

BREEDING AND GENETICS

Genotype-by-Environment Interaction Effects on Lint Yield of Cotton Cultivars Across Major Regions in the U.S. Cotton Belt

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ABSTRACT

Analysis of genotype (G)-by-environment (E) interactions and their influence on performance of cotton (*Gossypium hirsutum* L.) cultivars can help cotton breeders improve performance stability of cultivars across environments. Data from multi-location trials of the Regional High Quality Tests conducted as part of the USDA-ARS National Cotton Variety Tests during 2003 and 2009 were used to analyze G × E and relationships among test locations for megaenvironments. The trials were located in the Western, Plains, Central, Delta, and Eastern regions of the U.S. Cotton Belt. Effects of G × location for lint yield were either larger or comparable to the effects of G × year. The relationships among test locations were analyzed in GGE biplot and no clear megaenvironments were identified among test locations across years. Nevertheless, the locations of Las Cruces, NM in the Western and Lubbock, TX in the Plains test regions were identified as distinct from the test locations in the other areas. It was hypothesized that the environments in the U.S. Cotton Belt belonged to one megaenvironment with the areas in the Western and Plains as a subregion. The daily minimum temperature was significantly correlated to environment scores of the first principal component axis with r values

-0.41 and -0.30 for the early and late growing seasons, respectively. This result suggests that genetic improvement of cotton cultivars for tolerance to low temperature during the early and late growing season could increase yield stability.

Interaction of genotype by environment (G × E) is an important component in genetic variance analysis for quantitative traits in crops. Significant G × E component reduces correlations between genotype and phenotype values (Kang, 1998) and affects breeding for genetic improvement, especially for quantitative traits in crops. It was reported that 25 to 45% and 15 to 25% of the yield gain in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.), respectively, were attributed to G × E in a study of grain yield in these two crops during 1946 to 1977 (Simmonds, 1981). Significant G × E component necessitates multiple locations for performance tests in breeding programs, whereas the extent of genotypic effect relative to G × E component might reduce the number of environments necessary for performance tests.

In cotton, numerous studies regarding the genotypic and the G × E components have been conducted since the middle of the last century. In the analysis of four cultivars from National Cotton Variety Tests during 1960 and 1962, the ratio of G × location (L) component relative to genotypic component for lint yield was 2.3 (Abou-El-Fittough et al., 1969). The ratios of the component of G × E relative to genotypic component were less than 1.0 for fiber properties in the same study. In 12 location-year yield trials of cotton cultivars in South Carolina, the portion of sums of squares attributed to the total variation for E, G, and G × E were 90%, 2%, and 8%, respectively (Campbell and Jones, 2005). In a yield trial of 31 cotton varieties in three Mediterranean countries, the ratio of G × E component to genotypic component was 6.4 (Baxevanos et al., 2008a). Most recently, Meredith et al. (2012) reviewed six studies conducted worldwide between 1964 and 2011 for G × E effects on lint yield and fiber quality. The average attributes of E,

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G, and $G \times E$ to the total variation of lint yield was 86%, 5%, and 9%, respectively, in these studies. The average total variation of fiber strength was 44% and 16% due to G and $G \times E$, respectively, and that for fiber length was 27% and 17% due to G and $G \times E$, respectively. In summary of these previous studies, $G \times E$ effects were greater than genotype effects for lint yield, whereas the effects of $G \times E$ were usually small relative to genotypic effects for fiber properties.

Although significant $G \times E$ interactions warrant multiple location trials for stability, i.e., agronomic performance across different environments, analysis of relationships among test locations might identify the unnecessarily repeated locations and reduce the number of test sites. Test locations can be grouped into a so-called megaenvironment based on their close relationships in terms of $G \times E$ interactions for crop performance so that the test locations within a megaenvironment are homogeneous, whereas the variation among groups is maximized (Yan and Kang, 2003). These types of groups of test locations can be visualized by their separation in graphs using GGE biplot. However, in most cases, the identification of megaenvironments was not simple due to either unpredictable $G \times E$ interactions or year (Y) \times L interactions. In a test of 28 soybean (*Glycine max* L. Merr.) cultivars from 1997 to 1999 at three to four locations in Ontario, Canada, a complex megaenvironment was identified with unpredictable $G \times L \times Y$ interactions (Yan and Kang, 2003). In a study of sites for cotton trials of Delta and Pine in Spain during 1999 to 2006, Baxevanos et al. (2008a) analyzed eight 1-yr yield data sets for $G \times L$ interactions and two multiyear yield data sets for $G \times L \times Y$ interactions and revealed crossover $G \times L$ interactions, but not sufficient for megaenvironment differentiation. Bach et al. (2012) examined eight potato (*Solanum tuberosum* L.) clones and four cultivars at three locations in Ontario, Canada during 2009 and 2010 and found no significant megaenvironments for potato fiber components.

Highly unpredictable environments suggest the necessity to evaluate cultivars for both mean performance and stability of cultivars across environments. In cotton breeding, there were mainly two types of methods utilized to analyze stability performance in previous reports. El-Shaarawy (2000) evaluated 30 Egyptian cotton genotypes grown at five locations and identified four genotypes with high stability for lint yield based on a modified additive main effect and multiplicative interaction (AMMI) method.

Campbell and Jones (2005) assessed the effects of $G \times E$ interactions on agronomic performance using AMMI method in a series of cotton yield trials conducted in 12 location-year environments in South Carolina. In that study, two environments in South Carolina were identified to be appropriate for yield testing, although $G \times E$ was not large enough to detect megaenvironments. Blanche et al. (2006) analyzed performance stability by comparisons between transgenic cotton cultivars vs. conventional cultivars under multiple environments using GGE biplot. In another study, Blanche et al. (2007) compared GGE biplot stability values with other stability analysis methods and found correlations between GGE biplot stability values and other common stability indexes including cultivar superiority. In an analysis of seed cotton yield data from 31 cotton cultivars evaluated under multi-environments during 1999 and 2005, Baxevanos et al. (2008b) identified high correlations between GGE biplot indexes and seed cotton yield.

The major cotton producing regions in the U.S., the so-called Cotton Belt, are divided into four major geographic areas including the Southeast, Mid-South, Southwest, and West. The agricultural environments across the Cotton Belt are diverse, ranging from the humid Southeast coast to the desert Southwest and West. The National Cotton Variety Testing Program (NCVT) is a system that provides data from yield trials at locations representing these areas across the Cotton Belt. The Crop Genetics Research Unit of the USDA facilities at Stoneville, MS coordinates with the NCVT cooperators, including agricultural experiment stations of Alabama, Arkansas, Arizona, California, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, and Texas. The Regional High Quality Test (RHQ) is one of the regional cotton variety tests in the NCVT to identify genotypes with desirable combinations of lint yield and fiber quality. Data reported by RHQ between 2003 and 2009 were chosen in this study based on repetition of genotypes tested among locations within years and the number of genotypes common to the trials between two consecutive years. The objectives of this study were to analyze the effects of $G \times E$ on lint yield of cotton cultivars, analyze relationships among test locations across the Cotton Belt by grouping test locations into megaenvironments, and identify environmental factors affecting performance stability of cotton cultivars grown in the Cotton Belt. The determined relationships among test locations can be used to arrange a reasonable

distribution of test sites across the Cotton Belt and reduce duplicative locations. The information about environmental factors attributing to $G \times E$ effects will be useful for improving performance stability in cotton breeding programs.

MATERIALS AND METHODS

Data Source. Data analyzed in this study were obtained from 10 NCVT locations in the RHQ conducted between 2003 and 2009 (Table 1). The goal of RHQ program is to test high-quality cotton cultivars and elite strains across locations in the U.S. Cotton Belt for lint yield and fiber quality and select superior genotypes across environments of different regions. The means values in the tests were reported annually by the Crop Genetics Research Unit of USDA-ARS at Stoneville, MS (<http://www.ars.usda.gov/Business/Business.htm?docid=4357&modecode=64-02-15-00&page=2>). The 10 NCVT sites were located in five agric-climatic regions: Eastern, Delta, Central,

Plains, and Western. These sites differed substantially in terms of geographic locations, temperature, and rainfall (Table 1).

In the RHQ, the same set of cotton cultivars was tested at different locations each year, but different sets of cultivars were tested across years. Three to four cultivars in these sets were used as national standard cultivars that were planted every year among locations without change in a 3-yr cycle. The data analyzed in this study were derived from three of these 3-yr cycles, 2002 to 2004 in the first cycle, 2005 to 2007 in the second cycle, and 2008 to 2010 in third cycle. The years of 2003 and 2004 in the first cycle, 2005 and 2006 in the second cycle, and 2008 and 2009 in the third cycle were chosen for this study based on the maximum number of cultivars common between trials of different years. There were seven cultivars common between 2003 and 2004, eight cultivars common between 2005 and 2006, and six cultivars common between 2008 and 2009 in the three 3-yr cycles, respectively (Table 2).

Table 1. Site description of the Regional High Quality Tests during 2003 and 2009.

Location	Abbreviation	Region	Altitude (m)	Latitude (°)	Longitude (°)	Mean temperature (°C) ^z	Annual rainfall (mm)
Florence, SC	FLO	Eastern	45	34.2	-79.8	24.2	3788
Belle Mina, AL	BEL	Eastern	183	34.6	-86.9	22.8	3688
Jackson, TN	JAC	Eastern	42	35.8	-88.9	22.9	3760
Keiser, AR	KEI	Delta	70	35.7	-90.1	23.1	3664
Portageville, MO	POR	Delta	27	36.3	-90.2	23.9	3384
Stoneville, MS	STV	Delta	38	33.4	-90.9	22.7	3560
Bossier City, LA	BOS	Central	49	32.5	-93.7	25.4	3446
College Station, TX	COL	Central	97.5	30.6	-96.4	27.2	2821
Lubbock, TX	LUB	Plains	992	33.3	-103	23.8	1851
Las Cruces, NM	LAS	Western	1188	32.3	-107	24.4	1030

^z Mean temperature and annual rainfall were calculated from weather records between 10 April and 30 September in cotton growing seasons during 2003 and 2009.

Table 2. Cultivars common between two consecutive years in the 3-yr trial cycles.

2003-2004	2005-2006	2008-2009
Acala 1517-99 (Reg. no. CV-115)	PHY 72 Acala (PVP 200100115)	PHY 72
DP 458BR (PVP 9800206)	ST 4892BR	DP 555BR
ST 4892BR (PVP 200000253)	DP 555BR	Fibermax 9180B2F
DP 555BR (PVP 200200047)	Fibermax 960B2R (PVP 200500109)	Fibermax 1740 B2F
Fibermax 960LL	DP 455BR (PVP 200500052)	DP 161B2RF
DP 444BR (PVP 200300134)	DP 445BR (PVP 200400265)	MD25 (PI 659508, Meredith and Nokes, 2011)
Fibermax 832 (PVP 9800258)	Acala 1517-09R (PI 659506, Zhang et al., 2011)	
	Fibermax 960BR (PVP 200400224)	

Statistical Analysis. The General Linear Model procedure of the Statistical Analysis System (SAS Institute, Cary, NC) was used for analysis of variance in the trial data. A mixed model was used with genotype as fixed effect and environment and replication within environments as random effects. Because sets of genotypes were different in test years, the effects of $G \times E$, i.e., $G \times L$, $G \times Y$, and $Y \times L$, were analyzed using the data of genotypes common between the two consecutive years in the three 3-yr cycles. In doing so, Y , $G \times Y$, $Y \times L$, $G \times Y \times L$, and replicates within years were considered random effects.

GGE Biplot Software. Stability analysis was performed and visualized using GGE biplot software (Yan, 2001) as described by Yan and Kang (2003). GGE biplot is a window-based program that performs analysis of multi-environment trial data by a graphic approach. The main features of this software can be summarized as an approach to visualize cultivar evaluation in multi-environment trials by integrating genotype effects and $G \times E$ interactions after removing the environment main effect. GGE biplot was constructed by plotting the first principal component (PC1) and the second principal component (PC2) of singular values and eigenvectors for genotypes and environments.

The environments of the test locations were analyzed by the polygon view using a module in GGE biplot (Yan and Kang, 2003). In the analysis, a polygon was drawn to contain all cultivars within it. A set of lines perpendicular to each side of the polygon divided the biplot into sectors with environments falling into some of these sectors. The term winning cultivar was used to represent the cultivar with best performance in the environments falling into a sector. The winning cultivar could be viewed at a corner of the polygon for the environments falling into the sector. Environments falling into a sector with a winning cultivar at the corner of the polygon could be grouped. Different megaenvironments could be determined based on (1) different winning cultivars in sectors containing different environments and (2) apparent separation in groups of environments in the polygon view. The Arabic numbers in the graphs represented the tested cultivars. The genotypes ranked high for lint yield were distributed to the right side of the y-axis and the genotypes ranked high for yield stability were those near the x-axis.

To investigate environmental factors attributed to $G \times E$, Pearson correlations between the environment scores from GGE biplot and the means of the environment variables were calculated. The environment scores were calculated as tester eigenvectors from the

PC1, and the values were obtained from the log sheets of the software. In doing so, the growing seasons were artificially divided into early, middle, and late growing stages. Planting was conducted between the middle of April and May and harvest was conducted during the middle of September and October among locations. Therefore, the early growing stage (from 10 April to 31 May) included germination and seedling development; the middle growing stage (from 1 June to 20 Aug) included flowering and boll development; and the late stage (from 21 Aug to 30 Oct) included late boll development and boll opening.

RESULTS AND DISCUSSION

Genotype \times Environment Interactions. There were seven cultivars and six locations common in variety trials of 2003 and 2004, eight cultivars and eight locations common in variety trials of 2005 and 2006, and six cultivars and 10 locations common in variety trials of 2008 and 2009. Mean squares for interaction effects of $G \times Y$, $G \times L$, and $G \times Y \times L$ from analysis of variety trials in these 3-yr cycles are given in Table 3.

Both year and location contributed to variances of lint yield as indicated by significant effects of Y and L in all three trial periods. Significant $G \times L$ effects for lint yield indicate differential genotypic effects across locations that warrant multiple location trials for lint yield. However, genotypic effects were nine to 14 times larger than $G \times L$ effects in all trials based on mean squares. These results implied limited cross-over $G \times L$ interactions with the possibility of general adaptation for some genotypes across major regions in the Cotton Belt. The effects of $G \times Y$ were not significant for trials of 2003 and 2004 and 2005 and 2006, whereas the effects of $G \times L$ were significant for the trials of all three periods. $G \times Y$ interaction effect for lint yield was significant only in the trials of 2008 and 2009 and its mean square was similar with that of $G \times L$. Larger or comparable contribution of the variable location to variances of lint yield than that of the variable year would be expected because these trials were conducted at locations with wide environmental variations across major regions in the Cotton Belt. Similar results were reported in a previous study by Abou-El-Fittouh et al. (1969) using NCVT data during 1960 and 1962, in which the variance component of $G \times L$ was 10 times larger than the component of $G \times Y$. Effects of $G \times Y \times L$ interactions were not significant for the trials of 2005 and 2006, but significant for the trials of 2003 and 2004 and 2008 and 2009 periods.

Relationships Among the Test Locations Across Cotton Belt. Because the application of GGE biplot is designed to focus on G and G × E effects, only variables with significant effects of these two main components were appropriate for analysis using GGE biplot as suggested by Blanche et al. (2006) in a study of performance stability in cotton cultivars. In the current study, although the G × Y component was not significant for trials of 2003 and 2004 and 2005 and 2006, the effects of G, G × L, and G × Y × L components were highly significant in most trials between 2003 and 2009 (Table 3) indicating suitability for application of GGE biplot.

Because of highly significant Y × L interactions for all trials and G × Y × L interactions for trials of 2003 and 2004 and 2008 and 2009 (Table 3), simple repeat analysis of G × L interactions in different years might not be sufficient to confirm existence of megaenvironments. Instead, the jointed multiyear data sets were used to validate the existence of megaenvironments. The balanced data for lint yield, from seven cultivars evaluated at seven locations common between trials of 2003 and 2004 in the first 3-yr cycle, eight cultivars evaluated at eight locations common between the trials of 2005 and 2006 in the second 3-yr cycle, and six cultivars evaluated at 10 locations common between the trials of 2008 and 2009 in the third 3-yr cycle, were analyzed for the relationships among test locations across the Cotton Belt.

The principal components, PC1 and PC2, accounted for 82%, 76%, and 67% of the total variation in G and G × E components for lint yield in RHQ tests of 2003 and 2004, 2005 and 2006, and 2008 and 2009,

respectively (Figs. 1, 2, 3). The information content of the other principal components, PC3, PC4, PC5, and PC6, were all less than 1.0, which indicated suitability of PC1 and PC2 for analysis of environments in these trials. In the trials of 2003 and 2004, the polygon view revealed that there were four environments in a sector with ‘DP 555BR’ as the winning cultivar and the remaining environments in another sector with ‘DP 444BR’ as the winning cultivar (Fig. 1). Significant Y × L interaction was shown by the different relationships of Keiser, AR with other locations in 2003 and 2004. DP 444BR was the best performer in terms of lint yield although it was known as a late maturing cultivar. In the late seasons of 2003 and 2004, rainfall ranged between 15 to 318 mm, and average daily wind speed was lower than 24 km h⁻¹ with maximum wind speed, which was used as indication of storms in this study, lower than 80 km h⁻¹ for most locations. The absence of severe storms during the late seasons of this trial period might have attributed to the good performance of this late maturing cultivar. DP 555BR was the cultivar with highest instability for lint yield during this trial period. The yield of this cultivar ranged from 1655 to 1858 kg ha⁻¹ in College Station, TX, Stoneville, MS, Portageville, MO, and Bossier City, LA in 2004, but was only 1056 and 1364 kg ha⁻¹ in Lubbock, TX and Belle Mina, AL, respectively, in the same year (data not shown). The low minimum temperatures (13 to 14.5 °C) during the late seasons at Lubbock, TX, Keiser, AR, and Belle Mina, AL (Table 4) might have affected the yield of DP 555BR in these regions resulting in high instability.

Table 3. Mean squares of lint yield for common genotypes and locations between 2003 and 2004, 2005 and 2006, and 2008 and 2009^x.

Source	df 2003-2004	Lint yield × 10 ⁻⁴ 2003-2004	df ^y 2005-2006	Lint yield × 10 ⁻⁴ 2005-2006	df 2008-2009	Lint yield × 10 ⁻⁴ 2008-2009
Genotype (G)	6	65***z	7	118***	5	136***
Year (Y)	1	168***	1	65***	1	737***
G × Y	6	3.8	7	3.8	5	22***
Location (L)	5	258***	7	1168***	9	472***
G × L	30	7.0***	49	8.7**	45	15***
Y × L	5	66***	7	112***	9	192***
G × Y × L	30	4.5**	48	3.6	44	7.3***
Rep (Y)	10	4.1	10 (2)	20***	10 (5)	1.7
Error	256	2.4	366 (114)	5.5	349 (106)	2.6

^x There were six cultivars and six locations common between trials during 2003 and 2004, eight cultivars and eight locations common between 2005 and 2006, and six cultivars and 10 locations common between trials during 2008 and 2009.

^y There was one missing observation in each of 2005-2006 and 2008-2009 periods.

^z Values followed by ** and *** are significant at $p < 0.01$, $p < 0.001$, respectively.

Table 4. Means of weather parameters during the early, middle, and late seasons at 10 test locations during years 2003 to 2009.

Location	Growing season	Maximum temperature (°C)	Mean temperature (°C)	Minimum temperature (°C)	Precipitation ² (mm)	Relative humidity (%)	Wind speed (km/h)
Lubbock, TX	Early	27.4	19.6	11.8	82.1	51.3	21.2
	Middle	33.2	27.2	19.3	137	55.2	30.9
	Late	27.3	20.2	13.8	143	61.2	16.6
College Station, TX	Early	28.7	23.3	17.8	143	69.1	14.5
	Middle	34.4	28.9	23.4	229	68.0	11.3
	Late	30.8	25	19.2	243	67.7	10.4
Stoneville, MS	Early	27.2	19.0	15.7	207	78.7	4.63
	Middle	32.9	25.0	21.5	240	81.6	2.57
	Late	28.8	19.1	16.2	324	85.3	2.99
Portageville, MO	Early	24.1	19.0	13.8	185	—	31.1
	Middle	30.8	25.6	20.4	221	—	23.5
	Late	28.3	22.7	17.2	151	—	22.5
Las Cruces, NM	Early	29.2	20.1	11.0	21.8	—	—
	Middle	34.7	27.1	19.4	93.2	—	—
	Late	28.8	21.1	13.3	89.4	—	—
Keiser, AR	Early	26.1	20.3	14.7	216	—	—
	Middle	32.3	26.8	21.3	256	—	—
	Late	26.9	20.7	14.5	247	—	—
Florence, SC	Early	26.6	19.9	13.4	149	66.8	12.0
	Middle	31.8	26.5	21.1	342	74.8	9.22
	Late	27.4	21.8	16.3	213	74.2	10.2
Belle Mina, LA	Early	26.1	18.6	11.1	310	—	—
	Middle	31.4	24.7	17.9	236	—	—
	Late	28.5	20.8	13.0	234	—	—
Bossier City, LA	Early	27.6	21.7	15.8	200	74.7	11.5
	Middle	33.2	27.6	22.2	310	78.4	8.21
	Late	29.8	24.1	16.8	223	78.4	8.21
Jackson, TN	Early	25.2	19.0	16.8	227	69.1	11.6
	Middle	31.3	25.3	19.2	266	74.2	7.74
	Late	—	19.9	13.3	233	75.0	8.03

² Precipitation was defined as the rainfall during the growing seasons averaged over trials between 2003 and 2009.

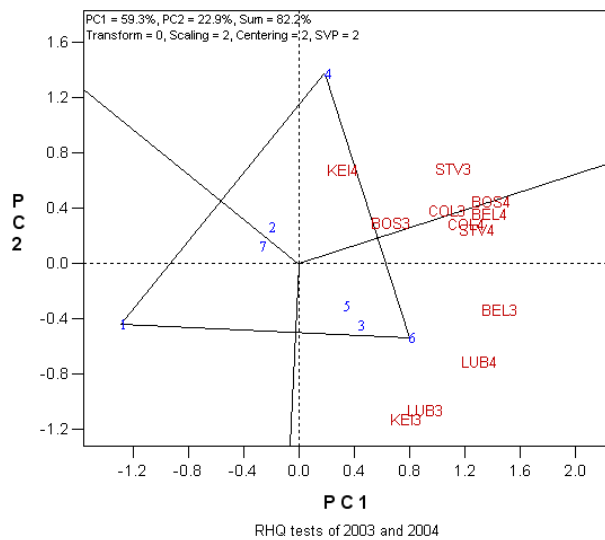


Figure 1. Polygon view of relationships among test locations in Regional High Quality Tests of 2003 and 2004. The Arabic numbers in blue represent cultivars: 1, Acala 1517-99; 2, DP 458BR; 3, ST 4892BR; 4, DP 555BR; 5, FM 960LL; 6, DP 444BR; 7, Acala 1517-09R. Uppercase letters in red represent test locations as shown in Table 1. The letters with Arabic number 3 are environments of 2003 and those with Arabic number 4 are environments of 2004. PC1 and PC2 are first and second principal components, respectively. Model parameters: Transform = 0, no transformation; Scaling = 2, heritability adjusted (Yan and Holland, 2010); Centering = 2, tester centered (G + GE); SVP = 2, tester metric (f = 0).

In the trials of 2005 and 2006, DP 555BR was the winning cultivar in the sector with environments of Las Cruces, NM and Lubbock, TX in both years and College Station, TX and Belle Mina, AL in 2006. ‘ST 4892BR’ was the winning cultivar in the sector with the environments of Belle Mina, AL and Stoneville, MS in 2005 and Keiser, AR and Portageville, MO in 2006 (Fig. 2). DP 445BR was the best performer in terms of lint yield and stability. Significant $Y \times L$ interaction was shown by the different relationships of Belle Mina, AL with other locations in 2005 and 2006.

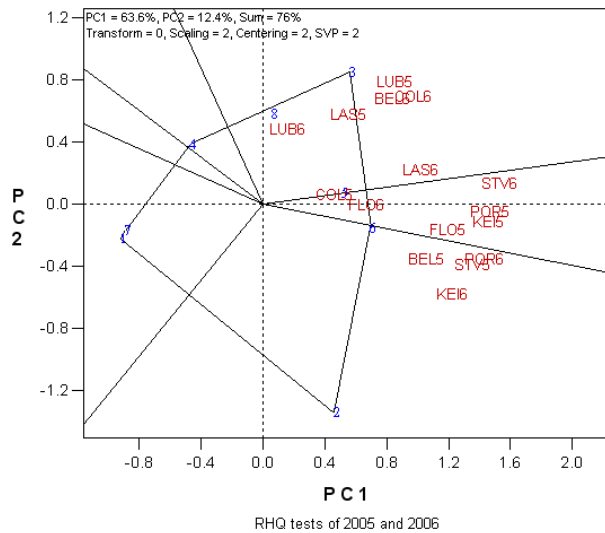


Figure 2. GGE biplot view of relationships among test locations in High Quality Regional Test of 2005 and 2006. The Arabic numbers in blue represent cultivars: 1, PHY72; 2, ST 4892BR; 3, DP 555BR; 4, FM 960B₂R; 5, DP 455BR; 6, DP 445BR; 7, Acala 1517-09R; 8, FM 960BR. Uppercase letters in red represent test locations as shown in Table 1. The letters with Arabic number 5 are environments of 2005 and those with Arabic number 6 are environments of 2006. PC1 and PC2 are first and second principal components, respectively. Model parameters: Transform = 0, no transformation; Scaling = 2, heritability adjusted (Yan and Holland, 2010); Centering = 2, tester centered (G + GE); SVP = 2, tester metric (f = 0).

In the trials of 2008 and 2009, ‘MD25’ was the winning cultivar in the sector with environments of College Station, TX and Portageville, MO in 2008 and 2009 and Bossier City, LA in 2008. DP 555BR was the winning cultivar in the sector with environments of Belle Mina, AL, Jackson, TN, Las Cruces, NM, and Lubbock, TX in 2008 and Bossier City, LA and Las Cruces, NM in 2009 (Fig. 3). ‘FM 1740B₂F’ was the best performer in terms of lint yield and stability. For the trials during 2005 and 2009, DP 555BR, ST 4892BR, and MD25 had high instability for lint yield. The exact causes of high instability for

lint yield of these cultivars are not clear, but there were a few severe tropical storms in the Central, Delta, and Eastern regions during the late seasons in 2005 and 2008. The poor weather conditions in the late seasons might have affected yield of these full season cultivars.

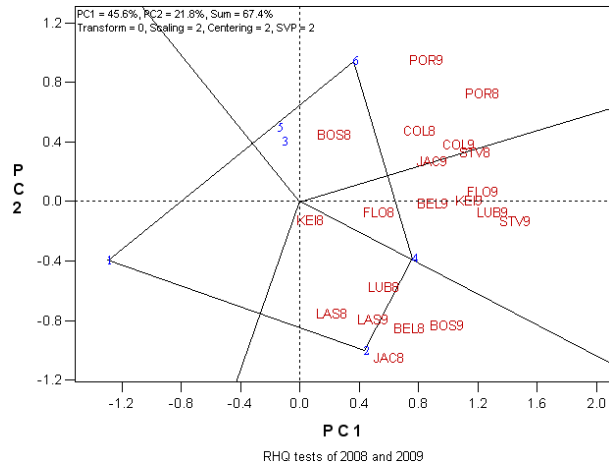


Figure 3. GGE biplot view of relationships among test locations in High Quality Regional Tests between 2008 and 2009. The Arabic numbers in blue represent cultivars: 1, PHY72; 2, DP 555BR; 3, FM 9180B₂F; 4, FM 1740B₂F; 5, DP161 B₂RF; 6, MD25. Uppercase letters in red represent test locations as shown in Table 1. The letters with Arabic number 8 are environments of 2008 and those with Arabic number 9 are environments of 2009. PC1 and PC2 are first and second principal components, respectively. Model parameters: Transform = 0, no transformation; Scaling = 2, heritability adjusted (Yan and Holland, 2010); Centering = 2, tester centered (G + GE); SVP = 2, tester metric (f = 0).

In summary of this analysis, although significant crossover $G \times E$ interactions were detected, they were not large enough to group environments into clear megaenvironments. In the polygon view of test locations of 2003 and 2004, half of the environments were at the edge of the DP 555BR and DP 444BR sectors. In the polygon views of test locations of 2005 and 2006 and 2008 to 2009, there were sectors of environments without clear winning cultivars. The failure to identify consistent relationships among test locations across years might be due to the unpredictable environmental factors of years and locations, as suggested by highly significant $Y \times L$ interactions (Table 3). However, the test locations of Las Cruces, NM in the Western region and Lubbock, TX in the Plains region were distinct from other locations in the Central, Delta, and Eastern regions. Although these two locations grouped with some unpredictable locations in different trial years, Las Cruces, NM

and Lubbock, TX were in the sectors separated from other environments except for the environment of Lubbock, TX in 2009 (Fig. 3). Although all test locations in the RHQ tests might belong to a single megaenvironment, the test locations of Las Cruces, NM and Lubbock, TX could be separated as a subregion. This relationship coincided with the geographic separation of locations according to elevations. Elevations of Lubbock, TX (992 m) and Las Cruces, NM (1188 m) were considerably higher than remaining locations (27-91 m). In addition, Lubbock, TX and Las Cruces, NM are located at a higher longitude (-103° to -107°) than all remaining locations (-80° to -96°).

Environmental Factors Affecting Yield Stability. To identify environmental factors contributing to $G \times E$ interactions for lint yield in the RHQ tests, correlations between the environmental scores of the first principal component (PC1) and weather variables were analyzed by Pearson correlation coefficients (Table 5). Among the three temperature variables, only the minimum temperature was detected to influence $G \times E$ effects with r values of -0.41 and -0.30 for the early and late growing seasons, respectively. The test locations of Las Cruces, NM, Belle Mina, AL, and Lubbock, TX had the lowest minimum temperature during the early and late growing seasons (Table 4). Therefore, these sites were the locations most attributed to the negative interactions. Abou-El-Fitouh et al. (1969) also identified minimum temperature significantly correlated with lint yield during boll development stage in the NCVT trials between 1960 and 1962. This result suggests that greater yield stability might be achieved by genetic improvement in cultivars for tolerance to low temperature at locations of Las Cruces, NM, Belle Mina, AL, and Lubbock, TX during seedling establishment, late boll development, and boll opening.

The other environmental factors, except for elevation, were not detected with significant correlations (Table 5). The significant contribution of elevation to yield stability is consistent with geographical separation by elevation of Las Cruces and Lubbock from other locations. The lack of significant correlation between the environmental scores and precipitation in this study might be caused by diverse field managements among test locations despite low precipitation for Las Cruces and Lubbock during the early growing season (Table 4). Application of irrigation during growing seasons at both locations negated precipitation as a major factor. The low correlation coefficients identified indicate that other factors such as biotic stress, soil fertility, etc., might have influence on yield stability of cotton cultivars across the Cotton Belt. Some of these factors have been analyzed for stability performance of cotton cultivars such as Verticillium wilt (*Verticillium dahlia* Kleb.) (Baxevanos et al., 2008a) and nitrogen fertilization (Boman et al., 1997). The analysis of these factors requires appropriate scores of stresses among collaborators of different test locations in future.

Implication in Breeding. One of the major goals in plant breeding is to improve performance stability of cultivars across environments. In the current study, based on lint yield in the trials conducted between 2003 and 2009 at different locations across the U.S. Cotton Belt, there were no consistent relationships among test locations across trial years. These results indicate unpredictable environmental factors affecting agronomic performance stability of cotton cultivars across environments in the Cotton Belt. Therefore, it is critical for cotton breeders to select superior genotypes with wide adaptation to diverse environments in variety trials of multiple test locations and different years.

Table 5. Pearson correlation coefficients (r) between the environmental scores of the first principal component axis (PC1) and the environmental variables at the early, middle, and late growing seasons during 2003 and 2009.

Growing season ^y	Maximum temperature	Minimum temperature	Mean temperature	Precipitation	Relative humidity	Wind	Elevation	Latitude	Longitude
Early	-0.04	-0.41***	-0.14	0.13	-0.44*	0.08	0.33*	0.04	-0.16
Middle	0.10	-0.22	0.12	-0.18	-0.44*	0.08			
Late	0.06	-0.30*	-0.07	-0.14	-0.33	0.04			

^y The early growing season, between 10 April and 31 May included germination and seedling development growth stages; the middle growing season, between 1 June and 20 Aug included squaring, flowering, and boll development growth stages; the late growing season, between 21 Aug and 30 Oct included late boll development and boll opening growth stages.

^z Values followed by * and ** are significant at $p < 0.05$, $p < 0.01$, respectively.

Cost for variety trials is one of the important factors to consider in breeding. Increasing the number of test locations and test years adds significant costs to breeding. Higher or comparable $G \times L$ effects to those of $G \times Y$ shown in this study imply that increase of test years would have at most the same effects as increasing number of test locations. Because significant $G \times Y$ effects for lint yield were detected in the trials of 2008 and 2009, a replication of variety trials across years is necessary, but extending trials beyond two years would not provide any advantage over increasing number of test locations across the major regions of the Cotton Belt. Reasonable distribution of test locations between megaenvironments could also reduce cost in variety trials. From the current arrangement of test locations in the RHQ tests, most test sites are in the Eastern, Delta, and Central regions with only one site for each of the Plains and Western regions. Because Las Cruces, NM and Lubbock, TX were identified distinct from other locations, more test sites in the Western and Plains regions are suggested for future yield trials. The duplicative test sites in the Eastern, Delta, and Central regions can serve as a back-up considering risky factors in cotton yield trials.

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DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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