IDENTIFICATION OF HEAT TOLERANCE IN UPLAND COTTON USING CHLOROPHYLL FLUORESCENCE

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Abstract

With global warming and climate changing, heat stress becomes one of the most serious factors adversely affecting upland cotton production in the US. Based on initial screenings, forty-four wild upland cotton accessions were identified with heat tolerance (Frederick 2006). Using chlorophyll fluorescence technique, ten elite accessions were selected from these forty-four selected accessions based on evaluation in a growth chamber. A field evaluation using chlorophyll fluorescence was conducted on these ten elite accessions and four commercial lines. We found that the top five accessions have higher chlorophyll fluorescence values than commercial lines throughout the growth season under high temperatures. Based on these results, the five elite accessions may have potential to be genetic materials for development of heat tolerant germplasm.

Introduction

Upland cotton (*Gossypium hirsutum* L.) is the most important cotton species accounting for 90% of total cotton production worldwide (Lu et. al 1997). Though cotton is a tropical or sub-tropical crop, the optimum temperature for cotton growth and photosynthesis is 20-30°C (Reddy et al. 1991). Temperature higher than 35°C would lead to inhibition of cotton growth and photosynthesis, especially in the reproductive stage (Bibi et al., 2008). However, the average maximum temperature during flower and boll development in the US belt is higher than 35°C. There is a negative association between high temperature and cotton yield .With every degree of daily maximum temperature in July increasing in Arkansas, lint yield decreased by nearly 50 kg/ha (Oosterhuis, 1999). Heat stress causes big losses to cotton production. Therefore we investigated the potential of wild resources as potential sources for heat tolerance breeding.

Absorbed light energy can be utilized in three ways: photochemistry, heat dissipation and emission of chlorophyll fluorescence. They compete with each other. A decrease in fluorescence demonstrates an increase in activity of the photochemical reaction (Bjorkman and Demmig, 1987). Chlorophyll fluorescence is a reliable tool for detecting photosynthetic activity. It is easy, quick and sensitive. Fv/Fm (the ratio of variable fluorescence to maximal fluorescence) is a stable parameter which is about 0.83 in normal condition. Under stress imposed by heat, cold or drought, plants would have a decreased Fv/Fm. Another reason for employing chlorophyll fluorescence technique is most of the wild accessions have difficulty in flowering and producing bolls and it is hard to measure yield and fiber quality. The objective of this research is to screen heat tolerance in wild upland cotton accessions and discover heat tolerant accessions as breeding materials using chlorophyll fluorescence.

Materials and Methods

1762 wild accessions of *G. hirsutum* were obtained from the USDA-SPARC cotton germplasm collection in College Station, Texas. Forty-four accessions were selected from those 1762 accessions using chlorophyll fluorescence in 2006.

Growth Chamber Test

Forty–four selected accessions and forty–four random accessions and two checks were grown in the greenhouse with 27 °C/ 23 °C day/night temperature and 60% relative humidity until plants were at the six to eight-leaf stage. Each entry consisted of three replications. The whole plant test was conducted in a growth chamber with 80% relative humidity and 14hrs/10hrs day/night photoperiod. Plants were pre-treated at 25 °C for 24hrs for acclimation and then they were heated at 45 °C for 24hrs followed by recovery at 25°C for 24hrs. Chlorophyll fluorescence was measured on the newest fully developed leaf at the end of each step using the opti-science modulated fluorometer.

Three experiments were performed on July 22nd, August 6th and 26th, 2010, respectively. The result was presented as the percentage of reduction of chlorophyll fluorescence values at the end of heat stress, R_1.
R_1=(F_0-F_1) /F_0 where R_1 (%) is the percentage of chlorophyll fluorescence reduction after heat treatment. F_0 is Fv/Fm after 24 hrs of pre-treatment; F_1 is Fv/Fm after 24 hrs of heat treatment.

Field Test

Top ten selected accessions and four checks (DP90, SG 747, FM 966 and Acala Maxxa) were included in the field test. A randomized complete block design with four replications was performed in the Plant Breeding Unit, E.V. Smith Research Center in Tallassee, Alabama. Cotton seeds were pre-treated with fungicide and sown at a distance of 3 seeds/ inch on May 6th 2011. Each plot consisted of one ten-feet long row. Chlorophyll fluorescence was measured from first square stage to the open boll stage and the readings were taken during the hottest hours (12pm~2pm) of a day. Eleven measurements were conducted on different days. Temperature and humidity conditions of each day were listed in Table 1.

Table 1. Temperature and humidity of measurement day.

Date	Temperature (°C)	Humidity (%)
6.22.2011	27.8	47
7.6.2011	33.3	50
7.13.2011	36.4	43
7.18.2011	37.5	41
7.29.2011	28.9	50
7.31.2011	33.3	60
8.10.2011	36.9	48
8.17.2011	35.9	41
8.22.2011	35.4	42
8.26.2011	36.4	30

All data were analyzed by SAS 9.2 using a GLM (general linear model). The main effects of group, accession, day and day×group interaction were included in the analysis (SAS, 2010).

Results and Discussion

Whole-Plant Growth Chamber Test

The results of growth chamber test are presented as group means of R_1 (%) and summarized in Table 2. The group mean of R_1 (%) for the 44 selected accessions was not significantly different from that of random and check groups (P-value=0.1798). The range of R_1 (%) for the selected group was from 21.10 to 26.98 while that for the random group was from 29.01 to 35.10 (Figure 1). However the top ten selected wild accessions with group mean of R_1 (%) 13.65 was significantly different from random and check groups (P-value=0.046). It was 17.19 % lower than fluorescence reduction of random and check groups. Whole accessions were sorted into four quartiles by R_1 (%) values. Twelve selected accessions were included in the top quartile (quartile with the smallest R_1 (%)) while eight random accessions appeared in this range. A different pattern was observed in the bottom quartile (quartile with the largest R_1 (%)) with appearance of six selected accessions, fifteen random accessions and the two checks (Figure 2). These results indicated a larger proportion of random group and checks had significant reduction of chlorophyll fluorescence than the selected group after heat treatment. In other words, selected accessions showed more tolerance to heat stress than random accessions and checks.

Table 2. Effect of heat treatment on R_1 (%) in the growth chamber.

Group	Group mean of R_1 (%) ± SE	Range of R_1 (%)
Selected	24.47±3.03	21.1~26.98
Top 10 selected	13.65±4.78	8.38~17.72
Random & checks	31.43±3.23	29.01~35.10
	Difference of LSmeans for R_1(%)	
Selected vs Random and checks	4.48±3.33 (p=0.1798)	
Top 10 selected vs Random and checks	17.19±8.23* (p=0.046)	

*indicate significant differences at the 0.05 level of probability.

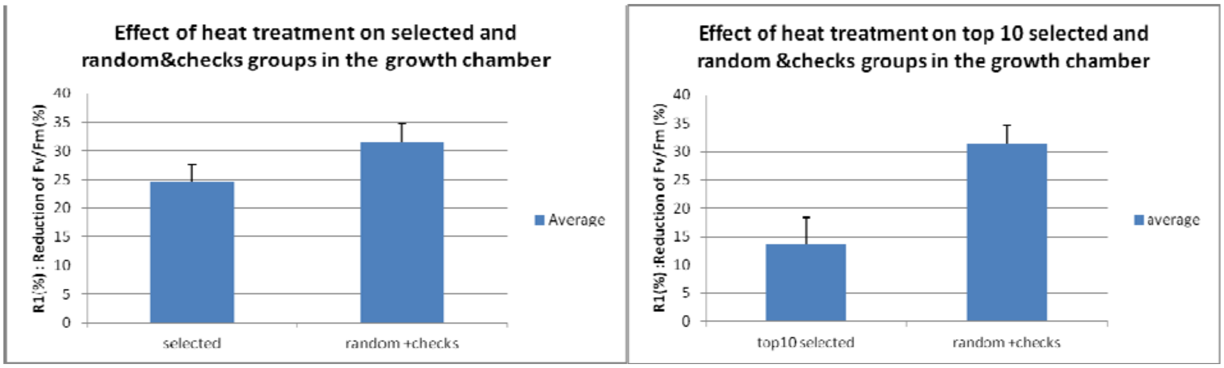


Figure 1.

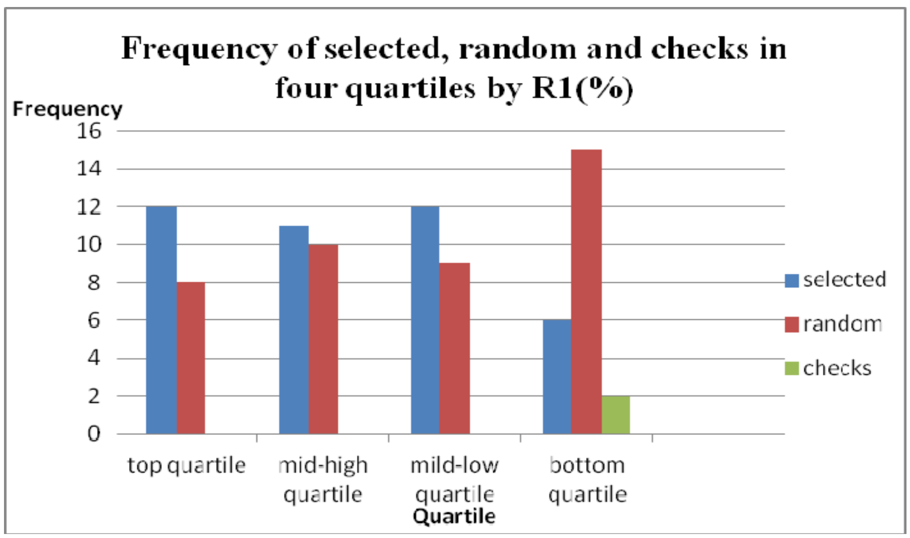


Figure 2.

Field Test

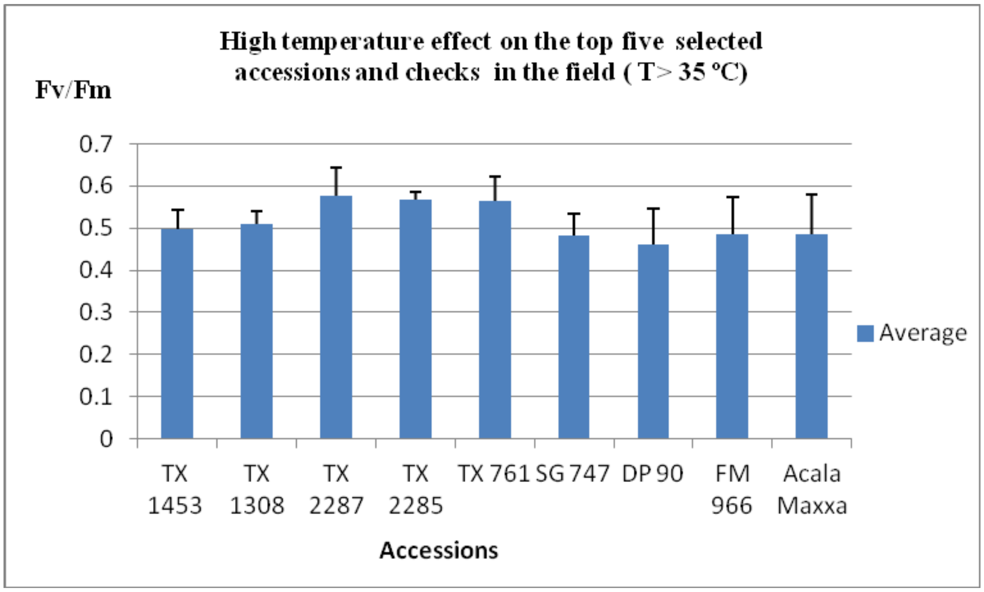
35 °C is the critical temperature for cotton growth. When temperature is higher than 35 °C, there will be heat stress on cotton plants. The temperature conditions were grouped into two types: high temperatures (T> 35 °C) and mild temperatures (T<35 °C). The average Fv/Fm for the top five selected accessions was 0.054 higher than checks under high temperatures which was significant (P-value<0.0001). However when the temperature was lower than 35 °C, the difference of Fv/Fm between the two groups was not significant (P-value=0.073) (Table 3). Although humidity may have an influence on heat tolerance, selected accessions appeared to consistently have higher chlorophyll fluorescence values than checks under heat stress conditions, especially accession TX 2287 and TX 2285. The average Fv/Fm values for those two were 0.575 and 0.568 respectively, around 0.1 higher than that of checks (Figure 3). When there was no heat stress, the range of fluorescence values for the two groups was similar. The five elite selected wild accessions are listed in Table 4. They are very diverse in origin and characteristics such as leaf color and leaf shape. Therefore they may have value as genetic resources for plant breeding.

Table 3. Effect of high temperature and mild temperature on Fv/Fm in the field.

	High temperature (T>35 °C)		Mild temperature (T<35 °C)	
	Group mean (Fv/Fm) ±SE	Range	Group mean (Fv/Fm) ±SE	Range
Selected (top five)	0.54±0.06	0.45~0.67	0.64±0.05	0.55~0.71
Checks	0.48±0.08	0.39~0.65	0.61±0.05	0.54~0.7

	Difference of LSmeans for Fv/Fm	
	High temperature (T>35 °C)	Mild temperature (T<35 °C)
Top five accessions vs. checks	0.054±0.011* (P<0.0001)	0.019±0.010 (P=0.073)

*indicate significant differences at the 0.05 level of probability.



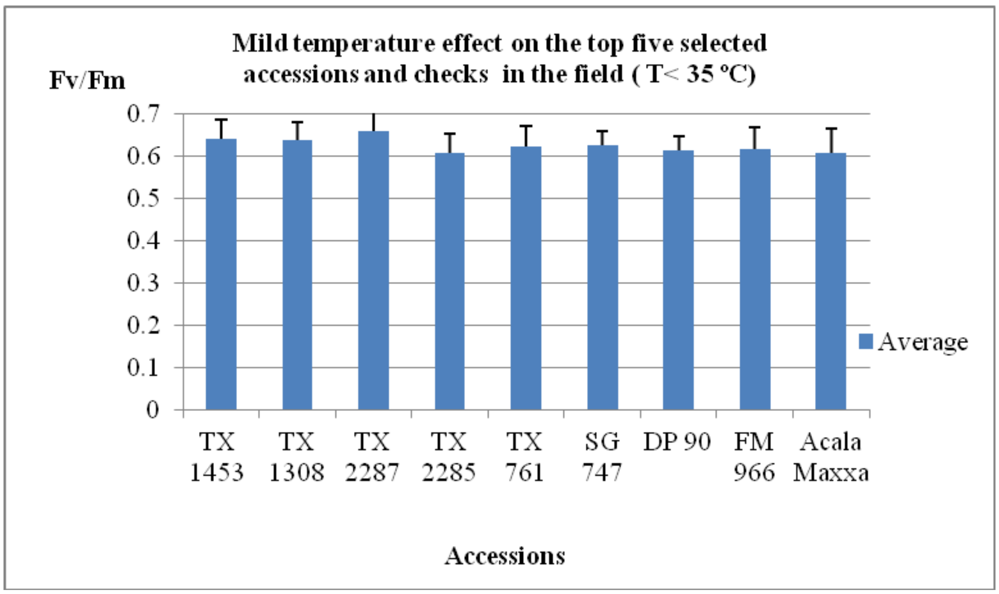


Figure 3.

Table 4. Characteristics of five selected wild accessions (www.cottondb.com).

Texas-No	PI-No	Country	State	Lint color	Leaf color	Leaf shape
1453	530133	Mexico	San Luis Potosi	white	green	normal
1308	381948	Argentina	--	white	green	normal
2287	501467	United States	Puerto Rico	white	green	okra
2285	501465	United States	Puerto Rico	white	green	normal
761	201597	Mexico	Veracruz	tan	green	super okra

Summary

Under the experimental conditions, although variations of fluorescence values existed among accessions, the top 10 selected accessions (wild accessions) appeared to be more tolerant to heat compared to random accessions and checks (commercial lines).This was also observed in the field test under high temperatures. Based on these results, the top five accessions may have potential to be genetic materials for development of heat tolerant germplasm. Further evaluations of heat tolerance with other measurements will be needed which includes membrane leakage measurement and protein and enzyme activity analysis.

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