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# MOLECULAR BIOLOGY AND PHYSIOLOGY

## Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes

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### ABSTRACT

**With global warming and climate change, high-temperature stress has become a major factor affecting crop growth and yield. Cotton (*Gossypium hirsutum* L.) crops in the United States experience periods of extreme high temperatures during flowering and boll development, but information is lacking on the physiological response of cotton to high-temperature stress and appropriate techniques to quantify this response. Our objective was to assess the effect of high- temperature stress on physiological processes and screen a diverse set of cotton cultivars for high-temperature tolerance. Growth-room studies were conducted to investigate the effect of high temperature on photosynthesis, quantum yield of photosystem II (PSII), and membrane leakage of four obsolete and four modern cotton cultivars. The results showed that the optimum temperature for photosynthetic carbon fixation of cotton was approximately 33 ºC and that photosynthesis in cotton decreased significantly at temperatures of 36 ºC and above. Similarly, increasing air temperature caused a decrease in quantum yield of PSII at 36 ºC and above indicating high-temperature stress. Membrane leakage was also significantly increased when temperature exceeded 33 to 35 ºC. The results showed that the 'Acala' genotypes were more tolerant to high temperatures compared to the other genotypes. Chlorophyll fluorescence and membrane leakage were practical and reliable techniques for quantifying tolerance to high temperatures. Photosynthesis was also sensitive but was not practical for screening large numbers of genotypes for temperature tolerance.**

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### INTRODUCTION

Although cotton originated in hot climates, it suffers under conditions of extreme high day temperatures (Oosterhuis, 2002), as evidenced by a correlation between high temperatures and low yields in Arkansas (Oosterhuis, 1999). Maximum growth measured as rapid, dry matter accumulation during the fruiting period in cotton occurs at day/night temperatures of 30/20ºC (Reddy et al., 1991). The optimum temperature for cotton enzymatic activity of glyoxylate reductase was reported to be 23.5 to 32 ºC (Burke et al., 1988); however, the average daily maximum temperature during boll development in the U.S. Cotton Belt is almost always above these optima.

Much research has been devoted to increasing heat tolerance in wheat (*Triticum aestivum* L.; Reynolds et al., 1994), cowpeas (*Vigna unguiculata* L.; Ismail and Hall, 1999), Kentucky bluegrass (*Poa pratensis* L.; Marcum, 1998), and English ivy (*Hedera helix* L.; Yeh and Hsu, 2004). In addition, public breeders have dramatically improved yields in Pima cotton (*Gossypium barbadense* L.) by increasing high temperature tolerance (Kittock et al., 1988). Other researchers have used in vitro pollen germination methods to screen cotton cultivars (Burke et al., 2004; Kakani et al., 2005; Liu et al., 2006). However, little is known about temperature tolerance in Upland cotton (*Gossypium hirsutum* L.) and about practical physiological methods that can be used in vivo to screen large numbers of cotton genotypes for high-temperature tolerance.

Photosynthesis is an important process that is detrimentally affected by extreme environmental conditions such as high temperature (Cothren, 1999). During the vegetative stage, extreme high temperatures can destroy components of leaf photosynthesis, thereby reducing $CO_2$ assimilation rates, damaging components of photosystem II (PSII) in the thylakoid membranes of the chloroplasts, and causing membrane damage (Hall, 2004). Inhibition of photosynthetic $CO_2$ fixation by high temperature has been documented in many plant species, including cotton (Perry et al., 1983). Photosynthesis has been used as a technique to assess high-temperature

tolerance in maize (*Zea mays* L.; Karim et al., 2000), and triticale (X *Triticosecale* Wittmack; Grzesiak et al., 2003); however, limited information exists about the use of photosynthesis as a screening technique for high-temperature tolerance in cotton.

Some light energy utilized in photosynthesis is lost as fluorescence mainly from the PSII reaction. The use of a pulse amplitude modulation fluorometer allows the continuous determination of quantum efficiency ($q_Q$) of PSII, known as the dark-adapted test, as well as the quantum yield ($q_E$) of PSII, known as the light-adapted test (Schreiber et al., 1986). There are many reports on chlorophyll fluorescence using the dark-adapted test ($q_Q = F_v / F_m$) where $F_v$ is the variable fluorescence and $F_m$ the maximum variable fluorescence upon application of continuous actinic light (Buwalda and Noga, 1994; Genty et al., 1989; Guo et al., 2004; Logan and Monson, 1999; Sayed et al., 1989). There is an increasing need for a rapid and nondestructive measurement of chlorophyll fluorescence, such as that measured in beech trees (*Fagus sylvatica* L; Bilger et al., 1995) by the use of the light-adapted test [$q_E = (F_m - F_s) / F_m$, where $F_s$ is the saturated variable fluorescence]. The quantum yield of PSII has been shown to be closely related with the efficiency of $CO_2$ assimilation (Genty et al., 1989) and has been used as a tool for investigating photochemical mechanisms underlying photosynthesis (Papageorgiou, 1975). Two objectives of this study were to assess the effect of high temperature on the quantum yield of PSII of cotton under ambient light conditions with a modulated chlorophyll fluorometer and evaluate the use of this technique for screening cotton cultivars for high-temperature tolerance.

Plants under high-temperature stress can exhibit changes in membrane fluidity (Sung et al., 2003). Cell membranes are important for the control of electrolyte movement in and out of the cell, and also provide a stable site for the binding and catalysis of enzymes. When plants are under high-temperature stress, the structure of membranes is altered, permeability increases, electrolyte leakage increases, and eventually the cell dies (Wang, 1988). Cell membrane damage and the leakage of solutes have been correlated to the severity of injury (Heckman et al., 2002). High- temperature tolerance in plants is attributed largely to resistance of cell membranes (Larcher, 1995) and membrane leakage has become a common method for measuring high-temperature tolerance in crop plants (Howarth et al., 1997; Kumar et al., 1999; Marcum, 1998; Rahman et al., 2003).

In this study, we hypothesized that photosynthesis and quantum yield of PSII would decrease with increasing temperature, membrane leakage would increase significantly, and these measurements can provide a means of evaluating high- temperature tolerance in cotton genotypes. Our objectives were to evaluate the effect of high temperature on each of these parameters and to use these techniques to screen a diverse set of obsolete and modern cotton cultivars for high-temperature tolerance.

## MATERIAL AND METHODS

**Plant material.** All measurements were made using cotton plants growing in large growth chambers (Model PW36, Conviron, Winnipeg, Canada) at the Altheimer Laboratory, University of Arkansas, Fayetteville, AR, during 2003 and 2004. Four obsolete cultivars (more than 30 years old) and four modern cultivars (planted commercially between 2000 and 2004) were used. Each modern cultivar was paired with an obsolete cultivar with similar genetics, i.e., Stoneville 213 (ST213) with Stoneville 474 (ST474), Rex with Suregrow 747 (SG747), Deltapine 16 (DP16) with Deltapine 33B (DP33B), and Acala SJ2 with Acala Maxxa (Table 1).

The genotypes were planted in 160 2-L pots filled with Sunshine potting media Mix #1 (Sun Gro Horticulture Distribution Inc., Bellevue, WA) in growth chambers (Conviron PGW36) (4 replications x 8 cultivars x 5 temperatures). The growth chambers were maintained at 30/20 ºC (day/night temperature), 75% relative humidity, and 12-h photoperiods. The photosynthetically active radiation (PAR) followed a typical diurnal pattern with the highest PAR (850 μmol/m$^2$/s) occurring between 1000 h and 1400 h. The plants were maintained at control conditions (30/20 ºC) until the appearance of floral buds (pinhead square stage). At this stage measurements of quantum yield of PSII, photosynthesis, and membrane leakage were taken between 1100 h and 1300 h using the fourth main-stem leaf from the terminal with one reading per leaf from 32 plants. After measurements were taken, the 32 plants used were removed and the day temperature was raised by 3 ºC. The temperature was raised from 30 to 42 ºC in 3 ºC increments during a 12-d period. The plants were acclimated to each temperature treatment for 3 d after which measurements were taken from 32 different plants each time. The plants were watered once daily with half-strength Peters

(Spectrum Group, St. Louis, MO) nutrient solution; additional water was applied as needed to maintain adequate soil moisture.

A preliminary study with six replications was performed with the cotton cultivar SG747 planted in 2-L pots filled with potting media as described above, in two different growth chambers. The purpose of this study was to test the techniques for measuring membrane leakage and quantum yield of PSII in cotton plants. The plants remained at 30/20 $^{o}$C until the appearance of the floral buds (pinhead square stage), after which they were divided into two sets and half moved into a different growth chamber at 35/25 $^{o}$C. After 3 d at this temperature regime, the temperature was raised to 40/30 $^{o}$C. Measurements were taken between 1100 h and 1300 h using the fourth main-stem leaf 3 d after the plants were exposed to each temperature regime.

**Measurement of photosynthesis**. Photosynthesis was measured using the LI-COR 6200 Portable Photosynthesis System (LI-COR Inc, Lincoln, NE). Measurements were taken between 1100 h and 1300 h using the fourth main-stem leaf from the terminal with one reading per leaf.

**Measurement of quantum yield of PSII of light-adapted leaves.** Quantum yield of PSII was measured using the light-adapted test of the modulated chlorophyll fluorometer OS1-FL (Opti-Sciences, Tyngsboro, MA). With this system, chlorophyll fluorescence is excited by a 660 nm solid-state light source with filters blocking radiation longer than 690 nm. The average intensity of this modulated light was adjusted from 0 to 1 μE. Detection was in the 700 to 750 nm range using a PIN silicon photodiode with appropriate filtering to remove extraneous light. Saturation of the photosystem being measured was provided by a filtered 35W halogen lamp (350–690 nm). For the light-adapted test (yield-test) an open-body clip held the fiber optic light guide steady at an angle of 50$^{o}$ to the leaf surface, while allowing the ambient light to fall onto the leaf surface. Measurements were taken between 1100 h and 1300 h using the fourth main-stem leaf from the terminal with one reading per leaf.

**Measurement of membrane leakage**. Membrane leakage was measured using an automatic conductivity meter (Applied Intelligent Systems Inc., Ann Arbor, MI). Single leaf disks (1-cm diameter) were excised with a leaf punch from the fourth main-stem leaf, one disk per treatment from similar interveinal areas, and placed into trays with individual cells containing 2 mL double deionized water. The electrical conductivity as a measure of cell leakage was read 48 h after the leaf disks were placed in the double deionized water at room temperature. The resulting electrical conductivity of the ion concentration in the solution depended on the leakage from the leaf disk.

**Statistical analysis**. The experimental design for the main experiment was completely randomized with four replications in a two-factor factorial arrangement of the treatments. The main factor was temperature and the sub-factor was "cultivars". Treatment differences were detected using analysis

**Table 1. Pedigree information for the obsolete and modern cotton cultivars evaluated for heat tolerance using quantum yield of PSII, membrane leakage, and photosynthesis**

| Cultivar | Area of Origin | Parent Lines |
|---|---|---|
| Obsolete[z] | | |
| Stoneville 213 | Mississippi River Delta | Stoneville 7 (1952) |
| Rex | Cotton Branch St., AR | BBR/2 x Empire |
| Deltapine 16 | Mississippi River Delta | DP Smoothleaf (1959) x DP 45 (1965) |
| Acala SJ2 | USDA ARS, Shafter, CA | AXTE-1 x NM2302 (1973) |
| Modern[y] | | |
| Stoneville 474 | Mississippi River Delta | (St 453) x (DES 119) |
| Suregrow 747 | Mississippi River Delta | Suregrow 125 |
| Deltapine 33B | Mississippi River Delta | Bt Coker x DP 5415 |
| Acala Maxxa | Western USA | T7538 x S4959 (1990) |

[z] More than 30 years old.

[y] Planted commercially between 2000 and 2004.

of variance (ANOVA). Data means were separated at probability values $\alpha \leq 0.05$. Because the temperature increments were equally spaced we used the orthogonal polynomial contrasts to test the significance of the linear and the quadratic response of each cultivar with increasing temperature. The statistical analysis was performed with the JMP 6 software (SAS Institute Inc., Cary, NC).

The preliminary study was arranged as a completely randomized design with six replications. The temperature data were analyzed using analysis of variance, and the data means were separated at probability values $\alpha \leq 0.05$. The statistical analysis was performed with the JMP 6 software.

## RESULTS

**Photosynthesis.** The effect of increasing air temperature on carbon fixation in Upland cotton cultivars is shown in Fig. 1. There was no significant cultivar-by-temperature interaction ($P = 0.0891$) (Table 2); therefore the main factor effects were tested. There was a significant effect for both factors, temperature ($P < 0.0001$) and cultivars ($P = 0.0006$).

When temperature increased from 30 to 33 °C, carbon fixation also increased significantly (Fig. 1). The effect of temperature was detrimental at 36 °C, with photosynthesis decreasing significantly with increasing temperature. The highest photosynthesis value, significantly different from all the other temperature regimes, was observed at 33 °C (15.4 μmole/m$^2$/sec). The significantly lowest value was observed at 42 °C (8.76 μmole/m$^2$/sec). Overall, photosynthesis in cotton decreased when the temperature increased to 36 °C and above, with a strong negative correlation ($R^2 = 0.97$) between photosynthesis and high temperatures.

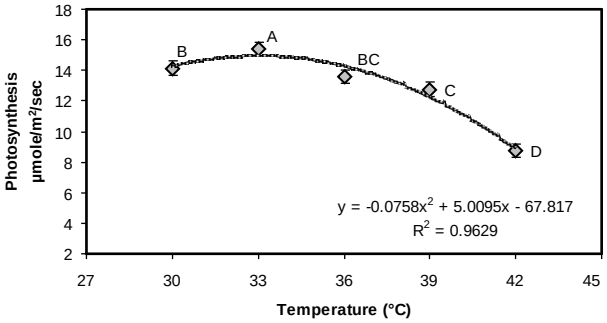


**Figure 1. Effect of increasing day temperatures in 3 °C increments on the photosynthetic carbon fixation of the eight cotton genotypes used under controlled environmental conditions. Least square means of all the cultivars with the same letter are not significantly different ($P \leq 0.05$).**

The cultivar effect on photosynthesis is illustrated in Fig. 2. The data revealed that the Acala cultivars showed numerically higher photosynthesis compared to all the other cultivars, and statistically higher photosynthesis compared to DP33B, ST213, and ST474. In general, the cultivars that were genetically related did not differ significantly, except for the two Stoneville cultivars, whereas the obsolete cultivar ST213 showed significantly higher photosynthesis compared to the modern cultivar ST474.

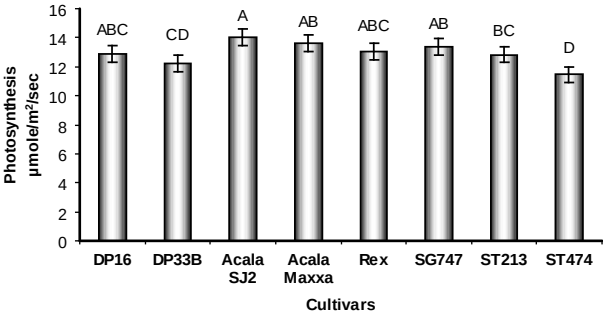


**Figure 2. Differences in the photosynthetic carbon fixation of the eight cotton cultivars. Least square means and standard errors (I) are shown for the $P \leq 0.05$ level. Columns with the same letter above them are not significantly different ($P \leq 0.05$).**

**Table 2. Analysis of variance and effect tests for photosynthesis**

| **Analysis of Variance for Photosynthesis** | | | | |
|---|---|---|---|---|
| **Source** | **DF** | **Sum of Squares** | **Mean Square** | **F Ratio** |
| **Model** | **39** | **1042.5625** | **26.7324** | **8.3183** |
| **Error** | **120** | **385.6399** | **3.2137** | **Prob > F** |
| **C. Total** | **159** | **1428.2024** | | **< 0.0001** |

| **Effect Tests for Photosynthesis** | | | | | |
|---|---|---|---|---|---|
| **Source** | **Nparm** | **DF** | **Sum of Squares** | **F Ratio** | **Prob > F** |
| **cultivars** | **7** | **7** | **90.27323** | **4.0129** | **0.0006** |
| **temperature** | **4** | **4** | **822.13313** | **63.9560** | **< 0.0001** |
| **cultivars*temperature** | **28** | **28** | **130.15610** | **1.4465** | **0.0891** |

**Quantum yield of PSII**. The data from the preliminary study using only cultivar SG747 showed that quantum yield of PSII was not affected significantly when the temperature increased from 30 to 35 ºC (Fig. 3); however, when the temperature was increased to 40 ºC the quantum yield of PSII decreased significantly. The percentage change for quantum yield of PSII at 35 ºC compared to 30 ºC control was 2%, whereas at 40 ºC the percentage decrease from the 30 ºC control was 49%. Using the equation for the quantum yield of PSII [$q_E = [(F_{ms} - F_s) / F_{ms}]$] we observed that increased air temperature decreased $q_E$ and thus increased the saturated variable fluorescence $F_s$.

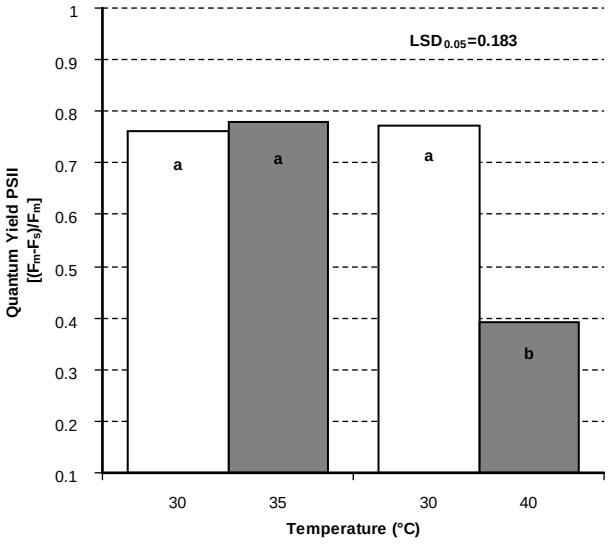


**Figure 3. Changes in quantum yield of PSII of cotton cv. SG747 due to increasing temperature with 5 ºC increments in the preliminary study. Pairs of columns with the same letter above them are not significantly different ($P \leq 0.05$).**

The effect of increasing air temperature on quantum yield of PSII in Upland cotton cultivars is shown in Fig. 4. Averaging all the cultivars in each temperature regime indicated that quantum yield of PSII decreased significantly with increasing air temperature. Increasing the temperature from 30 to 33 ºC did not affect the quantum yield of PSII significantly; however at 36 ºC and above, the quantum yield of PSII decreased significantly. Due to significant cultivar-by-temperature interaction ($P = 0.0294$, Table 3) we investigated the response of the quantum yield of each cultivar with increasing temperature (Fig. 5). The response was significant for cultivars DP16, DP33B, SG747, and ST213 showing that the quantum yield of PSII of these cultivars decreased linearly with increasing temperature. For these cultivars, the rates of decrease in quantum yield of PSII (slopes) were not significantly different (comparing the linear response of SG747 to ST213, DP33B, and DP16 the P-values were 0.0714, 0.2362, and 0.4377, respectively). In addition, the quantum yield of PSII of the two Acala cultivars, Rex, and ST474 was not significantly affected indicating greater tolerance to high temperature.

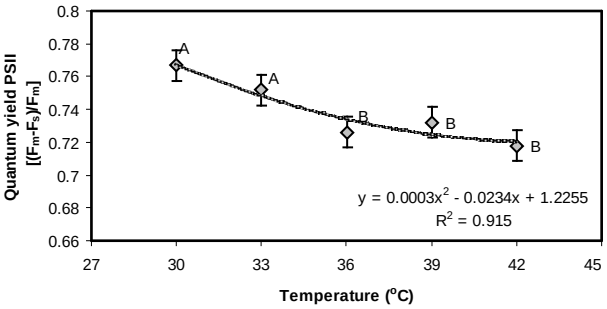


**Figure 4. Effect of elevated temperatures in 3 ºC increments on quantum yield of PSII of the eight genotypes used under controlled environmental conditions. Least square means of all the cultivars with the same letter are not significantly different ($P \leq 0.05$).**

**Table 3. Analysis of variance and effect tests for quantum yield of PSII**

| Analysis of Variance for Quantum Yield of PSII | | | | |
|---|---|---|---|---|
| **Source** | **DF** | **Sum of Squares** | **Mean Square** | **F Ratio** |
| **Model** | **39** | **0.15007738** | **0.003848** | **2.7825** |
| **Error** | **120** | **0.16595800** | **0.001383** | **Prob > F** |
| **C. Total** | **159** | **0.31603537** | | **< 0.0001** |

| Effect Tests for Quantum Yield of PSII | | | | | |
|---|---|---|---|---|---|
| **Source** | **Nparm** | **DF** | **Sum of Squares** | **F Ratio** | **Prob > F** |
| **cultivars** | **7** | **7** | **0.03490857** | **3.6059** | **0.0015** |
| **temperature** | **4** | **4** | **0.05016919** | **9.0690** | **< 0.0001** |
| **cultivars*temperature** | **28** | **28** | **0.06499961** | **1.6786** | **0.0294** |

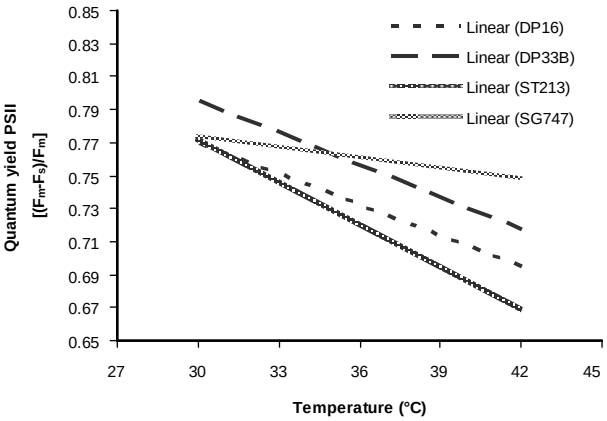


**Figure 5. Quantum yield of PSII as affected by increasing temperature from 30 to 42 ºC for each genotype that showed significant linear response (*P* = 0.05).**

**Membrane leakage.** The preliminary study using only cultivar SG747 showed that membrane leakage increased significantly when temperature increased from 30 to 35 ºC (Fig. 6). The increase in membrane leakage was significantly greater when the plants were subjected to 40 ºC. The percentage change at 35 ºC compared to the 30 ºC control was 35%, whereas at 40 ºC the percentage change from the 30 ºC control was 192%.

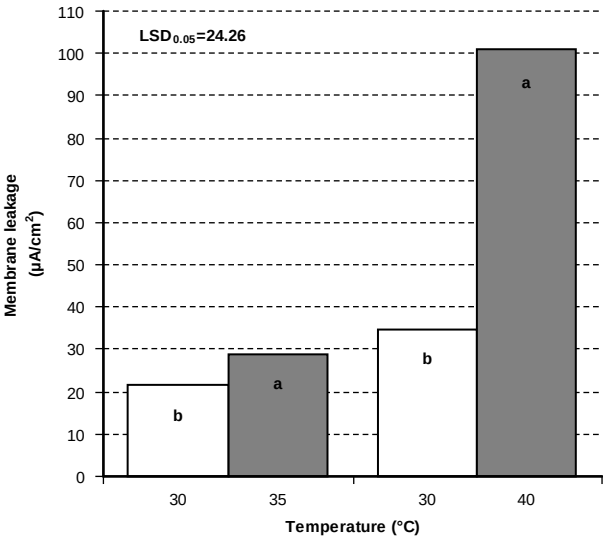


**Figure 6 Changes on membrane leakage cotton cv. SG747 due to increasing temperature with 5 ºC increments in the preliminary study. Pairs of columns with the same letter are not significantly different (*P* ≤ 0.05).**

The effect of increasing air temperature on membrane leakage in Upland cotton cultivars is shown in Fig. 7. Averaging all the cultivars in each temperature regime indicated that membrane leakage increased significantly when temperature increased from 30 to 33 ºC. The increase in membrane leakage continued with increasing temperature and became greater when temperature increased to 39 and 42 ºC. The statistical analysis revealed that there was a significant cultivar-by-temperature interaction (*P* = 0.002, Table 4), and therefore we investigated the response of membrane leakage of each cultivar with increasing temperature (Fig. 8). There was a significant linear response for DP16, DP33B, Acala SJ2, Rex, SG747, and ST474 showing that membrane leakage of these cultivars increased linearly with increasing temperature. For these cultivars the rates of increase in membrane leakage (slopes) were not significantly different. In addition, the membrane leakage on Acala Maxxa and ST213 did not significantly increase with increasing temperature, indicating a greater tolerance to high temperature.

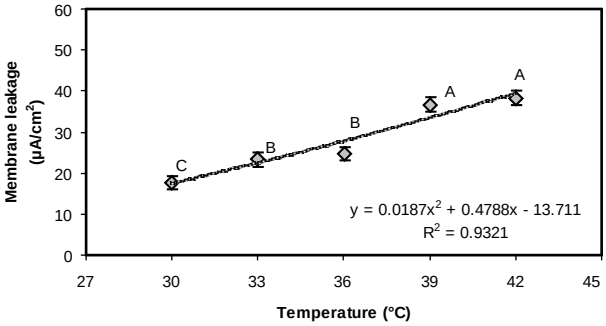


**Figure 7. Effect of elevated temperatures in 3 ºC increments on membrane leakage of the eight cotton genotypes used under controlled environmental conditions. Least square means of all the cultivars with the same letter are not significantly different (*P* ≤ 0.05).**

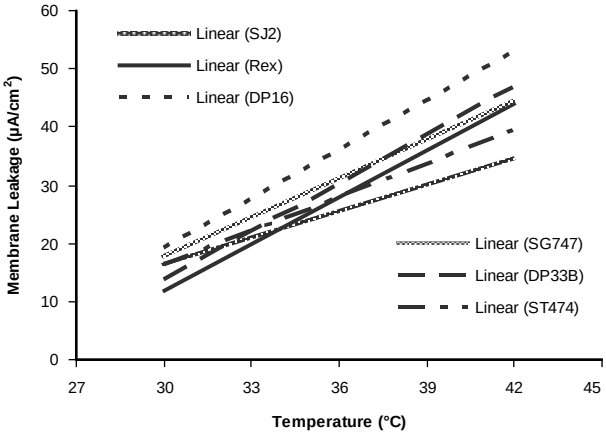


**Figure 8. Membrane leakage as affected by increasing temperature from 30 to 42 ºC for each genotype that showed significant linear response (*P* = 0.05).**

**Table 4. Analysis of variance and effect tests for membrane leakage**

| Analysis of Variance for Membrane Leakage | | | | |
|---|---|---|---|---|
| **Source** | **DF** | **Sum of Squares** | **Mean Square** | **F Ratio** |
| **Model** | **39** | **18377.475** | **471.217** | **5.4849** |
| **Error** | **120** | **10309.500** | **85.912** | **Prob > F** |
| **C. Total** | **159** | **28686.975** | | **< 0.0001** |

| Effect Tests for Membrane Leakage | | | | | |
|---|---|---|---|---|---|
| **Source** | **Nparm** | **DF** | **Sum of Squares** | **F Ratio** | **Prob > F** |
| **cultivars** | **7** | **7** | **2808.975** | **4.6708** | **0.0001** |
| **temperature** | **4** | **4** | **10327.663** | **30.0529** | **< 0.0001** |
| **cultivars*temperature** | **28** | **28** | **5240.838** | **2.1786** | **0.0020** |

**Cultivar screening.** Comparing the response to high temperature of all the cultivars using the three screening techniques revealed that the obsolete DP16 and modern DP33B cultivars did not differ significantly. The Rex and SG747, also similar parentage cultivars, did not show any significant difference in photosynthesis (Fig. 2) or membrane leakage (Table 4), but differed in quantum yield of PSII (Table 3) with the obsolete cultivar Rex being more temperature tolerant than the newer cultivar SG747. The obsolete cultivar ST213 showed more temperature tolerance than its modern counterpart cultivar ST474 in two of the three screening techniques: photosynthesis (Fig. 2) and membrane leakage (Table 4). The Acala genotypes showed greater high-temperature tolerance with higher photosynthetic rates than all the other genotypes (Fig. 2) and no significant temperature effect on quantum yield of PSII (Table 3). The only difference observed between the old and new Acala cultivars was that the newer cultivar Acala Maxxa showed greater tolerance to membrane leakage at high temperature. In these studies there was no correlation between membrane leakage ($R^2$ = 0.12) or quantum yield of PSII ($R^2$ = 0.13) with net photosynthetic carbon fixation. In addition, there was no interaction between membrane leakage and quantum yield of PSII.

## DISCUSSION

In these studies, carbon fixation in cotton decreased significantly at temperatures of 36 ºC and above. These results also indicated that the optimum for photosynthesis in cotton was approximately 33 ºC confirming previously reported data (Perry et al., 1983). Measuring photosynthetic carbon fixation is a promising way to characterize response to environmental stress such as high temperatures. In our studies the highest photosynthesis at high temperatures was exhibited by Acala Maxxa and Acala SJ2, followed by SG747, Rex, DP16, and DP33B. The two Stoneville cultivars performed poorly with ST474 showing significantly lower photosynthesis than the other genotypes. However, measuring photosynthesis in breeding trials with multiple entries would be laborious and not practical, as reported by Earl and Tollenaar (1999) for screening in maize.

Although researchers have studied the effect of temperature (Pettigrew at al., 1993), cultivar (Pettigrew and Meredith, 1994), and leaf age (Wullschleger and Oosterhuis, 1990) on photosynthesis, there are no reports of temperature on photosynthesis for a large number of Upland cotton genotypes. Reddy et al. (1998) studied the effect of temperature on growth and leaf characteristics of cotton, but not on photosynthesis. Zhenmin et al. (1997) assessed the photosynthetic rate of two Pima and one Upland cultivar in relation to heat stress, however only a single Upland genotype was used. To our knowledge, our data are the first report assessing high-temperature tolerance on a large number of different Upland cotton cultivars using photosynthesis via gas exchange techniques, as well as quantum yield of PSII and membrane leakage.

Increased day temperatures of 36 ºC and above caused significant decreases in the quantum yield of PSII in our studies. To our knowledge, this is the first record of measuring quantum yield of PSII in cotton via a modulated chlorophyll fluorometer under ambient light conditions to assess high-temperature tolerance in cotton. Among the cultivars tested, the quantum yield of PSII of Acala Maxxa, Rex, and

ST474 was not affected by elevated temperatures, indicating tolerance to high-temperature stress. The quantum yield of PSII for the other cultivars declined in a linear pattern with increasing temperature. The quantum efficiency of PSII measured with the dark-adapted test ($F_v$ / $F_m$) has been used widely as an indicator of environmental stress and as a screening method for resistance in plants such as in barley mutants (Georgieva et al., 2003); however, measuring quantum yield of PSII with the light-adapted test in our studies proved to be an easier and more practical way to screen multiple cultivars for high-temperature stress.

Similar to the above techniques, high temperatures were shown to have a detrimental effect on membrane leakage. Membrane leakage increased significantly at temperatures of 33 to 35 $^oC$ and above, thereby providing an even more sensitive technique than quantum yield of PSII for assessing high-temperature tolerance. Among the cultivars tested, the membrane fluidity of Acala Maxxa and ST213 was not significantly affected by increasing temperatures. Membrane leakage in the other cultivars increased in a linear pattern with increasing high temperature. This method of screening for high-temperature tolerance has been used in soybean (*Glycine max* L.; Martineau et al., 1979), cowpeas (*Vigna unguiculata* L.; Ismail and Hall, 1999), wheat (*Triticum aestivum* L.; Shanahan et al., 1990), holly (*Ilex opaca* Aiton; Ruter, 1993), turfgrass species (Wallner et al., 1982), and cotton (Rahman et al., 2003). Data from our studies support the findings of Rahman et al. (2003) that membrane leakage of cotton cultivars respond differently across increasing temperature regimes allowing screening for high-temperature tolerance. In addition, our studies corroborate these findings with two additional methods, photosynthesis and quantum yield of PSII, for evaluating temperature tolerance.

Among the techniques used, membrane leakage and quantum yield of PSII showed differential sensitivity to high temperature among different cotton cultivars, with the membrane leakage measurement being more sensitive to increasing temperature than quantum yield of PSII. Photosynthesis was also a sensitive technique for cultivar screening but would not be practical as a method of selection for a large number of cultivars.

Among the cultivars screened, the Acala genotypes showed the most tolerance to high temperature especially Acala Maxxa. This result was probably to be expected as the Acala cultivars were developed for production in semiarid and hot areas (Zhang et al., 2005). From a physiological point of view, the obsolete cultivar ST213 was more tolerant to high temperature than the modern ST474, whereas there was little difference among the other sets of obsolete and modern genotypes.

## CONCLUSIONS

In general, photosynthesis and quantum yield of PSII were detrimentally affected at temperatures of 36 $^oC$ and above, whereas measurements of membrane leakage showed an earlier significant plant response to high temperature at 33 to 35 $^oC$. These thresholds differed from those previously published for cotton. Differences in vapor pressure deficit as well as differences in adapted genetic material of cotton grown in hotter environments (e.g., temperature > 36 $^oC$ in Pakistan; Rahman et al., 2004) allow the crops in these locations to better tolerate high temperatures in comparison to Arkansas. All three techniques used in our study can be used for screening for high-temperature tolerance in cotton; however the easiest and more practical techniques were quantum yield of PSII and membrane leakage. Although measurement of photosynthesis proved to be sensitive to high temperatures, the measurement procedure was time consuming and not practical for cultivar screening for high-temperature tolerance. Among the cultivars tested, the Acala cultivars appear to be the most tolerant to high-temperature stress. Based on these results, our continuing work will focus on examining the effects of heat stress on fiber quality and yield.

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