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THE PHYSIOLOGICAL RESPONSE OF COTTON TO HIGH TEMPERATURE FOR GERMPLASM SCREENING

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Abstract

Variability in cotton yield is a major concern to cotton producers that has been associated with high temperatures and drought during boll development. Earlier work has indicated a strong negative correlation between high temperatures and low cotton (*Gossypium hirsutum*) yields in Arkansas. The current project was designed for field and growth room studies to (a) evaluate techniques for measuring temperature tolerance in cotton, and (b) to screen obsolete and modern cultivars for temperature tolerance. Preliminary results of these studies indicated that membrane leakage showed the most sensitivity for quantifying temperature tolerance in the field. In addition to membrane leakage, fluorescence also showed some sensitivity for quantifying temperature tolerance with the advantage that is much easier for field use. Similarly, measurement of catalase activity was sensitive but time consuming and more appropriate for laboratory analysis. The comparison of obsolete and modern cultivars did not show many significant differences, however the obsolete cultivars were more responsive to higher temperatures indicating more stress tolerance. This research will continue to evaluate genotypic temperature tolerance at higher temperatures (>30°C), and also to screen available cotton germplasm.

Introduction

One of the main problems cotton producers face today is variability in cotton yield. There are many factors that can affect the development of cotton yield, however, the main two are genetics and environment. Although water was considered the major limiting factor for crop production, irrigation has largely changed this concept. Temperature now is considered to be the major environmental factor affecting the development of cotton yield. Although cotton originates from hot climates, it does not necessarily grow best in excessively high temperatures. The ideal temperature range for cotton is reported to be 20 to 30°C (Reddy et al., 1991), with the optimum for photosynthesis at 28°C (Burke et al., 1988). There is a strong negative correlation between high temperatures and cotton yield (Oosterhuis, 1999) and there is evidence that cotton's metabolism decreases significantly at higher temperatures (Burke et al., 1988). From a physiological point of view, the ideal temperature range for cotton for optimal metabolic activity, also known as thermal kinetic window, is 23.3-32.2°C (Burke et al., 1988). However average daily temperature during boll development in the U.S. Cotton Belt are usually above 32.2°C. Most years in Arkansas, the cotton crop shows great potential mid-way through the season but fails dismally during August to achieve this potential, due to excessive temperatures, drought and periods of carbohydrates stress. In this project we focused on the effect of high temperatures on cotton for evaluating methods of assessing temperature tolerance in cotton for screening a select set of representative germplasm. Ultimately, the aim was to adopt a practical and accurate method to reliably screen the available cultivars for response to high temperatures in order to improve plant response to elevated temperatures for stabilization of yield. It was hypothesized that differences in response to high temperatures exist within the current cultivars and even more so in diverse germplasm lines. Furthermore, it was hypothesized that numerous physiological and biochemical parameters will be affected by elevated temperatures, but only a few of these will be both sensitive to high temperatures and easy to measure in large breeding trials. The objectives of this study were to quantify cotton response to high temperature in order to adopt a sensitive, reliable and practical technique to measure temperature tolerance in cotton and to screen a representative set of cultivars selected from the germplasm pools in the U.S.A.

Materials and Methods

Techniques for Measuring Temperature Tolerance

The techniques that were used for measuring temperature tolerance were chlorophyll fluorescence (FL) using a modulated fluorometer OS1-FL, membrane leakage (ML) using an automatic seed analyzer, canopy temperature using an infrared thermometer (physiological measurements) and antioxidant enzymes (catalase, glutathione reductase, peroxidase, ascorbate peroxidase) using the Anderson et al., 1992, technique. In addition, total active proteins (PR) using the Bradford (1976) method, and sugar alchohols (polyols) using an HPLC technique (biochemical measurements) were measured.

In 2003 three growth chamber studies and one field study were conducted. The first growth chamber study was conducted in the growth chambers in the Altheimer Laboratory, Fayetteville, AR. Cotton (*Gossypium hirsutum*) cv. Stoneville 213 and 474 planted in 2L pot-size filled with Sunshine mix and watered with half-strength Peter's nutrient solution. The growth chamber was maintained at 30/20°C (day/night) temperatures, at 80% relative humidity, and with 12h photoperiod. Measurements were started at the pinhead square stage using the fourth main-stem leaf. This study evaluated four different Fluorometer techniques. Fluorescence was measured using the Light- adapted test (using a fluorometer with the light-adapted clip), the

Dark-adapted test 1 (using the dark-adapted clip), the Leaf-tissue technique (using leaf punches 1.5 cm in diameter placed in a moistened environment and fluorescence measured with the dark adapted test), and the Dark-adapted test 2 (using a fluorometer with the dark-adapted clip on leaves covered with black bags).

In the second growth chamber study conducted in May 2003 different techniques were evaluated to quantify temperature tolerance under elevated temperatures. The cv. Suregrow 747 was planted in Sunshine mix and watered with half-strength Peter's nutrient solution. The plants were maintained at 30/20°C until the pinhead square stage, after which they were divided into two sets and half moved to 35/25°C. After three days at this temperature regime the temperature was raised to 40/30°C. Measurements were taken four days after the plants were placed in the elevated temperature, using the fourth main-stem leaf. Measurements were made of total active proteins (using the Bradford method), membrane leakage (using leaf-discs placed in 2 ml ionized water for 48 hours and measuring conductivity with an Automatic Seed Analyzer), chlorophyll fluorescence (using the light adapted test), and antioxidant enzymes (using the Anderson et al., 1992, technique).

Field Study

The field study to evaluate temperature tolerance in different cotton genotypes was planted in May 2003 in Fayetteville, AR, using a Randomized Complete Block design with six replications. The genotypes used were Stoneville 213, Deltapine 16, Rex, Acala SJ2 (Obsolete cultivars), and Stoneville 474, Deltapine 33B, Suregrow 747, Acala Maxxa (Modern cultivars). During the experiment temperature data was collected and measurements were taken within a temperature range of 28 to 32°C after the plants had entered the pinhead square stage. Measurements were made of total active proteins, membrane leakage, chlorophyll fluorescence, antioxidant enzymes, and polyols.

Results and Discussion

Techniques for Measuring Temperature Tolerance

The results from the first growth chamber study showed that three of the fluorometer techniques had similar and consistent results, while the fourth technique (Light-adapted test) had different but again consistent results (Figure 1). However, the light-adapted test was more applicable for field use. It was determined in a preliminary study with contrasting temperatures that 3-4 days were needed at a particular elevated temperature for plant acclimation before plant metabolic responses could be detected, i.e. by membrane leakage (ML), fluorescence (FL), proteins (PR) and catalase (CAT) (Figure 2). Among these four measurements (Figure 2) the results showed that ML and CAT were the most sensitive and accurate (Table 1) methods for quantifying temperature tolerance. However, ML was easier and more practical to measure in the field, while CAT was more time consuming and better suited for use in laboratory studies.

Screening of Cultivars

Field measurements of the selected eight cultivars with the three techniques (membrane leakage, fluorescence, and total active proteins) showed some genotypic variation, particularly at the higher temperatures, i.e. above 30.5 °C (Figure 3). Unfortunately, extremely high temperatures were not experienced in this 2003 field study. Measurements of sugar alcohols (polyols) as an indication of stress response to temperature showed clear genotypic differences (Figure 4). Old cultivars appeared to exhibit a greater increase in polyols at higher temperatures. Only the two Acala cultivars showed decreased polyols at higher temperatures. Although obsolete (old) and modern (new) cultivars did not show much significant difference in ML, FL, PR, polyols and gluatathione reductase with increased temperature, the obsolete cultivars were generally more responsive to higher temperatures indicating more stress tolerance (data not presented). For example, at the lower temperatures (27-28°C), old cultivars showed higher membrane leakage, whereas at the higher temperatures (30-31°C) they exhibited lower leakage. Similarly, old cultivars showed lower chlorophyll fluorescence (i.e. less stress) at higher temperatures. Old cultivars also showed lower protein levels at lower temperatures, but higher protein levels at high temperatures. For the polyols, old cultivars showed a larger increase at high temperatures indicating a better stress response. The antioxidant enzyme glutathione reductase also showed an increase in old cultivars at the higher temperature.

References

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Table 1. Contrasting sensitivity of techniques for measuring temperature tolerance

Temperature treatment	Measurements			
	Fluorescence	Membrane leakage µA/cm²	Proteins µg/mL	Catalase mM/g
30 °C	0.76 a †	21.4 b	1301.2 a	1702.4 a
35 °C	0.778 a	28.8 a	1038.6 b	1761.2 a
% of control	102.4 ±	134.5	100	103.5
30 °C	0.771 a	34.7 b	1011.5 a	1274.1 a
40 °C	0.393 b	101.1 a	763 a	255.3 b
% of control	50.96	291.6	75.4	20.03

† Numbers in a column followed by the same letter are not significantly different (P≤0.05).
± The higher temperature as a percentage of the 30°C control.

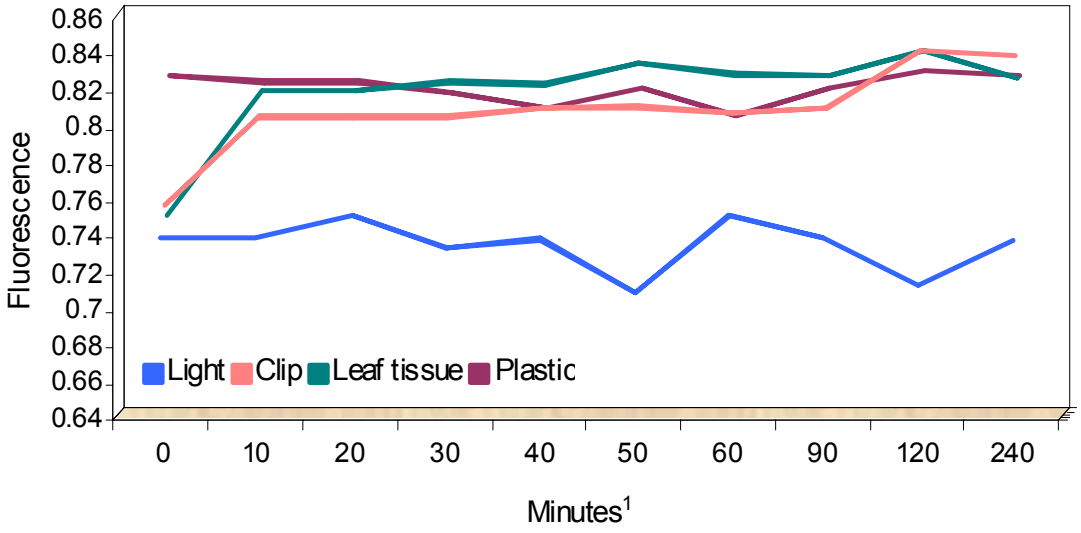


Figure 1. Measurements of fluorescence using four techniques; the light-adapted test [(Fms-Fs)/Fms] for the light technique, and the dark-adapted test (Fv/Fm) for the clip, leaf-tissue and plastic techniques. [1] The measurements were taken every ten minutes for one hour, then every 30 minutes until the second hour, and finally the last measurement was taken twelve hours later.

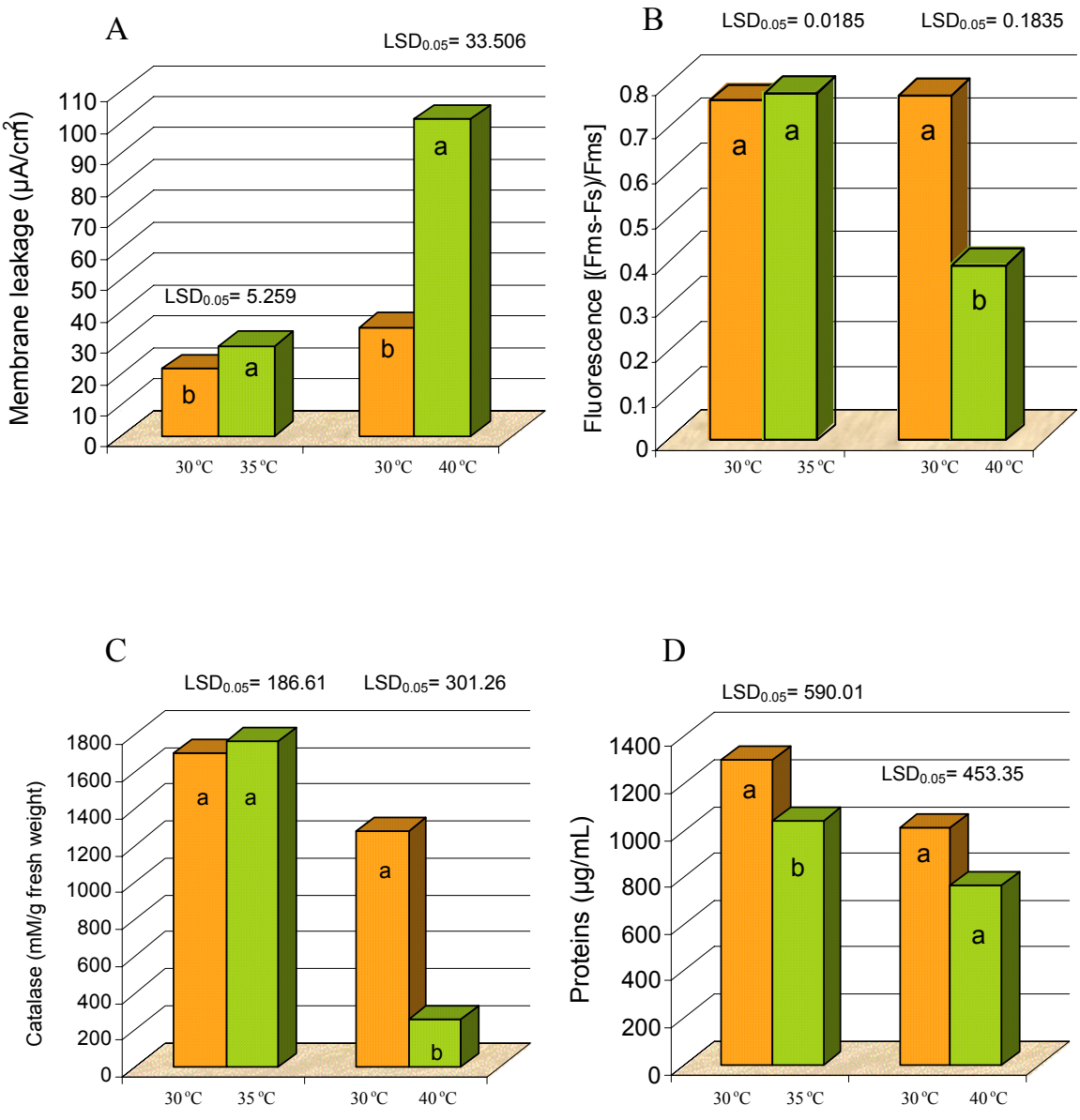


Figure 2. Effect of elevated temperatures on membrane leakage (A), fluorescence (B), total active proteins (C), and catalase (D). Pairs of columns superseded by a different letter are significantly different (P=0.05).

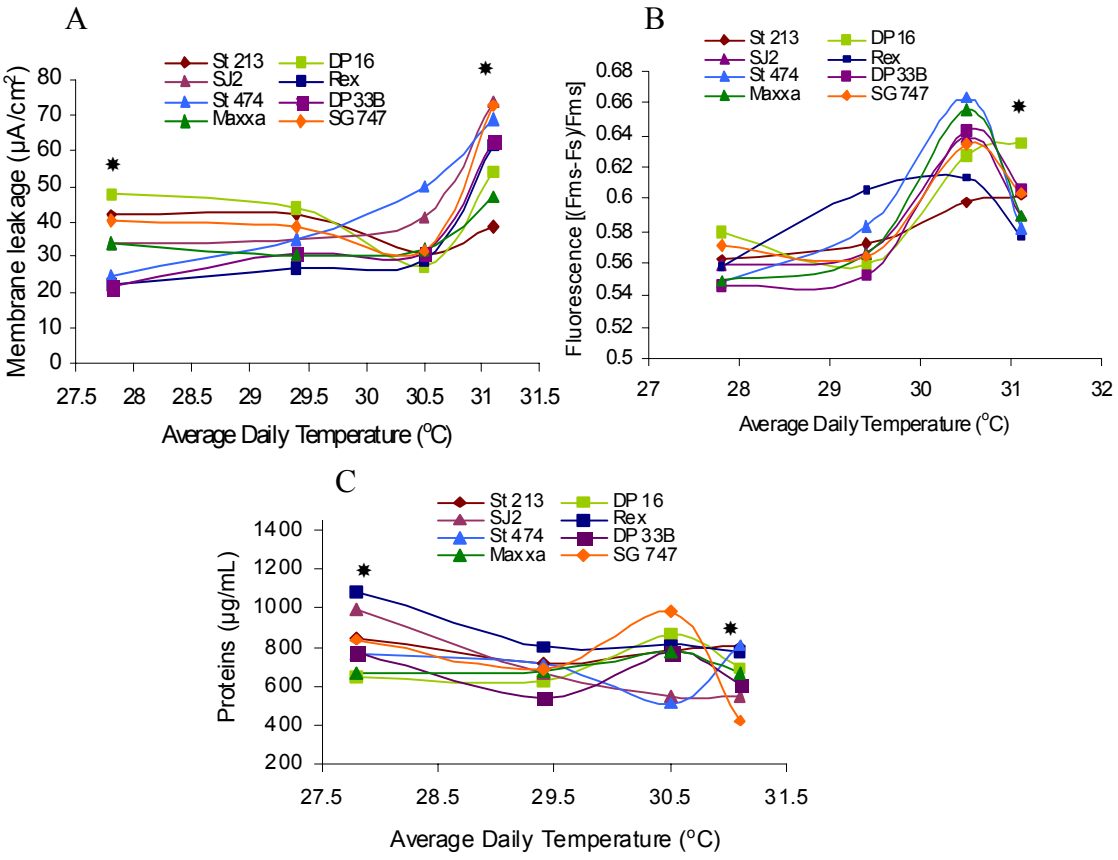
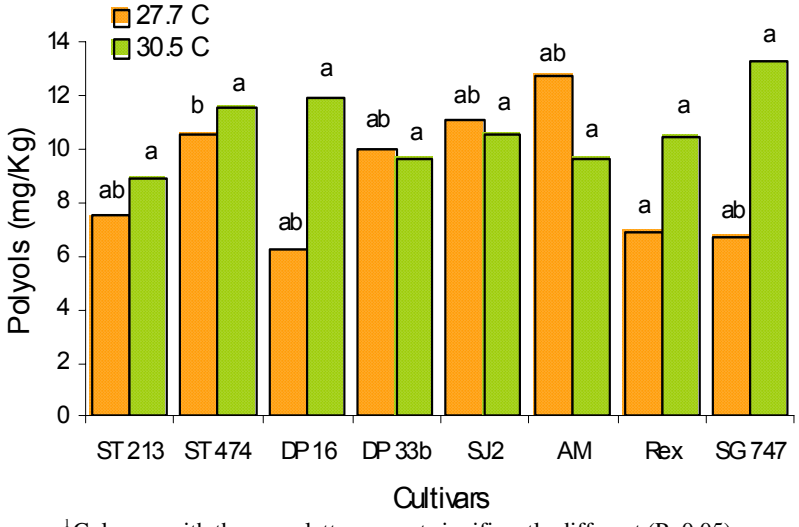


Figure 3. Field evaluation of genotypic responses to temperature as measured using membrane leakage (A), chlorophyll fluorescence (B), and total active proteins (C). ✸ Indicates significant differences between the cultivars (P=0.05).



[1]Columns with the same letter are not significantly different (P=0.05).

Figure 4. Polyols content of eight cultivars at two temperatures.