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Evaluation of chlorophyll fluorescence as a tool for the identification of drought tolerance in upland cotton

P. S. Longenberger · C. W. Smith · S. E. Duke · B. L. McMichael

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Abstract Chlorophyll fluorescence (CF) is one tool used by researchers to quantify plant water status during periods of limited water availability. The research reported herein was designed to evaluate a CF-based protocol as a tool for use in cotton, *Gossypium* spp. breeding programs for the identification of drought tolerant genotypes. Twenty genotypes were selected to represent diverse and distinct US germplasm pools. Replicated tests were performed in Lubbock and College Station, TX in 2006 and 2007. Dryland and irrigated treatments, as main plots, were applied in a randomized complete block design, split to genotypes. CF measurements were taken at mid-bloom and late bloom growth stages. Source leaf tissue was harvested at predawn and subjected to high temperature incubation with CF measurements subsequently taken hourly for 5 h. Drought stressed plants had not mobilized their carbohydrate reserves from their source leaves overnight and thus maintained cell viability and therefore higher CF values throughout the incubation and measurement period with the opposite being true for non-stressed plants. Fiber lint yield and fiber properties were measured for comparison with the CF data. Genotype × treatment effects complicated the classification of genotypic response to drought. Few and inconsistent correlations were found among CF values and lint yield or fiber properties. Data suggested that this procedure provides little potential in selecting plants for drought tolerance when plants are grown under field culture.

Keywords Chlorophyll fluorescence · Cotton · Drought tolerance

Abbreviations

CF	Chlorophyll fluorescence
CPCSD	California Planting Cotton Seed Distributors
CSIRO	Commonwealth Scientific and Industrial Research Organization
CSRL	Cropping Systems Research Laboratory
DP	Delta and Pine Land
DS	Drought susceptible
DT	Drought tolerant
ELO	Elongation

P. S. Longenberger · C. W. Smith
Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474, USA

P. S. Longenberger (✉)
Pioneer Hi-Bred International, Inc., Crop Genetics Research and Product Development, DuPont Agriculture and Nutrition, 982 New Holland Road, New Holland, PA 17557, USA
e-mail: Polly.Longenberger@pioneer.com

S. E. Duke
USDA-ARS, SPA, Southern Plains Agricultural Research Center, College Station, TX 77845, USA

B. L. McMichael
USDA-ARS, SPA, Plant Stress and Water Conserv. Lab, Lubbock, TX 79415, USA

FM	Fiber Max
HVI	High volume instrument
LB	Late bloom
MB	Mid bloom
MIC	Micronaire
SFC	Short fiber content
STR	Strength
Stv	Stoneville
TAM-MAR	Texas A&M multi-adversity resistance
UHM	Upper-half mean length
UI	Uniformity index

Introduction

Drought tolerance has come to the forefront of agronomic research in recent years due to dwindling irrigation reserves and increased costs associated with irrigation application (Gowda et al. 2007). Some level of water deficit stress is experienced by many crop plants grown with or without supplemental irrigation during most seasons even when meteorological drought conditions are not present. Therefore, all producers could benefit from the presence of drought tolerance.

Plant physiologists have suggested chlorophyll fluorescence as a means for understanding photosynthetic metabolism and thus identify plants, or at least genotypes, that vary in tolerance to moisture deficit. According to Maxwell and Johnson (2000), fluorescence analysis has become a powerful and widely used technique among plant physiologists and ecophysiologists. The value of fluorescence measurement lies in its relationship to photosynthesis since light absorbed by plants that does not drive the production of carbohydrates is dissipated as heat or re-emitted as light in the form of fluorescence. Physiologists and plant breeders now seek to relate CF measurements and genotype specific responses to stress.

Bajji et al. (2004) used CF yield (F_v/F_m) along with other physiological parameters to track improvements in drought tolerance due to selection of calluses after salt and PEG treatments. F_v/F_m was measured on hydrated excised leaves (control) or non-hydrated leaves (stressed) for 10 h under greenhouse conditions. Change in F_v/F_m was found to be reduced among progeny plants from selected callus.

Non-photochemical quenching of chlorophyll fluorescence (qN) increased in water stressed durum wheat, *Triticum turgidum* compared to control plants in a greenhouse experiment (Tambussi et al. 2002). Yield of quantum efficiency (F'_v/F'_m) decreased in water stressed plants while F_v/F_m remained unchanged among the stressed and control treatments. Massacci and Jones (1990) found similar results in apple, *Malus domestica*. qN increased in water stressed apple trees while F_v/F_m remained unchanged among stressed and well-watered controls.

Six normal leaf and two okra leaf upland cotton, *G. hirsutum*, genotypes were tested under dryland and irrigated conditions by Pettigrew (2004b). No differences were found among genotypes or between treatments for F_v/F_m. The okra leaf genotypes did have 14% greater F'_v/F'_m across treatments when compared to the normal leaf cottons. Higher photosynthetic rates per unit leaf area have been observed in okra leaf genotypes (Pettigrew 2004a).

Colom and Vazzana (2002) used chlorophyll fluorescence to clarify the ability of weeping lovegrass, *Eragrostis curvula* to grow and produce under drought stress. Two cultivars, one noted as being drought tolerant (DT) and the other drought susceptible (DS), were subjected to water stress and allowed to recover. F_v/F_m decreased among both cultivars during drought stress, but in the susceptible cultivar the reduction was much greater. After irrigation, both cultivars recovered within the same amount of time.

Chlorophyll fluorescence was found to be significantly and negatively correlated with a drought susceptibility index that was calculated based on yield from irrigated and dryland treatments with wheat, *Triticum* spp. (Ali Dib et al. 1994). The authors reported that fluorescence explained 62.4% of the drought susceptibility index of grain yield, thus supporting its use as a rapid tool for the identification of drought tolerant genotypes. Havaux and Lannoye (1985) studied fluorescence responses of DT and DS hard wheat *Triticum* spp. cultivars when leaf disks were subjected to rapid desiccation. Tolerant cultivars showed only minor changes in chlorophyll fluorescence while decreasing in susceptible cultivars. The authors supported the use of chlorophyll fluorescence as a screening tool in plant stress research.

Two DT and two DS corn, *Zea mays*, hybrids were evaluated under drought stressed and well watered conditions in the field with chlorophyll fluorescence (O'Neill et al. 2006). Both F_v/F_m and electron transport rate (ETR) were measured on three sampling

dates between 1100 and 1300 h. On the second sampling date drought stress was most pronounced and allowed for differentiation of genotypes. Under drought stress, both F_v/F_m and ETR were lower among the two DS lines compared with the DT lines. Under well watered conditions, the four lines could not be distinguished with F_v/F_m or ETR. The authors conclude that under water limited conditions chlorophyll fluorescence measurements can be used to classify corn hybrids according to their level of drought tolerance.

Burke (2007) developed a novel bioassay for the identification of drought stress in cotton that utilizes chlorophyll fluorescence to monitor cell viability under high temperature dark incubation. Differences between well watered and drought stressed plants can be established since, under stress, plants will not mobilize carbohydrate reserves overnight and will therefore maintain higher fluorescence values during high temperature dark incubation, with the opposite being true for well watered plants. Normal metabolic processes have been shown to be disrupted by drought leading to a reduction in the translocation of photosynthate from leaves to other plant tissues (Wilson et al. 1987).

Although sucrose and starch levels in dryland and irrigated tissue samples support the hypothesis behind the procedure, Burke (2007) notes that other factors contribute to the overall viability of plant tissues. These factors may include membrane composition, organic acid content, osmolyte accumulation, and stress protection protein synthesis.

In Burke's protocol, CF is not being used to monitor water stress responses on photosynthetic capacity but to monitor cell viability as it relates to carbohydrate concentration in source leaves. Timpa et al. (1986) found that four photoperiodic cottons could be characterized according to drought tolerance through organic acid and carbohydrate analysis. The four genotypes had been selected due to their response to water deficit. Two readily wilted, while the other two remain turgid during water deficit. Accumulation of carbohydrates under drought stress correlated with the visual observations. The wilt prone genotypes accumulated more carbohydrates in their leaf tissue than the turgid genotypes.

Burke (2007) detected treatment differences within 24 h of the termination of irrigation and 200–300 samples could be evaluated per day. The author cited concern for spacial variability issues when using the technique in the field. Samples taken 5 m apart on the same day differed but the sampling locations were consistent over 2 days of sampling. Burke noted the effect of leaf morphology when he compared four cotton genotypes. Three normal leaf cotton cultivars ('Fiber Max 989' (PVP no. 200500107), 'SureGrow 215' (PVP no. 200100155), and 'Deltapine 444' (200300134)) and one okra leaf cultivar ('Fiber Max 800' (PVP no. 200500110)) were each measured under dryland and irrigated conditions. Burke's procedure differentiated the four genotypes and highlighted potential differences between normal leaf and okra leaf response to drought stress. Fiber Max 800 showed the highest level of stress in the irrigation treatment and the lowest level of stress in the dryland treatment.

In this study, a novel chlorophyll fluorescence bioassay was evaluated for its utility in cotton breeding programs. Eighteen upland cotton genotypes from diverse germplasm pools, plus one pima, *G. barbadense*, cultivar, Pima S-7, and one *G. arboreum* genotype, unknown origin, were characterized via CF for their level of drought tolerance when grown under field culture. The objective of this study was to determine the feasibility of CF as a tool for drought tolerance evaluation in cotton breeding programs.

Materials and methods

Experimental design

Twenty genotypes described in Table 1 were planted in split plot arrangement of a randomized complete block design with irrigation treatment as main plots and genotypes as subplots. Planting dates were 28 April 2006 and 7 May 2007 at College Station and 15 May 2006 and 22 May 2007 in Lubbock. Precipitation at both locations delayed planting in 2007. Plots were single rows, 12.2 m × 102 cm, with four replications at College Station and single rows, 6 m × 102 cm, with four replications at Lubbock. The seeding rates and subsequent thinning were designed to establish 1 plant per 30 cm. Soil type was Belk clay at the Texas AgriLife Research Farm near College Station, Texas and Amarillo sandy loam at the USDA-ARS Crop Stress Research Lab (CSRL) in Lubbock.

Table 1 Year of release, region of adaptation, and developer for cotton genotypes planted in 2006 and 2007

Genotype	Year of release	Region of adaptation in USA	Developer
Acala 1517-99	1999	Western	New Mexico Agricultural Experiment Station
Acala Maxxa	1990	Western	CPCSD
All-Tex Atlas	1993	High Plains	All-Tex Seed Company
Deltapine 14	1941	Delta	Delta & Pine Land Company
Deltapine 491	2001	Delta	Delta & Pine Land Company
Deltapine 50	1984	Delta	Delta & Pine Land Company
Deltapine Acala 90	1981	Western	Delta & Pine Land Company
Fiber Max 832	1998	High Plains, Delta	CSIRO
Gossypium arboreum	NA	NA	Accession of unknown origin acquired and maintained by Cotton Improvement Laboratory, Texas AgriLife Research.
MD51ne	1991	Delta	USDA-ARS
Pima S-6	1984	Western	USDA-ARS
Paymaster HS 26	1983	High Plains	Paymaster Technologies
Phytogen PSC 355	2000	Mid South, Southeast	Phytogen Seed Company
Sure-Grow 747	1998	Mid South, Southeast	Sure-Grow Seed, Inc.
Stoneville 213	1962	Delta	Stoneville Pedigreed Seed Company
TAM 89E-51	Breeding line	Delta and Texas	Texas AgriLife Research
TAM 94L-25	2003	Texas	Texas AgriLife Research
TAM 96WD-69 s	2005	Texas	Texas AgriLife Research
Tamcot 22	2005	Texas	Texas AgriLife Research
Tamcot CAMD-E	1977	Texas	Texas AgriLife Research

NA denotes information not available

Irrigation treatments were no supplemental irrigation, or dryland (DL), and supplemental irrigation (IRR). These main treatments were applied as furrow irrigation applied as needed by visual estimate at College Station, while a drip irrigation system based on leaf canopy temperature termed BIOTIC was employed in Lubbock (Upchurch et al. 1996). College Station plots were irrigated on 15 June and 22 July 2006. No irrigation treatment could be established in 2007 at College Station because of frequent and adequate rainfall. The Lubbock irrigated plots received 0.6 cm day^{-1} as required by the BIOTIC irrigation system. Daily climatological data were recorded at both locations using automated weather stations located at the research sites. All other cultural practices were common for upland cotton production at each location, including fertilization, weed control, and biotic pest control. Five plants per subplot were sampled for CF values at the mid-bloom (MB) and late-bloom (LB) stages of growth. MB was approximately 2 weeks after blooming was initiated in greater than 50% of the upland cotton genotypes and LB was approximately 4 weeks after initiation of blooming. Four replications were measured per sub treatment and location at each sampling time with the exception of MB in Lubbock in 2007 where only two replications were measured. Sub treatments within each irrigation treatment were measured at each sampling time with the exception of MB and LB at College Station in 2007 when only DL plots were measured due to failure to establish an irrigation differential.

Lint yields were determined for College Station by harvesting plots with a one-row spindle picker modified for plot harvest on 2 October 2006 and 14 November 2007, while plots at Lubbock were harvested with a two-row plot stripper on 6 November 2006 and 22 October 2007. Sub samples of machine harvested yields, referred to as grab samples, were taken from two replications and ginned on a laboratory saw gin to determine gin turnout and provide lint samples for determination of HVI fiber properties. HVI measurements were

determined from the lint from each grab sample at Cotton Incorporated in Cary, NC.

Fluorescence bioassay

At predawn, a single-hole paper punch was used to harvest leaf tissue samples from the fifth main stem leaf (source leaf) of five plants per sub plot at each stage of growth. Leaf punches were placed in a 24-well plate half-filled with distilled water. Punches were transported to the lab and transferred to moistened filter paper lining a Pyrex dish and covered with Glad Clingwrap®. A speedball roller for Microseal® film was used to remove air bubbles and to ensure contact between the punches and the filter paper. An initial F'_v/F'_m (yield of quantum efficiency) measurement was taken with an OS1-FL modulated chlorophyll fluorometer (Opti-Sciences, Hudson, NH). The punches were incubated at 40°C in the dark and additional measurements were taken hourly for 5 h, generating F'_v/F'_m decline curves. Preliminary experiments (data not shown) indicated that this protocol would identify differences, if any, among genotypes or treatments and that such differences would by maximized at 5 h after the initial measurement. Therefore, only the final, or 5 h F'_v/F'_m measurement, will be reported.

Statistical analysis

Data Analyses were performed by location and by growth stage. Treatment structure was different at College Station in 2007 than other year-location combinations since frequent rainfall events did not allow for the establishment of an irrigated treatment. Data were analyzed with a mixed effects model in SAS software using the GLIMMIX procedure (SAS Institute 2004). The analysis included year, genotype, and treatment as fixed effects and replication as a random effect. Evaluation of normal probability plots of residuals and fluorescence data did not raise concerns of non-normal data distribution. A scatter plot of residuals versus expected fluorescence values indicated equality of error variances. Therefore, a gaussian distribution and identity link function were used in the analysis. Since an irrigated treatment was not established in 2007 at College Station, two interactions, treatment × year and treatment × year × genotype, could not be included in the College Station analysis.

Associations among CF, lint yield, and HVI fiber properties were estimated using Pearson correlation coefficients as determined by the SAS CORR procedure (SAS Institute 2004).

Results and discussion

Seasonal air temperatures during 2006 and 2007 were near long-term average for both locations, while precipitation at both locations was above the long term averages (data not shown). Frequent rain events during each growing season complicated the ability to establish stress conditions both in Lubbock and College Station.

The analysis of variance for F'_v/F'_m for the 20 genotypes evaluated indicated significant variation among genotypes, years, and their interaction in College Station at MB and LB growth stages (Table 2). The presence of the year × genotype interaction prevents the ability to separate and compare genotypic means averaged across years.

Analysis of variance for Lubbock at MB in 2006 and 2007 indicated that significant variation occurred for genotypes, years, and their interaction, and also for the interaction of irrigation treatments and years (Table 3). At LB there was significant variation for genotypes, years, treatments, and their interactions.

Gossypium arboreum and Pima S-6 had consistently high CF values in 2006 and 2007 at both CS and LUB (Tables 4, 5). TAM 89E-51 appears to be one of the more DS upland types in the test as it had high CF values. Acala Maxxa had low CF values indicating that it was one of the more DT upland types tested. Differences between upland genotypes were

Table 2 Variance analysis for F'_v/F'_m measurement taken after 5 h of incubation of 20 cotton genotypes at mid-bloom and late bloom grown under dryland and irrigated field conditions at College Station, TX in 2006 and 2007

Source	df	F'_v/F'_m (*F*)	
		MB stage	LB stage
Genotype	19, 174	12.43***	6.12***
Year	1, 174	12.32***	165.51***
Year × genotype	19, 174	4.70***	1.75*
Treatment	1, 6	2.21	2.78
Treatment × genotype	19, 174	0.53	1.01

* Significant at $P < 0.05$; *** Significant at $P < 0.001$

Table 3 Variance analysis for F'_v/F'_m measurement taken after 5 h of incubation of 20 cotton genotypes at mid-bloom and late bloom grown under dryland and irrigated field conditions at Lubbock, TX in 2006 and 2007

Source	df	F'_v/F'_m (*F*)	
		MB stage	LB stage
Genotype	19, 154	6.87***	12.80***
Year	1, 154	743.24***	144.94***
Year × genotype	19, 154	2.07**	1.38
Treatment	1, 6	0.59	21.80**
Treatment × genotype	19, 154	0.52	2.46**
Treatment × year	1, 154	7.32**	28.63***
Treatment × year × genotype	19, 154	0.48	0.62

** Significant at $P < 0.01$; *** significant at $P < 0.001$

difficult to establish as their CF values fell within a narrow range of values across locations and treatments.

The only location, growth stage combination with a significant treatment effect was LB in Lubbock. The mean CF values in both 2006 and 2007 were higher in the DL treatment plots. This result supported the expectation that higher CF values are associated with drought stress.

The inability of this CF protocol to separate genotypes without the interference of year and/or stress interactions severely limits CF as a selection tool for plant breeders seeking to select for drought tolerance.

Fluorescence and lint yield correlations

Lint yield is used commonly to compare cotton genotypes under drought stress conditions (Dumka et al. 2004; Pettigrew 2004b; Singh et al. 2006). If a strong correlation were to exist between chlorophyll fluorescence values and lint yield, then cotton breeders could use in-season fluorescence values to select drought tolerant genotypes without growing and harvesting all genotypes under evaluation. Significant correlations between lint yield and F'_v/F'_m values were found with seven of the 14 year–location–treatment–growth stage combinations, essentially half of the combination of sources of variation at each of the locations (Table 6). A correlation does not appear to be more likely to be found under DL or IRR conditions nor at mid-bloom or late bloom.

Table 4 F'_v/F'_m measurement taken after 5 h of incubation of 19 cotton genotypes at mid-bloom and late bloom growth stages grown under dryland and irrigated field conditions at College Station, TX in 2006 and dryland field conditions in 2007

Genotype	F'_v/F'_m					
	MB stage			LB stage		
	2006		2007	2006		2007
	DL	IRR	DL	DL	IRR	DL
Acala 1517-99	0.172	0.162	0.149	0.463	0.381	0.245
Acala Maxxa	0.162	0.165	0.144	0.488	0.446	0.243
AllTex Atlas	0.210	0.215	0.237	0.429	0.413	0.369
DP 14	0.222	0.201	0.193	0.464	0.375	0.330
DP 491	0.204	0.170	0.237	0.382	0.409	0.400
DP 50	0.186	0.154	0.147	0.473	0.361	0.401
DP 90	0.187	0.164	0.188	0.393	0.348	0.246
FM 832	0.167	0.184	0.226	0.413	0.390	0.344
G. arboreum	0.309	0.317	0.344	0.504	0.496	0.365
MD51ne	0.174	0.167	0.181	0.452	0.378	0.255
PM HS 26	0.196	0.164	0.193	0.468	0.402	0.353
PSC355	0.212	0.148	0.269	0.458	0.507	0.326
Pima S-6	0.276	0.224	0.538	0.671	0.615	0.477
SG 747	0.216	0.207	0.186	0.531	0.419	0.299
Stv 213	0.200	0.208	0.213	0.477	0.411	0.342
Tamcot 22	0.210	0.162	0.179	0.403	0.334	0.260
TAM 89E-51	0.234	0.212	0.291	0.506	0.548	0.345
TAM 94L-25	0.190	0.164	0.345	0.474	0.518	0.243
TAM 96WD-69 s	0.181	0.177	0.180	0.385	0.332	0.183
Tamcot CAMD-E	0.220	0.156	0.205	0.489	0.389	0.370
Mean	0.206	0.186	0.232	0.466	0.424	0.320
Standard deviation	0.036	0.039	0.092	0.064	0.076	0.072

Since all correlations between CF values and lint yield were negative the overall correlation across years, treaments, and locations was explored. The Pearson correlation coefficient at mid-bloom was −0.2622 and at late bloom was −0.7886. This may indicate the presence of a consistently negative relationship between lint yield and CF.

Fluorescence and fiber properties correlations

Cotton fiber quality is an important component indicating genotypic value and usually is measured when comparing genotypic performance across varied moisture regimes (Paterson et al. 2003; Stiller et al. 2004, 2005). The relationships between CF and fiber properties were explored using Pearson correlations.

Table 5 F'_v/F'_m measurement taken after 5 h of incubation of 19 cotton genotypes at mid-bloom and late bloom growth stages grown under dryland and irrigated field conditions at Lubbock, TX in 2006 and 2007

Genotype	F'_v/F'_m							
	MB stage				LB stage			
	2006		2007		2006		2007	
	DL	IRR	DL	IRR	DL	IRR	DL	IRR
Acala 1517-99	0.348	0.314	0.062	0.094	0.345	0.173	0.235	0.155
Acala Maxxa	0.287	0.202	0.064	0.062	0.347	0.169	0.221	0.105
AllTex Atlas	0.356	0.378	0.089	0.099	0.361	0.203	0.285	0.178
DP 14	0.328	0.268	0.071	0.051	0.386	0.204	0.270	0.174
DP 491	0.326	0.257	0.063	0.096	0.331	0.201	0.259	0.166
DP 50	0.336	0.223	0.046	0.094	0.371	0.251	0.285	0.231
DP 90	0.329	0.263	0.080	0.083	0.324	0.199	0.229	0.153
FM 832	0.379	0.351	0.069	0.092	0.335	0.209	0.218	0.154
G. arboreum	0.375	0.375	0.204	0.269	0.377	0.411	0.288	0.315
MD51ne	0.347	0.293	0.054	0.090	0.329	0.192	0.214	0.154
PM HS 26	0.319	0.299	0.092	0.094	0.369	0.184	0.270	0.152
PSC355	0.360	0.305	0.120	0.093	0.382	0.227	0.281	0.215
Pima S-6	0.432	0.406	0.152	0.159	0.560	0.450	0.372	0.266
SG 747	0.281	0.245	0.084	0.059	0.405	0.183	0.277	0.170
Stv 213	0.346	0.303	0.090	0.068	0.390	0.187	0.261	0.192
Tamcot 22	0.305	0.277	0.058	0.072	0.333	0.212	0.227	0.139
TAM 89E-51	0.384	0.337	0.082	0.065	0.421	0.265	0.358	0.215
TAM 94L-25	0.312	0.306	0.076	0.072	0.350	0.195	0.249	0.217
TAM 96WD-69 s	0.299	0.233	0.098	0.094	0.343	0.204	0.305	0.167
Tamcot CAMD-E	0.367	0.335	0.042	0.073	0.398	0.180	0.280	0.181
Mean	0.341	0.298	0.085	0.094	0.373	0.225	0.269	0.185
Standard deviation	0.037	0.054	0.038	0.047	0.052	0.074	0.042	0.048

If a genotype was drought stress resistant then one could reasonably expect that genotype to suffer less from periodic droughts and therefore produce a better fiber product and hence hold its economic value. However, that was not evident in these data. Micronaire (MIC), an indicator of fiber fineness and/or fiber maturity, measurements correlated with F'_v/F'_m values in 2007 at Lubbock at MB under DL conditions and at MB and LB under IRR conditions (Table 6). No significant associations were noted between MIC and CF at College Station.

Upper Half Mean (UHM) fiber length correlated with F'_v/F'_m values in 2006 at College Station MB and LB under DL and IRR conditions, respectively, and at Lubbock under DL conditions at the LB stage of growth. UHM length correlated with F'_v/F'_m values in 2007 at College Station at mid-bloom under DL conditions. Correlations between UHM and CF were positive and significant in these four cases and the non-significant correlations generally were positive. This relationship was not expected since higher CF values indicate stress and one would expect stress to shorten UHM fiber length. Short fiber content (SFC), i.e., the percent of fibers less than 12.7 mm, was positively and significantly correlated with F'_v/F'_m values in 2006 at the MB stage of growth in College Station.

Uniformity index (UI) correlated with F'_v/F'_m values in 2006 at College Station under IRR and DL conditions at MB and LB, and at the LB stage of growth at Lubbock. UI correlated with F'_v/F'_m values in 2007 in Lubbock under IRR conditions at mid-bloom. These correlation values were a mixture of positive and negative values, although the significant r values were all negative, suggesting that reduced CF values, which would indicate reduced stress, were associated with improved fiber length uniformity.

Table 6 Pearson correlation coefficients for micronaire (units), upper half mean length (mm), uniformity index (%), strength (kN m kg^{-1}), elongation (%), and short fiber content (%) versus F'_v/F'_m values measured at mid-bloom (MB) and late bloom (LB) for 19 cotton genotypes grown under irrigated (IRR) and dryland (DL) conditions at College Station, TX (CS) in 2005 and 20 cotton genotypes grown under irrigated and dryland conditions at College Station, TX and Lubbock, TX (LUB) in 2006 and 2007

Year	Location	Treatment	Stage	F'_v/F'_m (r)						
				Lint yield	MIC	UHM	UI	STR	ELO	SFC
2006	CS	DL	MB	−0.6598**	0.0905	0.1499	−0.5225*	−0.1601	0.3469	0.4924*
2006	CS	DL	LB	−0.6424**	−0.3797	0.5638**	0.0742	0.3837	0.0186	−0.1156
2006	CS	IRR	MB	−0.6808**	0.3650	−0.0237	−0.7207***	−0.2044	0.2161	0.6643**
2006	CS	IRR	LB	−0.4247	−0.1564	0.5088*	−0.0390	0.4033	−0.1124	−0.0483
2006	LUB	DL	MB	−0.1584	−0.0990	0.3500	0.2724	0.5750**	−0.2541	−0.2558
2006	LUB	DL	LB	−0.2903	−0.0954	0.4603*	0.3184	0.4644*	0.0075	−0.3156
2006	LUB	IRR	MB	−0.4206	−0.0019	0.1740	−0.2978	0.3270	−0.1322	0.0336
2006	LUB	IRR	LB	−0.6253**	0.2461	0.1381	−0.4640*	0.1918	0.0417	0.3482
2007	CS	DL	MB	−0.2514	−0.1167	0.6213*	0.1039	0.6735**	−0.2045	−0.2280
2007	CS	DL	LB	−0.0510	−0.2886	0.2811	0.1712	0.3325	0.2008	−0.1261
2007	LUB	DL	MB	−0.7901***	0.4855*	0.0438	−0.3577	0.1556	0.2592	0.1074
2007	LUB	DL	LB	−0.2055	−0.0456	0.1706	−0.0062	0.2708	0.2700	−0.0446
2007	LUB	IRR	MB	−0.8151***	0.4949*	−0.0045	−0.4459*	0.1269	−0.0513	0.3831
2007	LUB	IRR	LB	−0.5523*	0.4543*	0.1751	−0.1898	0.0285	−0.0305	0.2171

* Significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$

Fiber bundle strength (STR) measurements were positively and significantly correlated with F'_v/F'_m values in 2006 at Lubbock under DL conditions at both MB and LB, and at College Station in 2007 under DL conditions at MB, suggesting that as CF values increase, indicating stress, that STR increases. Higher STR values under stress were not expected unless stress could have caused a decrease in MIC which would result in an increase in the number of fiber in the cross section used to measure STR in the HVI system. This could result in higher STR reading although individual fiber strength may have remained unchanged. However, the ANOVA for MIC does not support this reasoning as irrigation treatment did not have a significant effect on MIC (data not shown).

Conclusions

The CF protocol described by Burke (2007) was not successful in discriminating among cotton plants grown with and without supplemental irrigation under field culture at College Station and Lubbock, Texas over two growing seasons. Results were complicated by genotype × treatment and year × genotype interactions. No consistent associations, as indicated by Pearson's correlation coefficients, were found between F'_v/F'_m values and lint yield or HVI fiber properties. This CF protocol does not appear to provide reproducible results needed by cotton breeders to select individual plants or strains exhibiting drought tolerance. The shortcomings of this physiological technique might be resolved under more arid conditions, such as those experienced in the desert Southwest production region. The protocol also should be evaluated anew when breeders identify upland cotton phenotypes with obvious and repeatable drought resistance.

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