Scientific Research Publishing

American Journal of Plant Sciences, 2017, 8, 517-532
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Genetic Diversity in the Environmental Conditioning of *Gossypium hirsutum* and *Gossypium barbadense* Cultivars

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How to cite this paper: Burke, J.J. (2017) Genetic Diversity in the Environmental Conditioning of *Gossypium hirsutum* and *Gossypium barbadense* Cultivars. *American Journal of Plant Sciences*, **8**, 517-532.
https://doi.org/10.4236/ajps.2017.83036

Received: January 14, 2017
Accepted: February 20, 2017
Published: February 23, 2017

Abstract

Enzyme adaptations to temperature occur constantly as temperature patterns modulate diurnally and seasonally. These adaptations entail qualitative and/or quantitative metabolic changes that often provide a competitive advantage, impact adjustment to new environments, and effect the survival of the species. Changes in isozymes or allozymes, changes in enzyme concentration, modification by substrate and effectors, and metabolic regulation of enzyme function without changing enzyme composition are all possible strategies for adaptation to changes in temperature. The degree of adaptation among cotton cultivars to a specific thermal regime may be difficult to determine from phenotypic responses of the plants. The present study evaluated the thermal sensitivity of *Gossypium hirsutum* L. and *Gossypium barbadense* L. cultivars following growth under distinct thermal environments. The metabolic fitness of *Gossypium hirsutum* L. and *Gossypium barbadense* L. cultivars showed that the *Gossypium hirsutum* L. cultivars grown in a 28˚C/20˚C day/night cycle tended to be better equipped to cope with a 16 h - 38˚C treatment than the same cultivars grown in a 38˚C/32˚C day/night cycle. The *Gossypium barbadense* L. cultivars, on the other hand, grown in a 38˚C/32˚C day/night cycle tended to be equipped to cope with a 16 h - 38˚C treatment than the same cultivars grown in a 28˚C/20˚C day/night cycle. The *Gossypium hirsutum* L. line TX 303 is an exception to these general trends as its responses were similar to the *Gossypium barbadense* L. St. Vincent and Pima S-7 cottons.

Keywords

Cotton, *Gossypium hirsutum* L., *Gossypium barbadense* L., Thermal Sensitivity, Adaptation

1. Introduction

Enzyme adaptations to temperature occur constantly as temperature patterns modulate diurnally, seasonally, or over centuries. These adaptations entail qualitative and/or quantitative metabolic changes that often provide a competitive advantage, impact adjustment to new environments, and effect the survival of the species. Changes in isozymes or allozymes, changes in enzyme concentration, modification by substrate and effectors, and metabolic regulation of enzyme function without changing enzyme composition are all possible strategies for adaptation to changes in temperature. The concept of thermal kinetic windows arose from a desire to investigate temperature stresses in plants and the realization of a lack of knowledge about how to identify the optimal temperatures for metabolism. Thermal kinetic windows of optimal enzyme function were defined as the temperature range over which the value of the apparent Michaelis-Menten Constant (Km) was within 200% of the minimum apparent Km value observed for the enzyme [1]. The 200% cutoff value was used because previous studies [2] [3] had reported that enzymes could function optimally with Km values within 200% of the minimum Km value. The purpose of the thermal kinetic window was to provide a general indicator of the range of temperatures in which the optimal temperature for metabolism was located. The temperature ranges comprising the thermal kinetic windows for wheat and cotton were 17.5˚C to 23˚C and 23.5˚C to 32˚C, respectively. Although the TKWs were 5˚C to 8˚C in breadth, it was shown that these plants were only within the optimal temperature range of their TKWs for a fraction of the growing season [1]. These initial observations called for a re-evaluation of our understanding of the temperature stresses experienced by plants in the field. To date, thermal kinetic windows have been reported for numerous species [1] [4]-[9].

Plants have developed a myriad of metabolic protection systems that protect the cellular machinery from thermal injury when exposed to sub- or super-optimal temperatures. Wang *et al.* [10] reported that molecular control mechanisms for abiotic stress tolerance are based on the activation and regulation of specific stress-related genes. They reported that these genes are involved in the whole sequence of stress responses, such as signaling, transcriptional control, protection of membranes and proteins, and free-radical and toxic-compound scavenging. Diurnal air temperature fluctuations of 10˚C to 20˚C are common occurrences throughout temperate geographical regions. Because Thermal Kinetic Windows are only 5˚C to 8˚C in breadth, plants may be outside their optimal thermal range a large portion of every day [1]. Under these varying thermal conditions the efficiency with which absorbed light energy is harvested by photosynthesis is altered by a regulatory mechanism that determines how much excitation energy is used and how much is dissipated as heat [11]-[16]. Thermally induced changes in carbon assimilation and metabolism require that the balance between absorbed light energy, heat dissipation, and photochemistry be maintained [17] [18]. Over-excitation of the photosynthetic apparatus stimulates photo-oxidative damage that inhibits photosynthesis and reduces crop produc-

tivity [19]. The dissipation of excess light energy does not come without cost to the plant. NADPH that could have been used in carbon fixation is diverted to the xanthophyll cycle for use in the over-excitation protection mechanism [20]. The utilization of NADPH by the xanthophyll cycle can lead to a loss in carbon fixation and reduction in the amount of stored reserves.

Domestication of cultivated *Gossypium* species resulted in four species: the Old World diploids, *G. arboretum* and *G. herbaceum*, and the New World allotetraploids, *G. hirsutum* and *G. barbadense* cotton. The New World cottons dominate cotton production with *G. barbadense* primarily produced in Central Asia, Egypt, India, China, Sudan, and the United States. *G. barbadense* cotton has long, strong and fine fibers favored by the textile industry, but because of relatively low yields only represents about of 10% of current cotton production [21]. *G. hirsutum* cotton represents approximately 90% of the world's cotton production and is grown in over 40 countries in tropical and temperate regions [21].

The present study developed a method to determine the "metabolic fitness" of a plant grown in distinct thermal environments, and used this assay to evaluate *G. hirsutum* and *G. barbadense* cotton cultivar adaptation to two distinct thermal environments. The assay provides insights into the impact of growth temperature on the ability of *Gossypium hirsutum* L. and *Gossypium barbadense* L. cultivars to respond to vegetative thermal challenges.

2. Material and Methods

2.1. Optimization of the Evaluation Protocol

Cultural Practices: Greenhouses

Cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) seeds were planted into 16 cm diameter pots containing 900 g of Sunshine Mix #1 soil (Sun Gro Horticulture Distributors Inc., Bellevue, WA). Three seeds were planted per pot and pots were placed on benches in a greenhouse set to provide a 28˚C/20˚C day/night cycle, and a second greenhouse was set to provide a 38˚C/32˚C day/night cycle. Plants were thinned to one plant per pot and grown throughout the year. 430 W high-pressure sodium lights (P. L. Light Systems, Beamsville. ON Canada) were used to maintain a 16/8h photoperiod. Nutrients were maintained by daily application with Peters Excel fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) through an automated watering system. Plants were grown under greenhouse conditions to the eight-leaf stage prior to analyses. Cotton cultivars evaluated included Coker 312, Pima S-7, Sea Island, St. Vincent, PM 2200, PM 2145, SG 521, PM 2326, and the Texas race stock T303.SureGrow 521 and Paymaster cultivars (PM) were purchased from local seed dealerships and the Texas race stock T303, Sea Island, St. Vincent and Pima S-7were obtained via the U.S. National Plant Germplasm system.

Cultural Practices: Field

The cotton (*Gossypium hirsutum* L.) cultivar PM2326 was planted in a North-South orientation using a John Deere 7300 Max Emerge 2 VacuMeter Planter. The conventional tilled plots were treated with Prowl (1 quart per Acre)

for weed control. Eight – 61 meter rows of PM 2326 BG/RR were planted in an irrigated and a second set planted in a rain fed plot. The irrigated plants received 5 mm of water per day from underground drip lines located on 1 m centers. Nine kilograms per hectare of 28-0-0 N-P-K fertilizer was applied during the pre-plant irrigation. The rain fed plants received a total of 94 mm of rainfall during the first 80 days following planting. The irrigated plants received a total of 494 mm of water from irrigation and rainfall during the first 80 days following planting. Air temperatures averaged a low temperature of 15.7˚C and a high temperature of 30.3˚C throughout the growing season.

Leaf Position Effects on Tissue Resistance to a High Respiratory Demand:

A leaf punch was taken from each of the main stem leaves at solar noon, placed on moistened 3 MM filter paper in a Pyrex baking dish, covered with Glad Cling Wrap, and placed in the dark in a VWR Model 2005 incubator (Sheldon Manufacturing, Inc., Cornelius, OR) set to 38˚C. Six replicate punches obtained from different plants were evaluated for each leaf position following a 16 h incubation at 38˚C. Photographs were taken of the punches using a Sony DSC-F707 Digital Still Camera. Chlorophyll fluorescence was analyzed using an Opti-Science OS1-FL Pulse Modulated Fluorometer (Tyngsboro, MA). In practice, the efficiency of quantum yield (Fv/Fm) was envisaged as a relative measure of the overall ability of a tissue to withstand an elevated respiratory demand.

Light Intensity Effects on the Efficiency of Quantum Yield (Fv/Fm):

The cotton cultivar Coker 312 planted as described above were placed on benches in a greenhouse with a 28˚C/28˚C day/night cycle. Plants were grown under greenhouse conditions to the eight-leaf stage. One set of three plants was moved under a shade cloth to reduce the radiation load on the plants to 60% of full sunlight. The effect of light intensity on the subsequent measurement of the efficiency of quantum yield (Fv/Fm) of cotton leaf tissues was evaluated on leaf discs taken at solar noon from the fifth main stem leaves of cotton grown in full sunlight, or grown in full sunlight and transferred to shade 24 h prior to analysis. Three replicate punches obtained from different plants were evaluated following a 16 h incubation at 38˚C using an Opti-Science OS1-FL Pulse Modulated Fluorometer. The efficiency of quantum yield (Fv/Fm) of photosystem II was used as a measure of the plant's ability to withstand a prolonged respiratory demand arising from the 16 h - 30˚C dark treatment.

Water-Deficit Stress Effects on the Efficiency of Quantum Yield (Fv/Fm):

The cultivar PM 2326 was used to evaluate the effect of water-deficit stress on the metabolic fitness of field-grown cotton. The plants were grown according the protocol outlined in the "Cultural Practices: Field" section above. A leaf punch was taken from the fifth main stem leaf, placed on moistened 3 MM filter paper in a Pyrex baking dish, covered with Glad Cling Wrap, and placed in the dark in a VWR Model 2005 incubator (Sheldon Manufacturing, Inc., Cornelius, OR) set to 38˚C. Five replicate punches obtained from different plants in the irrigated and rainfed plots were evaluated hourly following being placed in the 38˚C incubator. Chlorophyll fluorescence was analyzed using an Opti-Science OS1-FL

Modulated Fluorometer (Tyngsboro, MA).

Determination of Greenhouse Air Temperatures:

Greenhouse temperatures were determined using a HOBO Pro RH/Temp Data Logger (Onset Computer, Bourne, MA) placed within the cotton canopy. Relative humidity and air temperatures were measured every 30 seconds over a 24 h period. Daily relative humidity levels ranged from 25% to 41% throughout the study.

Data Processing:

Statistical significance between genotypes and treatments were analyzed with studentized t-test through the statistical applications of Social Science Statistics (http://www.socscistatistics.com/tests/Default.aspx). Graphs were created using KaleidaGraph Version 4.1.3.

2.2. Evaluation of *G. hirsutum* and *G. barbadense* Adaptation to Different Thermal Environments

Determination of the Efficiency of Quantum Yield (*Fv/Fm*) *of Cotton Grown in a Cool and Hot Environment*:

Four *Gossypium hirsutum* L. cultivars (PM 2200, PM 2145, SG 521, and T303) and two *Gossypium barbadense* L. cultivars (Pima S-7 and Sea Island) were grown in one greenhouse set to provide a 28/20˚C day/night cycle, and a second greenhouse set to provide a 38˚C/32˚C day/night cycle as described under "Cultural Practices". Leaf punches were excised at solar noon from the fifth main stem leaves when the plants reached the eight-leaf stage. Six replicate punches obtained from different plants were evaluated following a 16 h incubation at 38˚C using an Opti-Science OS1-FL Pulse Modulated Fluorometer.

Comparison of Metabolic Fitness Indices with TTC Cell Viability Determinations:

Two *Gossypium barbadense* L. cultivars (Pima S-7 and Sea Island) were grown in one greenhouse set to provide a 28/20˚C day/night cycle, and a second greenhouse set to provide a 38˚C/32˚C day/night cycle as described under "Cultural Practices". Leaf punches were excised from the fifth main stem leaves when the plants reached the eight-leaf stage. Six replicate punches obtained from different plants were evaluated following either a 16 h incubation at 38˚C or 16 h incubation at 22˚C using an Opti-Science OS1-FL Pulse Modulated Fluorometer. Following analysis of the photosystem II fluorescence yield, leaf punches from each temperature exposure were transferred to 6 ml of 0.1 M phosphate buffer, pH 7.0 containing 0.8% 2,3,5-triphenyltetrazolium chloride (TTC) and incubated in the dark at 32˚C for 24 h. Following the incubation, the TTC was extracted from the leaf punches with 95% ethanol according to the procedure described by Burke (1994).

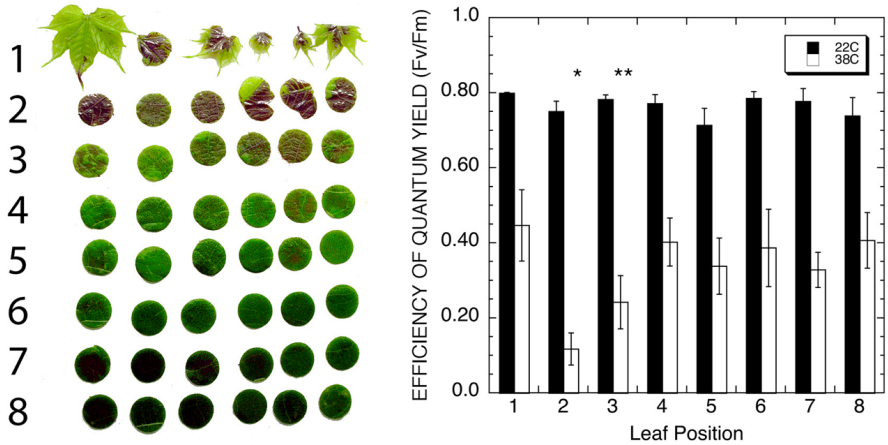
3. Results

3.1. Optimization of the Evaluation Protocol

Leaf Position Effects on Tissue Resistance to a High Respiratory Demand:

The chronological development of leaves takes a leaf from a metabolic sink as it first begins to develop, to a photosynthetic source as it expands and fully develops its photosynthetic potential, and gradually returning it to a metabolic sink as the ever developing canopy begins to shade the leaf from sunlight. It is reasonable to assume that these distinct developmental phases provide the leaf different metabolic tools with which to face environmental challenges. The present study evaluated the ability of leaves from different positions within the canopy to withstand a prolonged exposure to elevated temperature in the dark. These experiments were designed to help us determine if the ability to cope with the high respiratory demand might reflect the developmental stage of the leaf. Following a 16 h exposure to 38˚C, leaf punches taken from the first- to the eight-main stem positions showed differing levels of cellular injury (Figure 1). The most severe injury resulted in a darkening of the leaf punch from green to brown. The first main stem leaf showed partial injury as seen by the dark patches in the interveinal tissues (Figure 1). Leaf positions 2, 3, 7, and 8 exhibited potentially higher levels of cellular injury based upon the dark coloration. Leaf 4 showed minor cellular damage, and even less visual injury was seen in the punches from leaf positions 5 and 6.

The efficiency of quantum yield (Fv/Fm) of photosystem II chlorophyll fluorescence was used to further evaluate heat-induced injury in leaf punches from different leaf positions. High temperature-induced changes in chlorophyll fluorescence have been used as an indicator of thermal damage and correlates with the temperature at which leaves experience significant tissue necrosis [22]. The magnitude of the efficiency of quantum yield (Fv/Fm) decline following the 16 h - 38˚C heat treatment used to evaluate metabolic injury levels. Figure 1 shows a graph of metabolic fitness index determined for each main stem leaf position. High fluorescence levels were observed in the first leaf position compared with



* Significant $p < 0.05$, ** Significant $p < 0.1$

Figure 1. Photograph of replicate leaf samples following a 16 h - 38˚C heat treatment of the first eight main stem leaf positions. Main stem leaf positions are indicated by the numbers to the right of the punches. The graph shows the changes in the efficiency of quantum yield (Fv/Fm) associated with the different developmental stages of cotton leaf development. Error bars represent standard error values.

those of the second and third positions. The fluorescence values increased in leaf position four, and remained high in leaves five through eight. Based upon these findings, subsequent experiments were performed on leaves from the fifth main stem position.

Light Intensity Effects on the Efficiency of Quantum Yield (*Fv/Fm*):

A plant growing in sub- or supra-optimal temperatures will need to cope with excess incoming radiation because of metabolic limitations associated with thermal-induced enzyme inefficiencies. As the amount of light received by the plant increases, more metabolic resources are used to divert the radiation energy into heat, thereby protecting the photosystems from oxidation. NADPH, required for carbon fixation, is used by the xanthophyll cycle in the protection of the photosystems. Figure 2 shows the efficiency of quantum yield (Fv/Fm) of photosystem II of the fifth main stem leaves from plants grown in full sun, and from plants grown in full sun and covered with a shade cloth for 24 h prior to analysis. The shade cloth reduced incoming radiation by 60%. Efficiency of quantum yield (Fv/Fm) of photosystem II values of 0.8 are common for cotton leaves when removed from the plant. The leaves from the plant grown in full sunlight showed a reduction in efficiency of quantum yield from 0.8 to a value of 0.6. Plants that were moved to the shade prior to harvest maintained high efficiency of quantum yield (Fv/Fm) of photosystem II values throughout the test (Figure 2).

Water-Deficit Stress Effects on efficiency of quantum yield (*Fv/Fm*) *of photosystem II*:

Leaves of cotton grown under irrigated or rain fed conditions were evaluated for their efficiency of quantum yield (Fv/Fm) of photosystem II following exposure to elevated temperatures. The leaves from the irrigated plants showed a

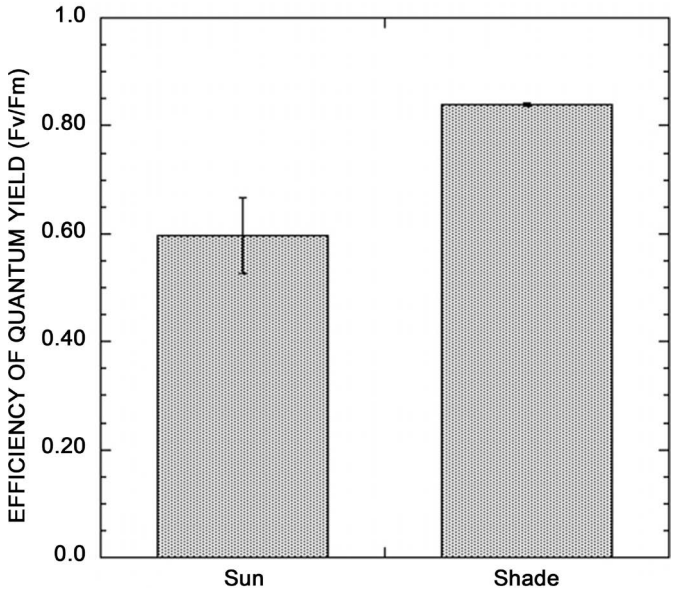
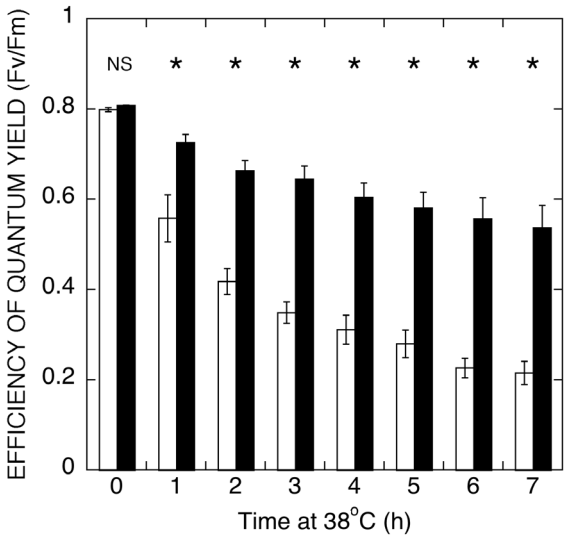


Figure 2. Graph of the efficiency of quantum yield (Fv/Fm) values for leaf samples from the fifth main stem leaf position of cotton plants exposed to full sunlight, and of cotton plants shaded for 24 h prior to sampling. Error bars represent standard error values.



* Significant $p < 0.05$, NS = Not Significant

Figure 3. Graph of the efficiency of quantum yield (Fv/Fm) values for leaf samples from the fifth main stem leaf position of cotton plants grown under irrigated or rain fed conditions. The leaves from the irrigated plants (open bars) showed a gradual decline in metabolic fitness with prolonged exposure to 38˚C. Leaves from the rain fed cotton (solid bars) also showed a decline in metabolic fitness over time; however, the rate of decline was less in the leaves of the rain fed plots compared with leaves from the irrigated plots. Error bars represent standard error values.

gradual decline in metabolic fitness with prolonged exposure to 38˚C (**Figure 3**). Leaves from the rainfed cotton also showed a decline in metabolic fitness over time; however, the rate of decline was less in the leaves of the rainfed plots compared with leaves from the irrigated plots. The efficiency of quantum yield (Fv/Fm) of photosystem II values were approximately two-fold higher in the leaves from the rainfed plots. These results show that adaptation to water-deficits will increase the metabolic fitness of the tissue.

3.2. Evaluation of *G. hirsutum* and *G. barbadense* Adaptation to Different Thermal Environments

Determination of the Metabolic Fitness of Cotton Grown in a Cool and Hot Environment:

Six cotton cultivars were grown in greenhouses set to two distinct thermal regimes. One greenhouse was set to provide a 28˚C/20˚C day/night cycle, and a second greenhouse set to provide a 38˚C/32˚C day/night cycle (**Figure 4**). Leaf temperatures closely tracked air temperatures in the greenhouse studies. Four of the cotton cultivars were the upland cottons (*Gossypium hirsutum* L.) PM 2200, PM 2145, SG 521, and the Texas race stock entry T303; and two of the cotton cultivars were the long-staple cottons (*Gossypium barbadense* L.) Pima S-7 and Sea Island. Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the white bars (**Figures 5-7**). Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the light grey bars. Leaf samples

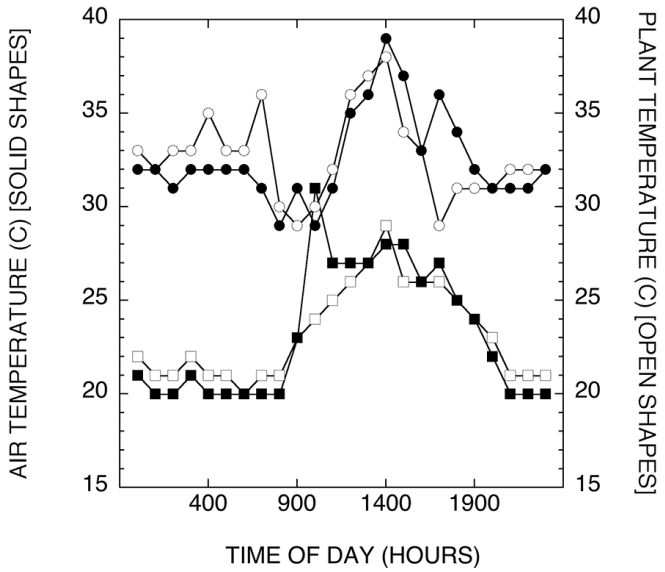
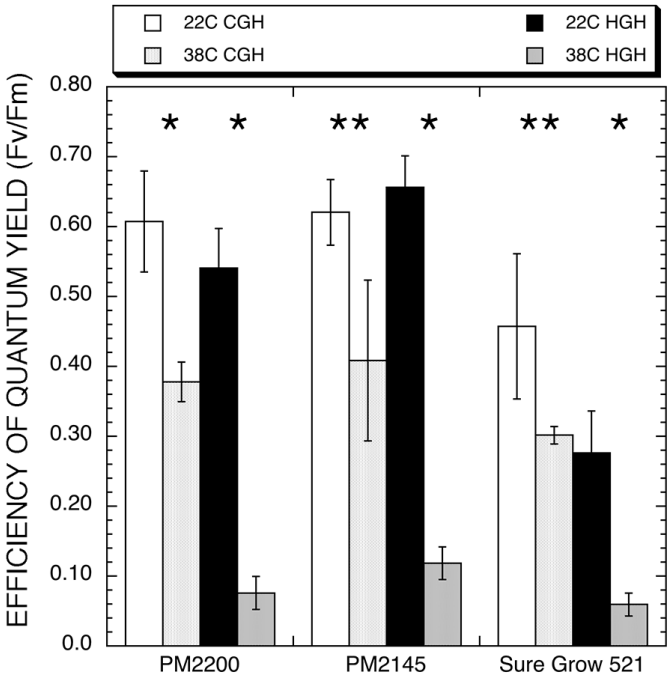
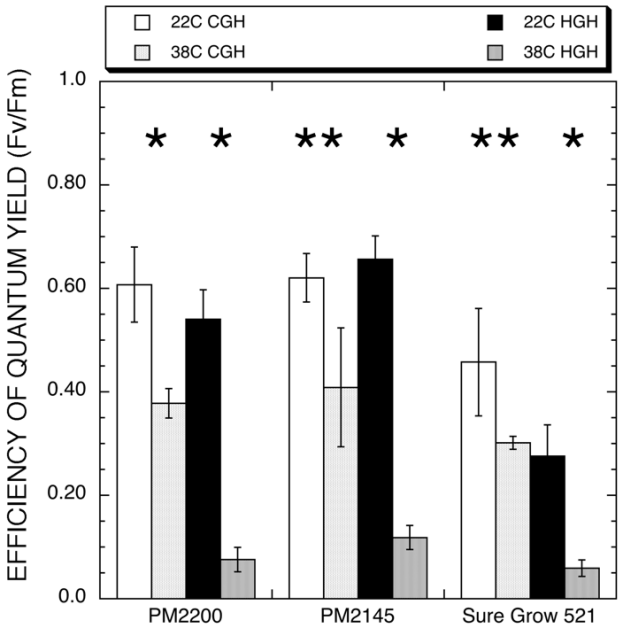


Figure 4. Graph of representative air temperatures experienced by the cotton plants in two greenhouses during the course of a 24 h period. One greenhouse was set to provide a 28˚C/20˚C day/night cycle (solid circles), and a second greenhouse was set to provide a 38˚C/32˚C day/night cycle (open circles).



* Significant $p < 0.05$, ** Significant $p < 0.1$

Figure 5. Graph of the efficiency of quantum yield (Fv/Fm) values of PM 2200, PM 2145 and SG 521 cotton cultivars following a 16 h - 38˚C heat treatment. Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the solid black bars. Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the solid white bars. Leaf samples from the 38˚C/32˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the solid grey bars, and leaf samples from the 38˚C/32˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the light grey bars. Error bars represent standard error values.



* Significant $p < 0.05$, ** Significant $p < 0.1$

Figure 6. Graph of the efficiency of quantum yield (Fv/Fm) values of Pima S-7, Sea Island and T303 cotton cultivars following a 16 h - 38˚C heat treatment. Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the solid black bars. Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the solid white bars. Leaf samples from the 38˚C/32˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the solid grey bars, and leaf samples from the 38˚C/32˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the light grey bars. Error bars represent standard error values.

from the 38˚C/32˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the solid black bars, and leaf samples from the 38˚C/32˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the dark grey bars.

Similar efficiency of quantum yield (Fv/Fm) of pho tosystem II patterns were observed for the PM220 and PM2145 cultivars (Figure 5). Efficiency of quantum yield (Fv/Fm) values of leaf punches maintained at 22˚C were a value of 0.6, while the efficiency of quantum yield values of the 38˚C treated leaf punches fell to approximately 0.4. Similar efficiency of quantum yield (Fv/Fm) of photosystem II values were seen between the leaf samples from the 28/20˚C day/night cycle maintained at 22˚C and the leaf punches from the 38/32˚C day/night cycle also maintained at 22˚C in the dark for 16 h. Low efficiency of quantum yield (Fv/Fm) of photosystem II values were observed in the leaf samples of the PM220 and PM2145 cultivars from the 38/32˚C day/night cycle maintained at 38˚C in the dark for 16 h. SG 521 leaf punches from either greenhouse had lower initial efficiency of quantum yield (Fv/Fm) of photosystem II in the 22˚C treatment compared with the PM220 and PM2145 cultivars (Figure 5). Despite SG 521 lower efficiency of quantum yields in the 22˚C treatment, fluorescence patterns similar to those of the PM220 and PM2145 cultivars were observed fol-

lowing the 38˚C treatments. These data show higher efficiency of quantum yieldvalues following the 38˚C treatment in leaves from the "cool" greenhouse, compared with leaves from the "warm" greenhouse.

Similar efficiency of quantum yield patterns were observed for Pima S-7, Sea Island and the T303 cultivars (Figure 6). Unlike the response of the upland cotton cultivars shown in Figure 5, higher efficiency of quantum yield values following the 38˚C treatment were seen in leaves from the "warm" greenhouse, compared with leaves from the "cool" greenhouse. It is interesting that the line TX 303 is classified as an upland cotton yet shows a stress phenotype similar to the *G. barbadense* lines evaluated.

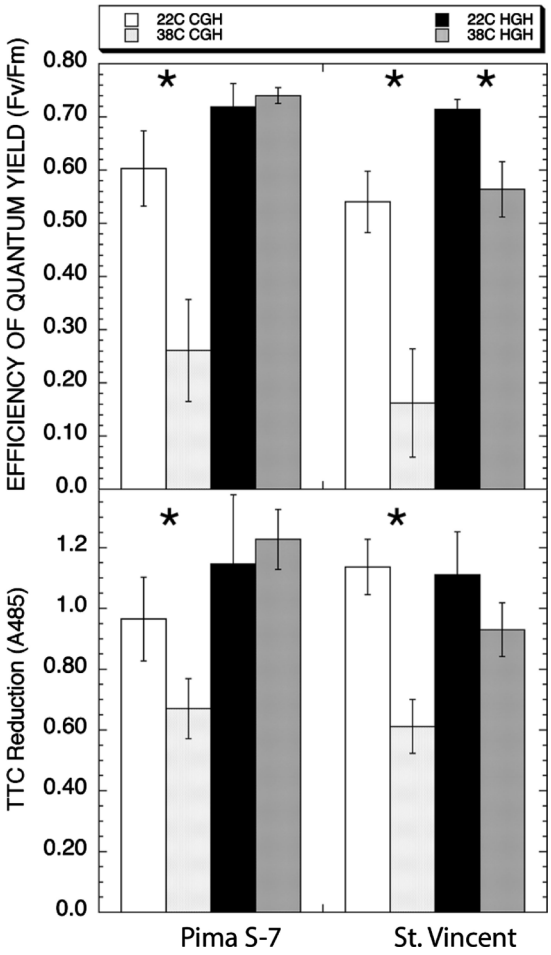
Comparison of the efficiency of quantum yield Indices with TTC Cell Viability Determinations:

Because this study used the efficiency of quantum yield of photosystem II as a relative measure of tissue viability, questions may arise as to the validity of this photosynthetic measurement being representative of the entire metabolism or fitness of the cell. To test the validity of using the quantum yield of photosystem II as a measure of the metabolic fitness of the cell, the efficiency of quantum yield values for Pima S-7 and Sea Island were compared with cell viability measurements obtained by using the viability stain 2,3,5-triphenyltetrazolium chloride. Figure 7 shows the comparison of efficiency of quantum yield values and TTC reduction for the same leaf punches. The efficiency of quantum yield values shown in Figure 7 show similar patterns to those described for these cultivars in Figure 6. Clearly, higher efficiency of quantum yield values following the 38˚C treatment were seen in leaves from the "warm" greenhouse, compared with leaves from the "cool" greenhouse. The TTC reduction levels mirrored the efficiency of quantum yield values for both cultivars and all treatments.

4. Discussion

Cotton is an essential crop grown in most tropical and subtropical regions of the world. Of the identified 50 species, four domesticated species (*G. hirsutum*, *G. barbadense*, *G. arboreum*, *and G. herbaceum*) have been studied extensively and considerable diversity identified [21]. The present study investigated the two New World tetraploid species *G. hirsutum* and *G. barbadense* as they were domesticated in different regions of the world. Although originally domesticated west of the Andes, our modern *G. barbadense* cultivars were developed from Sea Island cottons from Georgia and South Carolina [23]. *G. hirsutum*, on the other hand, was domesticated on the Yucatan peninsula in Mesoamerica [24].

Comparative studies reported by Reddy *et al.* [25] [26] evaluated vegetative and reproductive responses of a *G. hirsutum* cultivar (DES 119) and a *G. barbadense* cultivar (Pima S-6). The vegetative data showed that maximum stem elongation rates in *G. hirsutum* peaked under the 30C/22C day/night cycle, while the *G. barbadense* maximum stem elongation rate occurred under the 35˚C/27˚C day/night cycle. The reproductive responses to temperature showed that Pima cotton was more sensitive to high temperatures exemplified by reduced



* Significant $p < 0.05$

Figure 7. Graph of the efficiency of quantum yield (Fv/Fm) values and TTC reduction levels of Pima S-7 and Sea Island cotton cultivars following a 16 h - 38˚C heat treatment. Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the solid black bars. Leaf samples from the 28˚C/20˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the solid white bars. Leaf samples from the 38˚C/32˚C day/night cycle that were maintained at 22˚C in the dark for 16 h are represented by the solid grey bars, and leaf samples from the 38˚C/32˚C day/night cycle that were maintained at 38˚C in the dark for 16 h are represented by the light grey bars. Error bars represent standard error values.

fruiting branches compared to *G. hirsutum* under elevated temperatures.

The present study further analyzed plant vegetative responses when grown in sub-optimal or supra-optimal thermal environments. It describes studies to optimize the assay system, followed by characterization of plant responses to two distinct air temperature growing regimes. The relative sensitivities of the first-eight main stem leaf positions were investigated to determine which leaf positions were most heat resistant. The highest efficiency of quantum yield values were observed in the first and fourth through eight main stem positions (Figure 1). With the exception of the first leaf position, these results are not surprising based upon current knowledge of source-sink relationships and leaf development in cotton. The lack of injury to leaf position 1 was likely because of

poor contact between the leaf tissue and the filter paper based upon the pattern of injury seen in the photograph of the samples analyzed. The fifth leaf position is often referred to as "the most recently fully expanded leaf" and is a source leaf of photosynthate for the younger leaves [27]. Leaf samples were harvested at solar noon, not only to obtain tissue during maximum radiation load, but also to provide ample time for carbohydrate accumulation in the leaves [28] [29].

The efficiency with which absorbed light energy is harvested by photosynthesis is altered by a regulatory mechanism that determines how much excitation energy is used and how much is dissipated as heat under sub-optimal or supra-optimal thermal conditions [11] [12] [13] [14] [15]. When photosynthesis is limited the xanthophyll cycle protects against photo-oxidation of the pigment system by using the reducing power of NADPH. If the diversion of NADPH to this protective cycle limits the NADPH available for carbon fixation and the diversion of energy away from sugar biosynthesis makes plant tissues more susceptible to injury under elevated temperatures imposing a high respiratory demand on the cell, then the higher the light intensity the more susceptible the tissue should become to the high respiratory demand imposed by the efficiency of quantum yield assay. Cotton plants were exposed to full sun or shaded conditions prior to determination of the metabolic fitness index to determine if light intensity affected the ability of the tissue to withstand the prolonged respiratory demand associated with the elevated temperatures. The results shown in **Figure 2** are consistent with the hypothesis that the diversion of NADPH to the xanthophyll cycle may limit carbohydrate biosynthesis and result in a shortage of energy needed to meet the high respiratory demand of the assay. Based upon these findings, subsequent experiments were performed on leaves grown in full sunlight.

Water-deficit stress reduces plant growth and increases the amount of osmotic compounds found in cells. If the metabolic fitness assay measures in part the availability of cellular stored reserves to provide the energy needed to meet the high respiratory demand of the assay, then increased carbohydrates associated with water-deficit stress would be predicted to enhance the metabolic fitness of the tissue. The results shown in **Figure 3** support the hypothesis that increased osmolytes might enhance the metabolic fitness of the tissue. Leaf tissues from the water-deficit stressed plants maintained an efficiency of quantum yield value that was twice the level of the irrigated controls. These findings were the foundation for the development of a water-deficit stress bioassay [30].

The data provided in **Figures 1-3** provide assay guidelines that should be followed when evaluating developmental or genetic diversity in metabolic fitness in plants from different thermal environments. The optimal cotton tissue for analysis of metabolic fitness is the fifth main stem leaf from a plant that is adequately watered to avoid water-deficit stress, and that has received maximum irradiation. With the necessary parameters for the assay identified, the level of genetic diversity of upland cotton cultivars and long-staple cotton cultivars was investigated in well-watered plants grown in either a 28˚C/20˚C day/night cycle or a

38˚C/32˚C day/night cycle. Samples harvested at solar noon were evaluated and differences in metabolic fitness were observed depending upon the growth temperature and genetic background (Figure 5 and Figure 6). Most of the upland cottons grown in the 28˚C/20˚C environment were more resistant to the high respiratory demands of the assay than the same cultivars grown in a 38˚C/32˚C day/night cycle (Figure 5). Long-staple cotton cultivars, on the other hand, grown in the 38˚C/32˚C environment were more resistant to the high respiratory demands of the assay than the same cultivars grown in a 28˚C/20˚C day/night cycle (Figure 6). Unexpectedly, the TX 303 line exhibited temperature sensitivities similar to those of the *G. barbadense* lines evaluated in this study. It is interesting to speculate that with the origin of the *G. barbadense* being in northern Peru [21] that a possible introgression of genes from *barbadense* into TX 303 might have occurred in this line from Mexico. Clearly additional research is needed to further test this hypothesis. The data from the present study shows the usefulness of the metabolic fitness assay in identifying germplasm with enhanced metabolic performance when grown in specific thermal environments.

A concern associated with using the quantum yield of photosystem II as the metabolic fitness index is the question of whether this parameter truly reflects the metabolic response of the entire cell. The results of the comparison of the metabolic fitness index with the TTC cell viability assay support the use of the quantum yield of photosystem II as the metabolic fitness index (Figure 7). An advantage of the metabolic fitness assay over the TTC viability assay is the time required to obtain a measurement. The use of the pulse-modulated fluorometer allows immediate determination of the status of the tissue. The fluorescence assays only take a few seconds per sample. TTC, on the other hand, requires time for the uptake and reduction of the TTC, extraction of reduced TTC from the tissue, and subsequent measurement if TTC reduction levels with a spectrophotometer. This can add hours or days to the determination of tissue viability. Clearly, the ease of use of the pulse-modulated fluorometer is advantageous for screening large numbers of samples.

5. Conclusion

In summary, the present study describes an assay that provides information about the relative metabolic fitness of cotton grown in different thermal environments. The data also show the importance of avoiding water-deficit stress and providing high radiation levels to obtain experimental results about genetic diversity for distinct temperature optima whose interpretation is not confounded by osmoregulation or inadequate radiation loads.

Acknowledgements

The author thanks Jacob Sanchez and J.R. Quilantan for their excellent assistance.

References

[1] Burke, J.J., Mahan, J.R. and Hatfield, J.L. (1988) Crop-Specific Thermal Kinetic

Windows in Relation to Wheat and Cotton Biomass Production. *Agronomy Journal*, .**80**, 553-556. https://doi.org/10.2134/agronj1988.00021962008000040001x

[2] Somero, G.N. and Low, P.S. (1976) Temperature: A "Shaping Force" in Protein Evolution. *Biochemical Society Symposia*, **41**, 33-42.

[3] Teeri, J.A. and Peet, M.M. (1978) Adaptation of Malate Dehydrogenase to Environmental Temperature Variability in Two Populations of *Potentilla glaudulosa* Lindl. *Oecologia*, **34**, 133-141. https://doi.org/10.1007/BF00345162

[4] Anderson, J.V., Chevone, B.I. and Hess, J.L. (1992) Seasonal Variation in the Antioxidant System of Eastern White Pine Needles: Evidence for Thermal Dependence. *Plant Physiology*, **98**, 501-508. https://doi.org/10.1104/pp.98.2.501

[5] Burke, J.J. (1990) Variation among Species in the Temperature Dependence of the Reappearance of Variable Fluorescence Following Illumination. *Plant Physiology*, **93**, 652-656. https://doi.org/10.1104/pp.93.2.652

[6] Burke, J.J. and Oliver, M.J. (1993) Optimal Thermal Environments for Plant Metabolic Processes (*Cucumis sativis* L.). *Plant Physiology*, **102**, 295-302. https://doi.org/10.1104/pp.102.1.295

[7] Ferguson, D.L. and Burke, J.J. (1991) Influence of Water and Temperature Stress on the Temperature Dependence of the Reappearance of Variable Fluorescence Following Illumination. *Plant Physiology*, **97**, 188-192. https://doi.org/10.1104/pp.97.1.188

[8] Kidambi, S.P., Mahan, J.R. and Matches, A.G. (1990) Purification and Thermal Dependence of Glutathione Reductase from Two Forage Legume Species. *Plant Physiology*, **92**, 363-367. https://doi.org/10.1104/pp.92.2.363

[9] Mahan, J.R., Burke, J.J. and Orzech, K.A. (1990) Thermal Dependence of the Apparent Km of Glutathione Reductases from Three Plant Species. *Plant Physiology*, **93**, 822-824. https://doi.org/10.1104/pp.93.2.822

[10] Wang, W., Vinocur, B. and Altman, A. (2003) Plant Responses to Drought, Salinity and Extreme Temperatures: Towards Genetic Engineering for Stress Tolerance. *Planta*, **218**, 1-14. https://doi.org/10.1007/s00425-003-1105-5

[11] Bjorkman, O. and Demmig-Adams, B. (1995) Regulation of Photosynthetic Light Energy Capture, Conversion, and Dissipation in Leaves of Higher Plants. In: Schulze, E.D. and Caldwell, M.M., Eds., *Ecophysiology of Photosynthesis*, Springer-Verlag, Berlin, 17-47. https://doi.org/10.1007/978-3-642-79354-7_2

[12] Demmig-Adams, B. and Adams, W.W. III (1992) Photoprotection and Other Responses of Plants to High Light Stress. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**, 599-626. https://doi.org/10.1146/annurev.pp.43.060192.003123

[13] Demmig-Adams, B., Adams, W.W. III, Logan, B.A. and Verhoevan, A.S. (1995) Xanthophyll-Cycle Dependent Energy Dissipation and Flexible Photosystem II Efficiency in Plants Acclimated to Light Stress. *Australian Journal of Plant Physiology*, **22**, 249-260. https://doi.org/10.1071/PP9950249

[14] Horton, P. (1987) Interplay between Environmental and Metabolic Factors in the Regulation of Electron Transport in Higher Plants. In: Bihhens, J., Ed., *Progress in Photosynthesis Research*, Vol. 2, Martinus Nijhoff Publishers, Dordrecht, 681-688. https://doi.org/10.1007/978-94-009-3535-8_161

[15] Horton, P. and Ruban, A.V. (1992) Regulation of Photosystem II. *Photosynthesis Research*, **34**, 375-385. https://doi.org/10.1007/BF00029812

[16] Ruban, A.V. and Horton, P. (1999) The Xanthophyll Cycle Modulates the Kinetics of Nonphotochemical Energy Dissipation in Isolated Light-Harvesting Complexes,

Intact Chloroplasts, and Leaves of Spinach. *Plant Physiology*, **119**, 531-542. https://doi.org/10.1104/pp.119.2.531

[17] Drake, B.G., Gonzalez-Meler, M.A. and Long, S.P. (1997) More Efficient Plants: A Consequence of Rising Atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 609-639. https://doi.org/10.1146/annurev.arplant.48.1.609

[18] Pammenter, N.W., Loreta, F. and Sharkey, T.D. (1993) End Product Feed-Back Effects on Photosynthetic Electron Transport. *Photosynthesis Research*, **35**, 5-14. https://doi.org/10.1007/BF02185407

[19] Horton, P. (2000) Prospects of Crop Improvement through Manipulation of Photosynthesis: Morphological and Biochemical Aspects of Light Capture. *Journal of Experimental Botany*, **51**, 475-485. https://doi.org/10.1093/jexbot/51.suppl_1.475

[20] Niyogi, K.K. (1999) Photoprotection Revisited: Genetic and Molecular Approaches. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 333-359. https://doi.org/10.1146/annurev.arplant.50.1.333

[21] Wendel, J.F., Brubaker, C.L. and Seelanan, T. (2010) The Origin and Evolution of *Gossypium*. In: Stewart, J.M., Oosterhuis, D.M., Heitholt, J.J. and Mauney, J.R., Eds., *Physiology of Cotton*, Springer, Dordrecht, 1-18. https://doi.org/10.1007/978-90-481-3195-2_1

[22] Bilger, H.-W., Schreiber, U. and Lange, O.L. (1984) Determination of Leaf Heat Resistance: Comparative Investigation of Chlorophyll Fluorescence Changes and Tissue Necrosis Methods. *Oecologia*, **63**, 256-262. https://doi.org/10.1007/BF00379886

[23] Hutchinson, J.B. and Manning, H.L. (1945) The Sea Island Cottons. *Empire Journal of Experimental Agriculture*, **13**, 80-92.

[24] Brubaker, C.L. and Wendel, J.F. (1994) Re-Evaluating the Origin of Domesticated Cotton (*Gossypium hirsutum*: Malvaceae) Using Nuclear Restriction Fragment Length Polymorphisms (RFLPs). *American Journal of Botany*, **81**, 1309-1326. https://doi.org/10.2307/2445407

[25] Reddy, K.R., Hodges, H.F., McKinion, J.M. and Wall, G.W. (1992a) Temperature Effects on Pima Cotton Growth and Development. *Agronomy Journal*, **84**, 237-243. https://doi.org/10.2134/agronj1992.00021962008400020022x

[26] Reddy, K.R., Reddy, V.R. and Hodges, H.F. (1992b) Temperature Effects on Early Season Cotton Growth and Development. *Agronomy Journal*, **84**, 229-237. https://doi.org/10.2134/agronj1992.00021962008400020021x

[27] Sasek, T.W., DeLucia, E.H. and Strain, B.R. (1985) Reversibility of Photosynthetic Inhibition in Cotton after Long-Term Exposure of Elevated CO_2 Concentrations. *Plant Physiology*, **78**, 619-622. https://doi.org/10.1104/pp.78.3.619

[28] Warner, D.A. and Burke, J.J. (1993) Cool Night Temperatures alter Leaf Starch and Photosystem II Chlorophyll Fluorescence in Cotton. *Agronomy Journal*, **85**, 836-840. https://doi.org/10.2134/agronj1993.00021962008500040011x

[29] Warner, D.A., Holaday, A.S. and Burke, J.J. (1995) Acclimation of Carbon Metabolism to Night Temperature in Cotton. *Agronomy Journal*, **87**, 1193-1197. https://doi.org/10.2134/agronj1995.00021962008700060026x

[30] Burke, J.J. (2007) Evaluation of Source Leaf Responses to Water-Deficit Stresses in Cotton Using a Novel Stress Bioassay. *Plant Physiology*, **143**, 108-121. https://doi.org/10.1104/pp.106.087783