SCRENNING COTTON CULITVARS FOR ABIOTIC STRESS TOLERANCE USING REPRODUCTIVE AND PHYSIOLOGICAL PARAMETERS

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Abstract

Cotton, an important fiber crop, is highly sensitive to environmental stresses. Increases in temperature above the optimum, particularly during flowering and boll-filling, is detrimental to yield and fiber quality. Developing rapid and inexpensive screening tools for abiotic stress tolerance is therefore needed and will be beneficial to breeding programs and selection of cultivars for a niche environment. In this study, several reproductive and physiological parameters were employed as screening tools for abiotic stress tolerance among 38 cotton cultivars. Pollen-based parameters such as in vitro pollen viability (PV) and pollen germination (PG) at optimum and high temperatures were studied to determine variability among cultivars. Photosynthesis and other physiological parameters such as cell membrane thermostability (CMT), chlorophyll stability index (CSI), total chlorophyll, and carotenoid content, and biophysical parameter such as canopy temperature depression (CTD) were measured during the flowering period. Cumulative heat stress response index (CHSRI) of each cultivar, calculated as the sum of individual stress responses derived from reproductive, photosynthetic, and physiological parameters, were used to distinguish differences among the cultivars. Cultivars were classified as heat-sensitive, intermediate, and heat-tolerant for both physiological and pollen-based traits. Based on reproductive heat stress response index (RHSRI), seven cultivars (NG 1511 B2RF, CG3428B2RF, DP0912B2RF, DG 2530B2RF, DG2595B2RF, DP1321B2RF and LA122) have been classified as heat-tolerant, while 21 and 10 identified as intermediate and heat-sensitive, respectively. Similarly, based on physiological heat stress response index (PHSRI), 16 cultivars were classified as heat-sensitive, 17 as intermediate, and 5 as heat-tolerant (PHY367WRF, NITRO44B2RF, DP 1321 B2RF, PX532211WRF and DP1048B2RF) among the 38 cultivars studied. Poor correlation between physiological and pollen-based traits revealed that reproductive and physiological traits are different among cultivars and breeding programs should pay consideration to both traits. Traits identified could serve as useful screening tools in cotton breeding programs and cultivars identified should be considered as potential candidates in breeding programs aimed to develop suitable genotypes to cope in the present and projected warmer climates. Also, cultivar-dependent relative scores, based on physiological and reproductive parameters, will be vital in selecting cultivars for a niche environment to cope with abiotic stresses at both vegetative and reproductive stages.

Introduction

With rapid changes in climate as a result of continued projected changes in carbon dioxide and other greenhouse gases, high temperature stress has become one of the prime factors exerting a major influence on crop production. Global surface air temperatures have increased by 0.6°C during the last century and climate models project increase of 1.4 - 5.8°C by the end of the current century (Houghton et al., 2001). Along with projected higher temperatures, extreme events such as warmer days and a concurrent decrease in diurnal temperature ranges are projected to occur more frequently in the future climates (Dai et al., 2001).Cotton an important fiber crop, is highly sensitive to such environmental stresses. Among cotton growing areas across the world, unexpected periodic episodes of extreme heat stress, and likely during the time of flowering and boll-filling, will result into lower boll set, reduced lint yields, and poor fiber quality. Hence, crop scientists in the future will have to face the challenge of growing crops in a much different environment than today because of projected changes in climate.Crop production and productivity is highly sensitive to changes in climate and weather conditions. One of the more practical and economic ways to overcome negative effects of heat stress in cotton is to identify and/or develop tolerant cultivars.With improved physiological techniques and genomic tools, it is becoming easier to manipulate traits for abiotic stress tolerance. Understanding plant responses to abiotic stress at the whole plant level and developing rapid and inexpensive screening tools for stress tolerance are sought after areas these days.

Materials and Methods

An experiment, comprising of 38 cultivars/entries from the 2012 Mississippi State University cotton variety trials arranged in a randomized complete block design with four replications, was conducted at the R. R. Foil Plant Science Research Center, Mississippi State University, Mississippi State (lat. 33º 28´ N, long. 88 º 47´ W). Cultivars were planted May 25, 2012 on rows spaced 1 m apart and standard cultural practices applied as specified by the Mississippi State Extension service. All measurements were taken during the peak of flowering, about 60 DAS from July 25 to August 1, 2012. Among reproductive parameters, in vitro pollen viability (PV) and pollen germination (PG) were studied to determine variability in response among cultivars. One to two recently opened flowers from 10-15 plants/cultivar/plot at anthesis were randomly collected between 08.00 and 10.00 h during the flowering period. Pollen grains were collected from these flowers and distributed uniformly on the solidified germination medium using a tiny, clean, bristle paint brush. The Petri dishes were then covered and incubated in an incubator (Precision Instruments, New York, NY) at temperature treatments of 30 and 38 °C. Total pollen grains and number of pollen grains germinated were counted using a Nikon SMZ 800 microscope (Nikon Alphaphot YS microscope; Nikon Instrument, Kangava, Japan).Viability of pollen was tested using 2% concentration of 2, 3, 5-triphenyl tetrazolium chloride (TTC) stain in deionized water as described by Aslam et al. (1964). A modified pollen germination medium (Reddy and Kakani, 2007) was used for pollen germination.

Leaf net photosynthesis (Photo) and stomatal conductance (SC) were measured between 10.00 and 14.00 h during cloud free days, on the third or fourth fully expanded leaf from the top of plants using portable photosynthesis system (Model LI-6400, LI-COR, USA). Apart from photosynthetic parameters, physiological parameters such as cell membrane thermostability (CMT), chlorophyll stability index (CSI), total chlorophyll, carotenoid content, and canopy temperature depression (CTD) were also measured during flowering. Leaf pigment content and chlorophyll stability index (CSI) was measured by taking two sets of leaf samples collected from five fully expanded leaves for each cultivar during the same period. Five leaf discs of 2.0 cm^2, from each sample were collected randomly and placed in vials containing 4 ml of dimethyl sulphoxide for chlorophyll (Chl) extraction. Absorbance of the extract was measured using a Bio-Rad ultraviolet/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA) at 470, 648, and 663 nm to calculate concentrations of Chl a, Chl b, and carotenoid content (Chapple et al., 1992). The chlorophyll stability index (CSI) was determined according to Sairam et al. (1997). Accordingly, another set of leaf discs was collected from each cultivar and incubated at 56°C in a temperature- controlled water bath for 1 h. The set of tubes was brought to 25°C and the Chl content was measured from the heat-treated samples as described previously. The CSI was estimated as the ratio of Chl content in heated leaf (56°C) to that in fresh leaf expressed as a percentage. The leaf CMT in cotton cultivars was assessed according to the procedure described by Martineau et al. (1979). Canopy temperature depression measurements were taken during flowering period, where leaf temperature of five, fully expanded leaves from each cultivar and the respective air temperatures were measured between 12.00 and 13.00 h (cloudless, bright days) using a handheld infrared thermometer (Model OS533E-OMEGASCOPE; OMEGA Engineering, Inc., Stamford, CT).

Individual response index (IRI) of each parameter was calculated as the value for a cultivar divided by the maximum value observed over all other cultivars. Reproductive heat stress response index (RHSRI) of each cultivar, was then calculated as the sum of individual response index derived from reproductive parameters, i.e. in vitro pollen viability and pollen germination. Similarly, physiological heat stress response index (PHSRI) of each cultivar, was also calculated as the sum of individual response index derived from photosynthetic parameters (photosynthesis and stomatal conductance), pigments (total chlorophyll and carotenoids), CMT, CTD, and CSI to distinguish differences among the cultivars and to classify them as heat-sensitive, intermediate and heat-tolerant to high temperature.

Cultivars were classified based on their cumulative response index value. Standard deviations SD_R, and SD_P were calculated for reproductive and physiological parameters, respectively, based upon differences found in respective cumulative index values. Each cultivar was classified based on its reproductive parameters and standard deviation (SD) as low, intermediate, and high heat tolerant (Equations 1, 2, and 3).

Low heat tolerant = [(minimum RHSRI)] - [(minimum RHSRI + 1.5 SD)] **(1)**
Intermediate = [(minimum RHSRI + 1.5 SD)] – [(minimum RHSRI + 3.0 SD)] **(2)**
High heat tolerant = > [(minimum RHSRI + 3.0 SD)] **(3)**
Similarly, cultivars were also classified as either low, intermediate, or high for heat tolerance based on physiological parameters and SD (Equations 4, 5, and 6).

Low heat tolerant = [(minimum PHSRI) - [(minimum PHSRI + 1.5 SD)] **(4)**
Intermediate = [(minimum PHSRI + 1.5 SD)] – [(minimum PHSRI + 3.0 SD)] **(5)**
High heat tolerant = > [(minimum PHSRI + 3.0 SD)] **(6)**

Results and Discussion

Cultivars differed significantly for all physiological and pollen-based parameters measured. Photosynthesis among the cultivars ranged from 16.7 (DP 0912B2RF) to 41.93 µmol m^{-2} s^{-1} for (CT12214) with an overall average of 30.19. Similarly, the CMT (%) values ranged from 15.58 (MON11R136B2R2) to 40.37 (PX532211WRF) with an overall mean value of 25.38 µmol m^{-2} s^{-1} for the 38 cultivars tested, indicating a wide range in variation among cultivars. Total chlorophyll content was highest for PX5322111WRF (38.05) and lowest for MON11R136B2R2 (28.37) with a mean value of 33.14. Values for CTD also suggested a wide range of variability among cultivars with the highest (4.33) and lowest (1.20) values for DP1048B2RF and DP0920B2RF, respectively. DP1321B2RF (51.31%) recorded the highest pollen germination at 30º C while the lowest was recorded for PX433906WRF (23.18%).However at 38 º C, cultivar DG2595B2RF (41.2%) recorded the highest and FM1944GLB2 (15.39%) resulted in least pollen germination. Pollen viability among cultivars ranged from 43.8 (DP1137B2RF) to 90.89% (DP1359B2RF) with an average of 59.82%.The values for SLA were highest for PX433915WRF (199.58), whereas DG2595B2RF (155.9) produced the lowest value.

Cumulative heat stress response indices (CHSRI) of each cultivar, calculated as the sum of individual stress responses derived from reproductive and photosynthetic parameters, including CMT, CTD, and CSI, were used to distinguish differences among the cultivars and to classify them as sensitive , intermediate, and tolerant to high temperature (Figure 1 and 2). Cultivars evaluated also differed for individual and cumulative reproductive as well as physiological response indices. Since there was no correlation between physiological and pollen-based traits, the cultivars were classified based on physiological and reproductive parameters. Classification of 38 cotton cultivars into heat tolerant groups based on cumulative reproductive and, physiological stress response indices is summarized in (Table 1). Based on RHSRI, only 7 cotton cultivars (NG 1511 B2RF, CG3428B2RF, DP0912B2RF, DG 2530 B2RF, DG 2595 B2RF, DP1321B2RF, and LA122) were classified as heat-tolerant while 21 and 10 were intermediate and heat-sensitive, respectively. Similarly, based on PHSRI, 14 cultivars were classified as heat-sensitive, 19 as intermediate, and only five could be categorized as heat-tolerant (PHY367WRF, NITRO44B2RF, DP1321B2RF, PX532211WRF, and DP1048B2RF).

Conclusions

Cultivars identified as heat tolerant should be considered as potential candidates for use in breeding programs and in the selection of cultivars for a niche environment. Based on reproductive as well as physiological heat stress parameters among the 38 cultivars, DP1321B2RF has been identified, and would be expected to perform better under heat stressed environments than the other cultivars evaluated.

Table 1. Classification of 38 cotton cultivars into heat and heat and drought tolerant groups based on cumulative reproductive and, physiological stress response indices.

Heat Stress Response Index –Reproductive			**Heat Stress Response Index –Physiological**		
Low	**Intermediate**	**High**	**Low**	**Intermediate**	**High**
NITRO 44 B2RF (1.23)	BX 1346GLB2 (1.50)	DG 2595 B2RF (1.77)	DP 0920 B2RF (3.74)	AM1550 B2RF (4.22)	NITRO 44 B2RF (5.05)
DP 1137 B2RF (1.26)	PHY 375 WRF (1.50)	DP 1321 B2RF (1.79)	CG 3428 B2RF (3.76)	DP 1133 B2RF (4.42)	DP 1321 B2RF (5.08)
DP 1048 B2RF (1.27)	DP 1044 B2RF (1.50)	DG 2530 B2RF (1.79)	MON 11R136B2R2 (3.80)	UA 222 (4.42)	DP 1048 B2RF (5.15)
PX433915WRF (1.29)	UA 222 (1.51)	LA 122 (1.81)	BX 1348GLB2 (3.99)	DP 1219 B2RF (4.46)	PHY367WRF (5.18)
DP 1219 B2RF (1.33)	FM 1944GLB2 (1.53)	DP 0912 B2RF (1.82)	DP 0912 B2RF (4.00)	DG 2595 B2RF (4.49)	PX532211WRF (5.72)
DP 1034 B2RF (1.37)	DP 1133 B2RF (1.55)	CG 3428 B2RF (1.82)	DG 2530 B2RF (4.12)	DG 2570 B2RF (4.51)	
DP 1359 B2RF (1.40)	PX4339CBWRF (1.56)	NG 1511 B2RF (1.86)	HQ 210 CT (4.21)	NG 1511 B2RF (4.57)	
PX532211WRF (1.40)	DP 0920 B2RF (1.57)		DP 1137 B2RF (4.22)	PX4339CBWRF (4.67)	
PX433906WRF (1.44)	CG 3787 B2RF (1.57)		ST 5288B2F (4.24)	FM 1944GLB2 (4.72)	
ST 5288B2F (1.45)	NG 5315 B2RF (1.58)		DG 2610 B2RF (4.32)	PX433906WRF (4.74)	
	CT12214 (1.61)		DP 1359 B2RF (4.32)	NG 5315 B2RF (4.80)	
	HQ 210 CT (1.61)		PHY499WRF (4.36)	DP 1044 B2RF (4.80)	
	DG 2570 B2RF (1.61)		PHY 375 WRF (4.37)	LA 122 (4.85)	
	MON 11R136B2R2 (1.64)		CG 3787 B2RF (4.38)	BX 1346GLB2 (4.86)	
	AM1550 B2RF (1.65)			PX433915WRF (4.87)	
	PHY499WRF (1.67)			DP 1034 B2RF (4.87)	
	DP 1311 B2RF (1.69)			UA48 (4.88)	
	DG 2610 B2RF (1.69)			CT12214 (4.89)	
	BX 1348GLB2 (1.70)			DP 1311 B2RF (4.92)	
	PHY367WRF (1.72)				
	UA48 (1.72)				
10	**21**	**7**	**14**	**19**	**5**

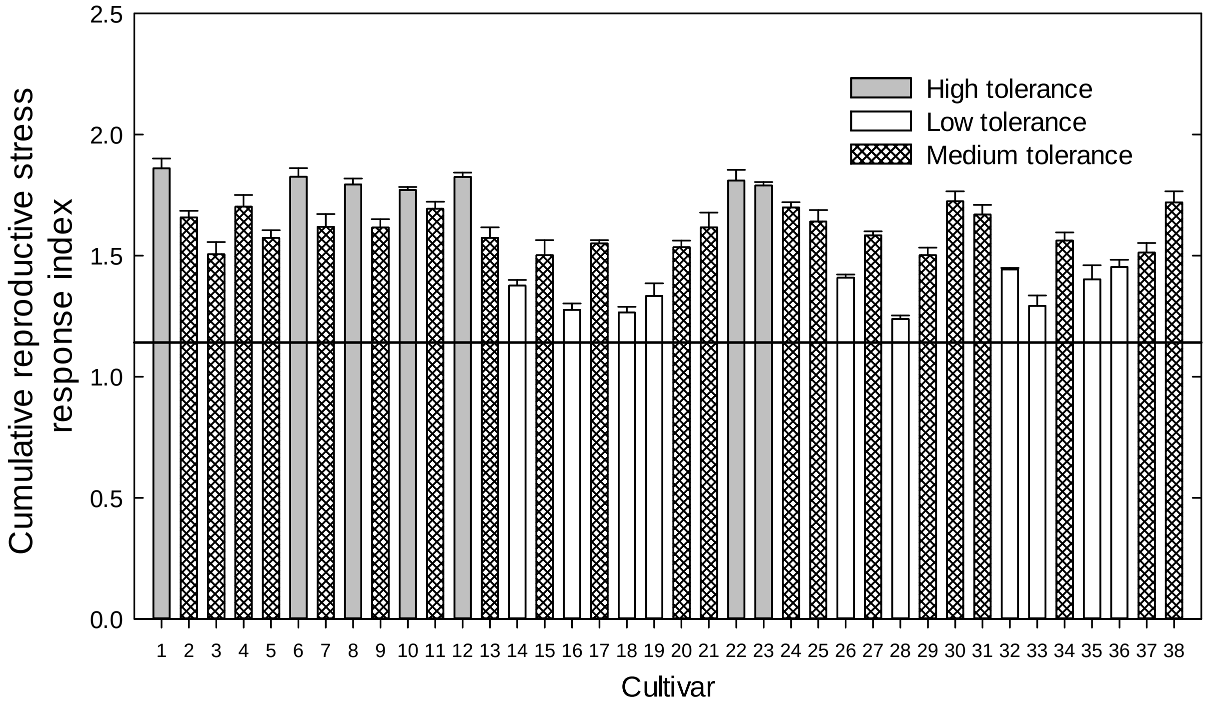

Figure 1. Classification of 38 cotton cultivars based on cumulative reproductive stress response indices.

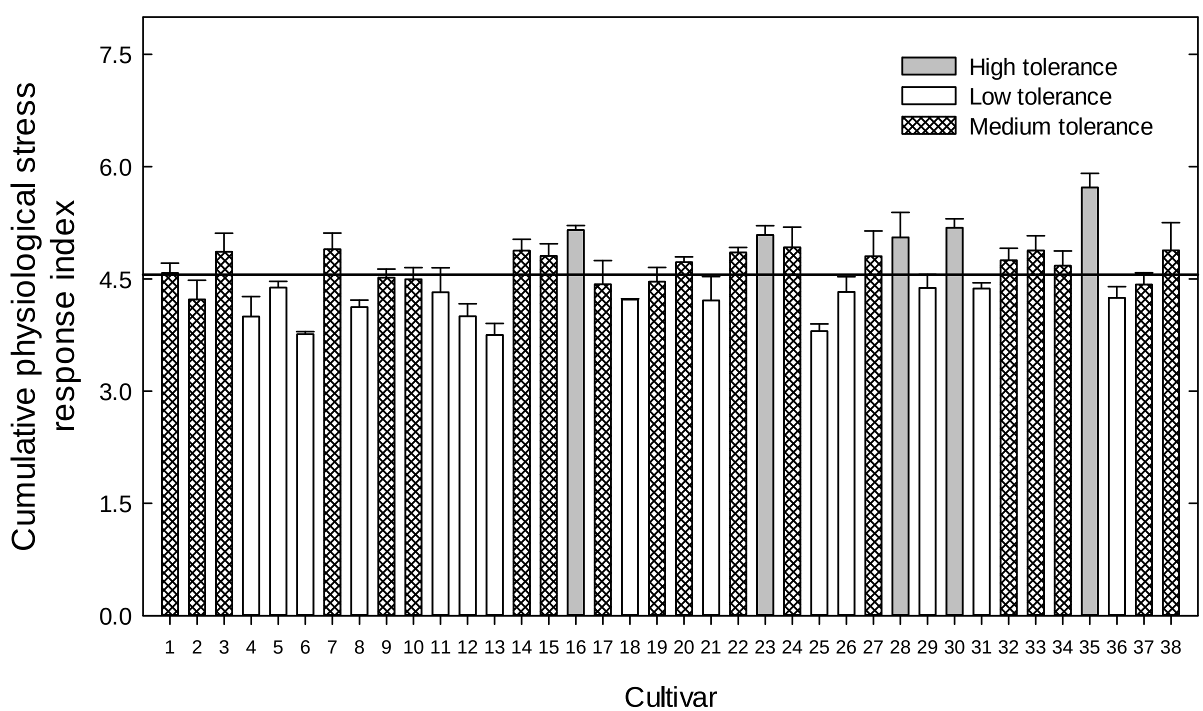

Figure 2. Classification of 38 cotton cultivars based on cumulative physiological stress response indices.

Acknowledgments

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