Identifying Cotton Cultivars for Hotter Temperatures

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Summary

High temperature stress adversely affects the growth, development and ultimately yield of cotton. This paper presents a general outline of studies evaluating methodologies that measure the ability of cotton to tolerate high temperatures. Simple methodologies that measured plant response to high temperature stress at different scales (field to laboratory) are being assessed for their ability to detect differences between cotton cultivars. Preliminary measurements on known heat tolerant and susceptible cultivars taken at a whole plant, leaf, cell and at the individual gene levels suggest that there is indeed variation among cultivars, and there is opportunity to detect differences in their ability to withstand high temperatures with simple laboratory tests. These methodologies are being further evaluated to establish their value in identifying thermotolerant cotton cultivars from a broader range of cotton germplasm for potential use in cotton breeding programs.

Key words

Cultivar screening, high temperature tolerance, heat stress, yield, photosynthesis, membrane integrity, enzyme viability, gene expression

Introduction

Cotton has an optimal thermal kinetic window of 23 to 32^{o}C in which metabolic activity is most efficient (Burke *et al.* 1988). High temperatures (>35^{o}C) throughout the growing season are commonplace among the cotton production areas of Australia and may adversely affect the growth and development potential of the crop and ultimately yield (Hodges *et al.* 1993). This may be attributed to lower performance of photosynthetic and respiratory enzymes under high temperature stress (Reddy *et al.* 1991) or cell membrane damage (Sullivan 1971). Thermotolerant cotton plants possess mechanisms to buffer the effects of

short term high temperature stress against changes in the viability of these enzymes and mechanisms to prevent or compensate for tissue damage.

Temperature regulation in the plant is also facilitated by heat dissipation through transpiration in the leaves. Low or unreliable water availability exaggerates temperature stress in the leaves also leading to photosynthetic decline. It is likely that future climates for cotton production will have an increased number of hot days and there maybe more instances of limited water availability to compensate for increased evaporative demand (McRae *et al.* 2007). Hence, efforts to reduce water use in cotton cropping systems must be supplemented by the development of strategies aimed at developing cotton cultivars with superior thermotolerance during drought conditions.

A series of field and glasshouse experiments were conducted to evaluate methodologies for determining of the ability of cotton cultivars to tolerate high temperatures under a range of different high temperature stresses as well as assessing their applicability for screening larger populations of cultivars. High temperature thermotolerance was determined through a series of screening assays and measurements at a crop, whole plant, leaf, cell and individual gene level, thus providing a multi-scale analysis of thermotolerance in cotton (Figure 1). Working across scales allowed us to validate these methodologies by establishing whether differences detected at one level translated to differences at others.

Methodology

High temperature stress under field conditions varies both daily and seasonally and is generally confounded by concurrent conditions of high light, low humidity and drought stress. Furthermore, biotic stresses such as insect, disease and weed pressures may contribute to low yields under field conditions (Hearn and Fitt 1992). Due to these extensive environmental variables in the field, laboratory assays have been traditionally used to determine thermotolerance in various plant species (Blum and Ebercon 1981; Porter *et al.* 1995; Steponkus and Lanphear 1967; Sullivan 1971). These assays can be rapid, cost effective and repeatable as a defined and isolated high temperature stress can be generated to screen multiple generations under identical environmental conditions. However, there are few reports on whether tolerance assumptions based on these assays, are applicable to field conditions.

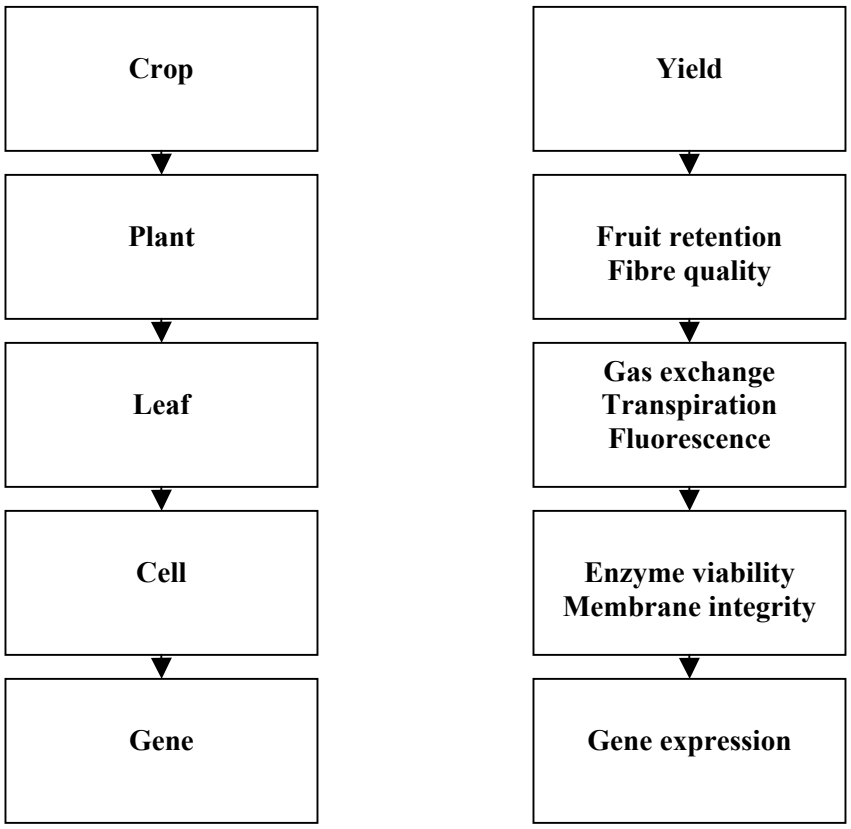

Figure 1 Schematic diagram of multi-scale analysis of methods for screening for thermotolerance in cotton.

In this study we assessed a number of experimental approaches (assays) for their ability to detect differences between cultivar Sicot 53, known to have a relatively high level of thermotolerance and the less tolerant cultivar Sicala 45. To assess their tolerance a number of approaches were implemented to generate heat stress, including;

- Field tents (made from clear Solarweave ® plastic) that were erected above the crop canopy to raise air temperatures under the fabric, but still facilitate airflow down the rows
- Growth cabinets to increase the ambient air temperature circulating around the plants
- Water baths to subject leaf material to high temperature stress in the laboratory

Specifically the measurements on these plants and leaf material collected from these plants used to detect cultivar differences that encompassed the multi-scale approach described above were;

- Crop and plant level measurements: yield, fruit retention and fibre quality
- Leaf level physiological measurements: leaf photosynthesis, stomatal conductance, transpiration and fluorescence using a Li-6400 portable photosynthesis system

- Cell level biochemical assays: enzyme viability test measuring the degree impaired function of respiratory enzymes in the mitochondria and cell membrane structural integrity test that measure the degree of membrane leakage
- Gene level measurements: an estimate of the relative expression of the Rubisco activase gene, a chaperone for the Rubisco enzyme and hence a primary determinant of carbon assimilation in the plant

Results and Discussion

In this paper we present examples of some results of our studies comparing methodologies to detect differences in heat tolerance at the crop (yield), cell (cell membrane integrity) and gene level (Rubisco activase expression) (Figure 1).

Crop level measurements: Yield determination

Lint yield was significantly higher for plants grown under ambient field conditions, compared with plants grown under short-term exposure to high temperature stress ($P<0.001$) under tent 1 (Figure 2), placed over the crop on the 4th February 2006. Lint yield was not different for plants grown under ambient field conditions compared with plants grown under short-term exposure to high temperature stress under tent 2 ($P=0.306$), placed on a separate part of the crop to tent 1 on the 24th February 2006. Lint yield was also significantly higher for plants grown under tent 2, in comparison to plants grown under tent 1 ($P<0.001$). This indicates that the exposure to high temperature stress under tent 1 was sufficient to illicit a heat stress response, however the exposure to high temperature stress under Tent 2 was not sufficient to illicit a stress response.

There was no significant cultivar differentiation in yield under ambient field (control) conditions (Figure 2) ($P=0.274$), thereby suggesting that both cultivars are equally suited to the growing conditions and equally affected by non-target selection pressures throughout the season. There was also no significant cultivar difference in lint yield under both tent 1 and tent 2 conditions.

Yield is a primary tool for screening plant populations in the field. Differences in yield can describe the broad-scale suitability of one cultivar to a specific growing region, thereby ensuring high yields for producers. This approach in this study was not successful in identifying specific high temperature thermotolerance. The reason for this is that yield analysis comprises a multitude of morphological, physiological and biochemical responses to environmental stimuli and can be variable across seasons and within populations.

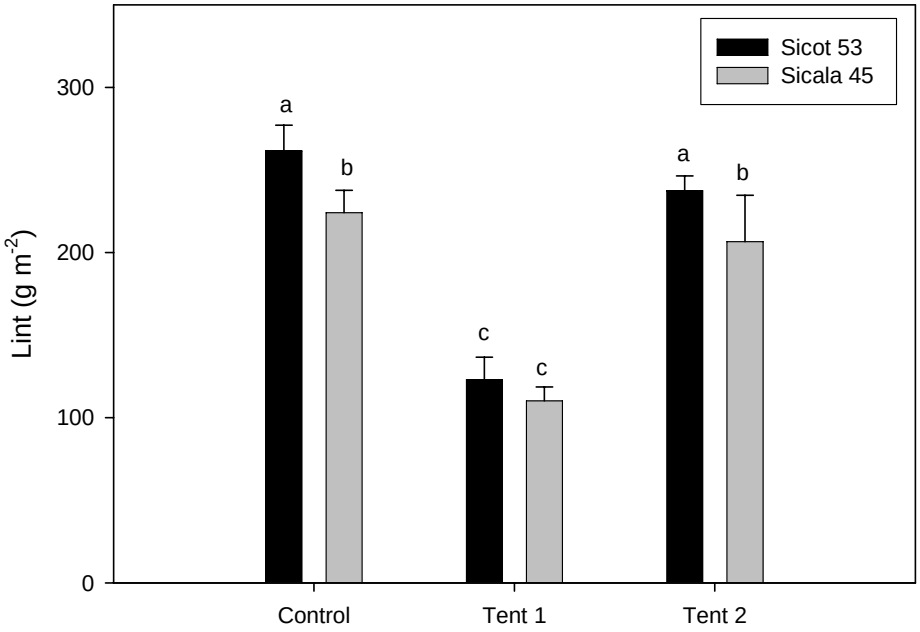

Figure 2 Yield of Sicala 45 and Sicot 53 cotton cultivars under field conditions at the Australian Cotton Research Institute, Narrabri, NSW. Standard error bars are shown for P≤0.05 level. Data points with the same letter above them are not significantly different.

Leaf level biochemical assays: Cell membrane structural integrity

The degree of cell leakage attributed to high temperature stress may be estimated by exposing leaf tissue to high temperatures and measuring the electrical conductivity of the exudate. Exposure to high temperature stress (>40^{o}C) generated using water baths, increased the relative electrical conductivity of the solution containing incubated cotton leaf tissue. This is consistent with the findings of Bibi *et al*., (2008) and may be attributed to changes in the cell membrane structure, resulting in a decrease in membrane integrity, leakage of the cell cytoplasmic contents and an overall limitation of the efficacy of electron carriers and enzymes associated with the membrane, which are vital for efficient photosynthetic function (Taiz and Zeiger 2006).

The relative electrical conductivity of the solution varied significantly between cultivars Sicot 53 and Sicala 45 (P<0.001). Membrane leakage increased sigmoidally with increasing temperatures for both cultivars (Figure 3). The critical temperature at which 50% membrane leakage occurred was higher for Sicot 53 (46^{o}C) than for Sicala 45 (39^{o}C) (Figure 3). Sicala 53 was best able to maintain cell structural stability at 45^{o}C, thereby indicating a higher inherit level of thermotolerance than cultivar Sicala 45. Sicala 45 incurred a high rate of

membrane leakage between 35 and 40°C which was not evident in cultivar Sicot 53. The quantity of membrane leakage was not distinguishable between cultivars in the control (25°C) or excessively high temperature (65°C) treatments (Figure 3). Hence, the membrane leakage assay may be used to distinguish thermotolerance between different cultivars, provided that an adequate temperature is used, but this temperature is sufficiently low to distinguish between cultivars. Sicot 53 consistently shows a greater level of thermotolerance than Sicala 45. Hence, laboratory determination of cell membrane integrity of field grown cotton tissue may be a viable method of screening for thermotolerance in cotton cultivars.

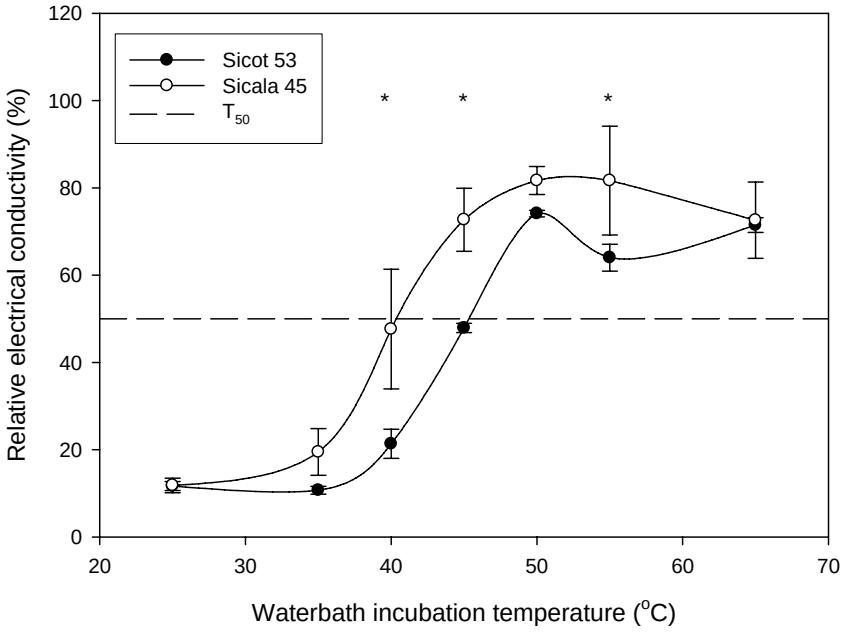

Figure 3 Relative electrical conductivity (%) of leaf tissue of cotton cultivars Sicot 53 and Sicala 45, grown under field conditions at the Australian Cotton Research Institute, Narrabri, NSW in the 2005-2006 growing season and incubated for 2 hours at different temperatures (25, 35, 40, 45, 50, 55, 65°C) in a temperature controlled water bath. Asterix represent significant differences in relative electrical conductivity (%) between cultivars Sicot 53 and Sicala 45, at a 95% confidence interval.

Gene level measurements: Rubisco activase expression

Molecular biology has been widely adopted for the identification of gene up and down-regulation in response to biotic and abiotic stress. Each organism has a template of DNA encoding for the functionality of proteins. Under a time course of high temperature stress, genes within this template are up regulated or down regulated (dependent on function), which can be quantified by real-time polymerase chain reaction (PCR).

There was a significant interaction between temperature treatment and cultivar for relative expression of Rubisco activase at 1 h after the imposition of high temperature stress

(*P*=0.002) (Figure 4). The relative expression of Rubisco activase was not significantly different in the leaf tissue of Sicot 53 plants grown under optimal (32^{o}C) and high temperature (42^{o}C) conditions. This indicates that Sicot 53 is able to maintain enzyme function and subsequent energy production under short term (1 h) high temperature stress, indicating a high level of thermotolerance. Conversely, the relative expression of Rubisco activase in Sicala 45 leaf tissue was significantly decreased under high temperature conditions. This may indicate severe protein denaturation and subsequent decrease in overall plant function, hence contributing to a relatively low level of thermotolerance.

Molecular screening tools for thermotolerance provide a protein specific analysis of cultivar specific responses to high temperature stress. Hence, specific enzymes in overall physiological processes, such as photosynthesis can be targeted for screening criteria, or genetic improvement. These methods are rapid, with a short lag time between multi-generational analyses and readily repeatable. However, these processes are relatively expensive in comparison to physiological screening mechanisms. It would be beneficial to identify a range of cultivar specific thermally responsive genes but then develop rapid biochemical or physiological tools to rapidly screen large populations for breeding programs.

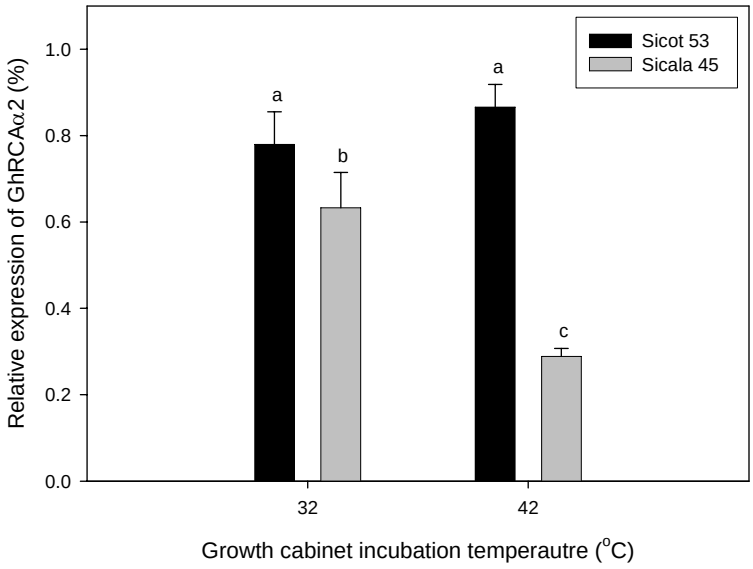

Figure 4 Relative gene expression of Rubisco activase in leaf tissue of cotton cultivars Sicot 53 and Sicala 45, after 1 hour at 32^{o}C (control) or 42^{o}C (heat) in the growth cabinet at the Australian Cotton Research Institute, Narrabri, NSW. Least square means and standard errors are shown for P≤0.05 level. Data points with the same letter above them are not significantly different.

Conclusion

High temperature stress adversely affects multiple physiological and biochemical pathways contributing to the growth and development, and ultimately yield of cotton. Although breeding programs have generally focused on yield as a cultivar selection tool, there exists potential for the development of stress specific screening tools for rapid identification of superior cotton cultivars. The broad scale nature of yield and photosynthesis as stress screening tools deems them relatively unrepeatable under field conditions. However, these tools may be applied to assess broad-spectrum production of cotton cultivars in specific growing regions. Care must be taken to account for the large influence of environmental variability on these results.

The membrane integrity test is a quick and reliable laboratory assay that can be used to estimate the relative thermotolerance of cotton cultivars under high temperature stress. This assay may hence have potential to be employed to screen a large number of cotton cultivars for thermotolerance under field conditions

Molecular biology provides significant opportunities for the determination of gene specific effects of high temperature stress on the functionality of biochemical pathways of plants. There is great potential for further evaluation of stress genes in cotton, and for subsequent determination of the cultivar specificity of these genes. Once identified, real-time PCR is a relative complex and expensive, yet repeatable method of screening for thermotolerance. However, thermotolerance in field grown cotton is most likely multi-genetic and hence specific gene investigations need to be supplemented with plant and crop physiology measurements to ensure that differences detected at the gene level translate to significant differences in real growing conditions.

This paper presents an array of measurements and assays that can be implemented to field grown and glasshouse grown cotton plants, for the determination of thermotolerance. All of these assays indicated that Sicot 53 is relatively more thermotolerant than Sicala 45, thereby justifying the validity of each level of analysis. These methods can be applied to a broad range of genetic material, in a diverse range of environments, from which a cultivar-specific thermotolerance index can be devised. This index may offer opportunity to be applied to other abiotic stresses such as cold, high light and drought conditions. Tolerant cultivars can then be incorporated into current breeding programs to ensure that cotton yields are maintained under high temperature stress.

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