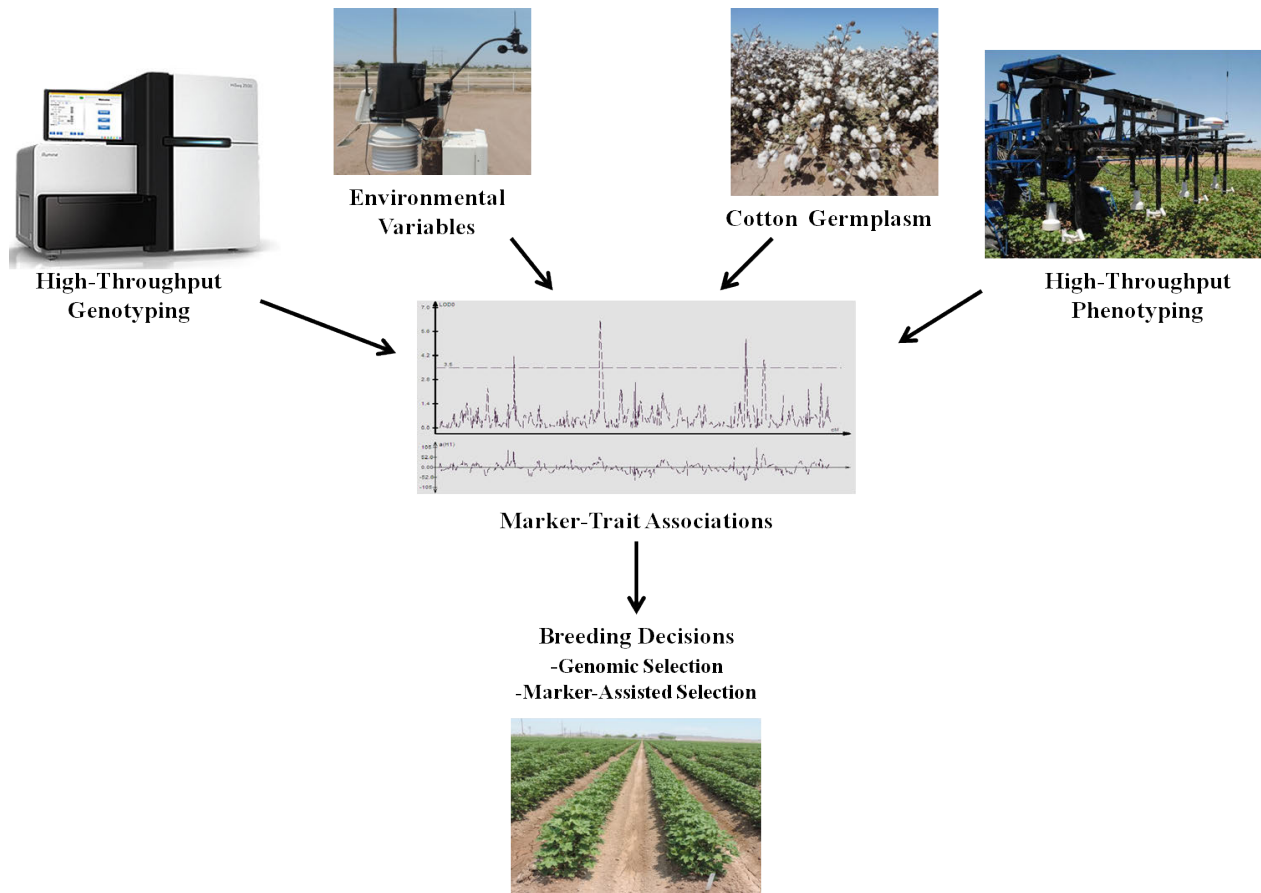
Figure 2. Overview of cotton genetic improvement. Hi-Seq 2500 image from www.illumina.com.

PERSPECTIVES

There has been a tremendous amount of discussion on the selection for high WUE in plant breeding programs centered on the development of stress-resilient cultivars, especially for rainfed (dryland) environments. However, the debate continues on whether total yield harvested per unit of water used over the growing season is the ideal target trait for crop improvement in water-limited environments. In a review by Blum (2009), he gave clarity to the term WUE and posited why this trait should not be targeted when breeding for high yield under drought stress. Notably, Blum highlighted experimental findings from a number of studies to argue that selection for high WUE will ultimately result in the development of cultivars that are lower yielding with reduced tolerance to drought. Alternatively, he proposed that plant breeders should instead be selecting for constitutive and stress adaptive traits that contribute to the effective use of water (EUW) by maximizing the capture of soil moisture for transpiration and minimizing water

depletion from soil evaporation. With that to consider, selection should be targeted on traits that increase EUW for transpiration such as reduced nonstomatal transpiration, more vigorous seedling growth, increased osmotic adjustment, and deeper root systems.

We can also apply Blum's theory of EUW to the development of cotton cultivars that are both heat- and drought-tolerant. Among the potentially many negative phenotypic consequences that can result from the co-occurrence of both stressors, an increase in leaf temperature and reduction of photosynthesis are almost certain (Carmo-Silva et al., 2012). Therefore, stomatal conductance needs to be maintained at some reasonable level to allow for the evaporative cooling effect from transpiration and reduction of stomatal and non-stomatal limitations on photosynthesis (Carmo-Silva et al., 2012; Radin et al., 1994). In cotton, it has been shown that the highest yielding cotton cultivars grown under irrigated, hot, dry conditions tend to also have the highest stomatal conductance (Cornish et al., 1991; Lu et al., 1994, 1998; Radin et al., 1994; Ulloa et al., 2000). If we can maintain a minimal level of

transpiration to offset the negative phenotypic effects from both heat and drought stresses while limiting unnecessary water loss, it might be possible to select for higher yield under the combination of high temperature and drought conditions.

If breeders continue to select for high WUE, defined by Condon et al. (2004) as selecting for varieties that limit transpiration, there will likely be an increase in the incidence of yield losses when a combination of both stresses occurs in a farmer's field. However, moisture levels in most cotton production areas do not support maximum transpiration rates during high temperature. To circumvent this strategy, we need to start thinking about particular developmental stages where high EUW is crucial, such as germination and pre-bloom, and what stages need sufficient transpiration to withstand high temperature—for example, during peak bloom. This type of action will likely come from a transgenic event that can be activated upon an environmental cue or chemical application (Corrado and Karali, 2009; Padidam, 2003). Although this technology has not seen widespread adoption in crop plants, it is a technique that, when combined with breeding for locally adapted, high yielding cotton cultivars, might provide farmers the most resilient cotton plant to withstand both high temperature and drought.

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