The *Journal of Cotton Science 16:42–52 (2012)*
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MOLECULAR BIOLOGY AND PHYSIOLOGY

Interactions Between Irrigation Regimes and Varieties Result in Altered Cottonseed Composition

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ABSTRACT

The value of whole cottonseed and cottonseed products has increased as demand has grown from the dairy and food related industries. Although cottonseed composition has previously been documented to be affected by variety, planting date, and irrigation, interactions between varieties and irrigation regimes have not been adequately described. Six different varieties were planted on two planting dates and grown under irrigated or dryland conditions to document how varieties interacted with irrigation regimes to impact various seed composition traits. Variety was a major source of variation for all the seed composition traits quantified. Individual seed mass variation among varieties could explain some of the seed composition variation observed, particularly for protein. For many of these seed composition traits, variety also interacted statistically with irrigation regimes to impact trait expression. Most seed composition traits of the varieties responded in the same direction to irrigation, but there was sufficient variety variation in the response that significant interactions were produced. 'ST 5599BR' often exhibited a different irrigation response compared to the other varieties, particularly for the different fatty acid components. These results indicate that a pairing of varieties and management practices could be utilized to help achieve desired seed composition traits. Although lint production is the primary economic incentive for cotton producers, improved cottonseed composition offers an important, consistent, and reliable secondary revenue stream for producers.

Prior to the recent run-up in cotton (*Gossypium hirsutum* L.) prices during the 2010-2011 marketing season, the price received for lint had remained relatively flat for many years and was comparatively lower than price appreciation for some other agricultural commodities such as maize (*Zea mays* L.) and soybean (*Glycine max* L.), which have seen considerable price appreciation in recent years. Tempering this disappointment for cotton producers in the lint price has been the steady appreciation in the value of whole cottonseed.

The market for whole cottonseed and cottonseed products has expanded as multiple industries have recognized the benefits cottonseed and cottonseed products offer their operations. The dairy industry is partial to whole cottonseed because of its high protein (35%) and oil (30%) composition (Arieli, 1998). Cottonseed meal is also used as a feed supplement for ruminants. The use of both products can be limited by the gossypol content of cottonseed (Bernardi and Goldblatt, 1980). The distribution of saturated and unsaturated fatty acids within the oil component of cottonseed produces a relatively stable frying oil while providing some health benefits (O'Brien and Wakelyn, 2005). These traits make the oil desirable for use by both food processing and restaurant industries. Variety variation in seed composition traits has been well established over the years by the National Cotton Variety Trials (USDA, 2009) and studies reported in books (Cherry and Leffler, 1984; Tharp, 1948) and journals (Cherry, 1983; Dowd et al., 2010; Kohel and Cherry, 1983; Lawhon et al., 1977; Lukonge et al., 2007; Pandey and Thejappa, 1975; Pons et al., 1953; Stansbury et al., 1953, 1954; Turner et al., 1976). Despite this genetic variation, cotton breeders have devoted little effort toward breeding for modified seed composition due to their almost singular focus upon improving lint yields and fiber quality and lack of sufficient economic motivation for improving these traits. Environmental influences further contribute to variations in seed composition (Cherry, 1983; Dowd et al., 2010; Pettigrew and Dowd, 2011; Pons et al., 1953; Stansbury et al., 1953, 1954; Turner et al., 1976). For example, the amount of water available to the crop during the growing season has a profound effect on seed composition (Pettigrew and Dowd, 2011; Pons et al., 1953; Stansbury et al., 1953, 1954).

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Although seed genetics can be accurately defined, environmental influences can be complex. Multiple factors often operate separately or in combination to determine trait expression for a given environment. Recently, Pettigrew and Dowd (2011) were able to document a cause-and-effect relationship between the amount of water available during crop development and changes in seed composition. Planting dates were also found to affect seed composition, but multiple environmental factors could vary between planting dates and possibly contribute to the planting date response. Cotton plants with different genetic makeup can also respond differently to these various environmental influences. Although the contribution of any genetic-by-environment interaction might be small relative to the main effects, as for example appears to be the case for cottonseed oil fatty acid composition (Dowd et al., 2010), it is important to understand if different cotton varieties respond differently to particular environmental influences. Previous results from Pettigrew and Dowd (2011) showed significant variety-by-irrigation interactions were detected for many of the seed composition traits, but they were not fully explored because the principle focus of that research was to describe how the main effects of planting date and irrigation altered cottonseed composition. In this research, the objectives were to more fully elucidate the variety effect on cottonseed composition and document how different varieties can interact with irrigation regimes to impact cottonseed composition.

MATERIALS AND METHODS

Field studies were conducted at Stoneville, MS on a Dubbs silt loam (fine-silty, mixed, active, themic Typic Hapludalfs) during the years 2005-2008. The treatments in this study included six cotton varieties, two irrigation regimes, and two planting dates. The varieties 'DP 445BR', 'DP 555BR', 'FM 800BR', 'FM 960BR', 'ST 4892BR', and 'ST 5599BR' were grown each year of the study and represented a diversity of maturities and breeding programs. Seed of DP 445BR and DP 555BR were provided by Delta and Pine Land Co., Scott, MS and Bayer CropScience, Research Triangle Park, NC, provided the FM 800BR, FM 960BR, ST 4892BR, and ST 5599BR seed. Irrigation regimes consisted of irrigated and dryland conditions. Three furrow irrigation applications occurred in 2006, two applications occurred in 2007, and four applications occurred in 2008, with approximately 2.54 cm of water being applied during each irrigation event. The planting date comparison included plantings occurring approximately during the first week of April (Early) and the first week of May (Normal). Specific dates for the early planting were 30 March 2006, 2 April 2007, and 31 March 2008. Normal planting dates were 2 May 2006, 27 April 2007, and 6 May 2008. Plot size consisted of four rows spaced 1-m apart and 18.3-m long. Other specific cultural practices have been described in earlier reports (Pettigrew, 2010; Pettigrew and Dowd, 2011).

The experimental design utilized in this study was a randomized complete block with a modified split-split treatment arrangement. Irrigation regimes were the main plots, planting dates were the split plots, and cultivars were the split-split plots. Irrigation regimes were replicated in three blocks. Within each block, there were two replications of planting date for each block × irrigation combination. Cultivars were randomly assigned within each irrigation × block × planting date combination. All treatments and cultivars were randomly assigned the first year of the study and then remained in their initial location throughout the duration of the study.

After defoliation of the crop, a 50-boll sample was hand-harvested from one of the inner plot rows of each plot, avoiding the ends of the row. This boll sample was subsequently ginned on a 10-saw laboratory gin, saving and weighting the lint and seed. The seed saved from these boll samples was utilized for the seed composition analyses.

Seed Preparation. Approximately 75 g of non-delinted seed from each plot were cracked by milling for several seconds in a blender. This partially cracked seed was then sifted through a series of # 4 (4.75 mm opening) and # 12 sieves (1.70 mm opening). Material collecting on the # 4 sieve was re-milled through the blending process increasing the length and intensity of the grinding and then was resifted. Dehulled kernels and larger kernel pieces were collected on the surface of the # 12 sieve and were ground in a food chopper to pass through a # 20 sieve (0.85 mm opening). Ground kernel samples were then stored in the dark at -20 °C until subsequent analyses.

Compositional Assays. Specific details concerning the various extractions techniques and assay procedures have been reported previously in detail elsewhere (Pettigrew and Dowd, 2011), so only a brief synopsis of these methods is included here. Crude oil was extracted from the dry ground kernel tissue with petroleum ether (CAS #8032-32-4) and, after evaporation of the solvent, was quantified gravimetrically. To determine the distribution of fatty acids in the oil, the oil was extracted with hexane (CAS #110-54-3), and the recovered glycerides were converted to fatty acid methyl esters by heating with 0.5 N methanolic base (a product of Sigma-Aldrich-Supelco, Bellefonte, PA). Individual fatty acid esters were separated and measured by gas chromatography on a polar capillary column. Gossypol enantiomers were extracted from the meal by forming a Schiff's base complex with *R*-(-)-2-amino-1-propanol (CAS #35320-23-1) and detecting the resulting diastereomeric complexes by High Pressure Liquid Chromotography (HPLC), based on AOCS Recommended Practice Ba 8a-99 (AOCS, 1998). Nitrogen was determined by combustion with a nitrogen analyzer. Protein was calculated by multiplying the nitrogen level by 6.0, which is the conversion factor appropriate for cottonseed based on reported amino acid distributions (Dowd and Wakelyn, 2010). Soluble sugars were extracted and derivatized in a solution containing pyridine (CAS #110-86-1), hexamethyldisilazane (CAS #999-97-3), and trifluoroacetic acid (CAS #76-05-1). This mixture was heated at 70 °C to convert the sugar's hydroxyl groups to trimethylsilyl ethers. The sugars were then separated and quantified by gas chromatograph on a non-polar capillary column.

Statistical analyses were performed by analysis of variance (PROC MIXED, SAS Institute, 1996). Because all irrigation, planting date, and cultivar treatments remained in their original location each year of the study, years were treated as repeated measurements when conducting a combined analysis across years. Random effects used in this model for the comparison across years were block × water; rep × water(block); block × rep × planting(water); block × rep × cultivar(water × planting); and year × block × rep. Irrigation, planting date, and cultivar means were averaged across years when statistically important interactions were not detected. Means were separated by use of a protected least squares difference (LSD) at $P \leq 0.05$. The irrigation × variety interaction was expressed in the tables by presenting the irrigation regime difference for each variety. LSDs for comparing these differences were computed to help explain the varying irrigation responses among the varieties.

RESULTS AND DISCUSSION

Data from this research were collected during three distinct growing environments. Specific weather data for each year of this study have been summarized and presented in an earlier publication (Pettigrew and Dowd, 2011). In general, the weather during the 2006 growing season was relatively typical for the Mississippi Delta. Rainfall during July in 2007 was unusually high compared with the rainfall for this month in the other years of the study. In contrast, June and July of 2008 were hot and dry, followed by an extraordinarily amount of precipitation during September of 2008 because of hurricane Gustav. Because of these contrasting climatic patterns, the year effect was the largest statistically significant source of variation for almost every trait that was measured (Table 1). The variety effect was the second strongest statistically significant source of variation. Years also significantly interacted with planting date, irrigation regime, and variety for most of the traits due to the diverse growing environments prevailing during these years (Table 2). However, f-values for the interactions with years were small relative to the main effects; consequently, the variety means and the variety × irrigation interaction means were averaged across years. A significant irrigation × planting interaction was also detected for some seed composition traits. However, most of these interactions were small and were related to the different maturities of the varieties (e.g., late maturing DPL 555BR often responded differently to planting date than the other varieties). Therefore, we will not discuss irrigation × planting date interactions in this report.

Variety differences were detected in the individual seed mass data (Table 2). DPL 555BR had the smallest seed mass (21% smaller than the next smallest variety) and FM 960BR had the greatest seed mass (3% greater than the next largest variety). The seed mass increase associated with irrigation (Pettigrew, 2010) was consistent across the varieties as there was no significant variety × irrigation interaction. Total seed gossypol levels are known to be strongly affected by both variety and environment (Pons et al., 1953). In this experiment, considerable genetic variability was detected for this trait with DPL 444BR, ST 4892BR, and ST 5599BR having greater concentrations than the other three varieties. Irrigation was found to increase gossypol level for all varieties (Table 2). The irrigation response, however, was greater for the DPL 444BR and Stoneville varieties than it was for the other varieties.

Table 1. Analysis of variance table containing sources of variation, degrees of freedom, and mean square values for cottonseed gossypol, oil, protein, carbohydrate, and fatty acid concentrations.

Source of[z] Variation	df	Total Gossypol	% (+) Gossypol	Crude Oil	Protein	Total Soluble Carbohydrates	Saturated Fatty Acids	Unsaturated Fatty Acids
Block	2	0.0571	1.7514	53.7028	55.7362	0.8608 *	1.3789	0.1342
Rep(Block)	3	0.0045	2.5497	6.3517	1.6641	0.3642	0.2566	0.5727
Water	1	5.3074 ** [y]	621.2349 **	515.0484 *	1427.7299 *	8.5627 **	56.1538 **	47.2797 **
Planting	1	0.1621 *	1.3394	59.3630 **	6.2841	8.4392 **	1.7842 *	0.9788
Water*Planting	1	0.3962 **	40.0090 **	61.1256 **	48.5489 **	2.5854 **	0.0030	0.3089
Variety	5	3.6278 **	1069.1012 **	367.3316 **	323.1516 **	6.7500 **	241.0757 **	234.8762 **
Water*Variety	5	0.0990 **	7.8067 **	2.7263	1.2059	0.2127 **	1.0874 **	1.1665 **
Planting*Variety	5	0.0322 *	8.7515 **	3.9832	4.2550	0.2810 **	0.1637	0.1862
Water*Planting*Variety	5	0.0157	2.6467	1.1394	1.7732	0.0903	0.5159	0.5244
Year	2	5.3465 **	396.6885 **	369.0560 **	374.5595 **	135.6824 **	343.2273 **	196.3852 **
Year*Water	2	0.2204 **	6.7672 *	21.5226 **	82.1196 **	7.0652 **	0.3902	1.1324 *
Year*Planting	2	0.4721 **	106.7278 **	2.7203	23.6636 **	9.3400 **	1.4867 **	0.7475
Year*Water*Planting	2	0.1157 **	53.0884 **	1.3215	1.8184	0.8342 **	0.7631 *	0.2242
Year*Variety	10	0.0875 **	15.4658 **	3.1530 *	4.0394 *	0.9977 **	1.1995 **	1.4922 **
Year*Water*Variety	10	0.0278 **	9.1498 **	3.0729 *	2.7217	0.0770	0.3462	0.3183
Year*Planting*Variety	10	0.0283 **	2.5290	2.7094	2.5324	0.0688	0.4329	0.4108
Year*Water*Planting*Variety	10	0.0096	1.5539	2.2721	1.2969	0.0477	0.1790	0.1750

[z] Random effects used in this model were block*water; rep*water(block); block*rep*planting(water); block*rep*variety(water*planting); year*block*rep. Nested effects denoted with parentheses (i.e. rep(block) denotes rep within block).

[y] *, ** denote significance at the 0.05 and 0.01 levels, respectively.

Table 2. Effect of varying varieties and irrigation regimes on cottonseed gossypol, crude oil, and protein concentrations.

Variety	Irrigation Regime	Total Gossypol	% (+) Gossypol	Crude Oil	Protein	Seed Mass
		g kg^{-1}	%[z]	g kg^{-1}	g kg^{-1}	mg seed^{-1}
DPL 444BR	Dryland	11.2	65.5	313	376	91
DPL 555BR		8.7	61.3	280	410	72
FM 800BR		9.0	56.8	328	368	101
FM 960BR		9.4	58.9	335	353	105
ST 4892BR		13.3	62.6	306	374	94
ST 5599BR		12.3	67.5	334	356	99
DPL 444BR	Irrigated	13.5	62.3	339	339	94
DPL 555BR		10.4	58.2	296	375	74
FM 800BR		10.4	54.8	350	334	104
FM 960BR		11.2	56.8	360	315	107
ST 4892BR		16.3	61.1	324	340	97
ST 5599BR		15.4	64.9	356	315	102
LSD 0.05[y]		0.5	0.6	8	8	2
	LSD 0.05[x]	0.7	0.7	14	14	2
P > *F* [w]		0.01	0.01	0.40	0.86	0.90
DPL 444BR	Irrigation Difference	2.3	-3.2	26	-37	3
DPL 555BR		1.7	-3.1	16	-35	2
FM 800BR		1.4	-2.0	22	-34	3
FM 960BR		1.8	-2.1	25	-38	2
ST 4892BR		3.0	-1.5	18	-34	4
ST 5599BR		3.1	-2.6	22	-41	3
LSD 0.05[v]		1.0	1.0	20	20	3

[z] Percentage of the total gossypol.

[y] LSD for comparing varieties within irrigation regimes.

[x] LSD for comparing irrigation regimes within varieties.

[w] Probability level for significance of the interaction between irrigation regimes and varieties.

[v] LSD for comparing the differences between the dryland and irrigated regimes among the varieties.

The ratio of the gossypol isomers is generally thought to be strongly determined by genetics with little influence of environment (Rayburn et al., 2000). Significant varietal differences in the % (+) gossypol were apparent in the study (Table 2). Of the varieties studied, ST 5599BR had the largest % (+) gossypol content, with FM 800BR having the lowest % (+) gossypol content. For all varieties, irrigation reduced the levels of % (+) gossypol. Both Deltapine varieties, however, had the greatest irrigation induced reduction in % (+) gossypol and these varieties had significantly greater reductions than the reductions observed for FM 800BR and ST 4892BR.

Varietal differences on the order observed in prior reports (Kohel and Cherry, 1983; Lawhon et al., 1977; Stansbury et al., 1954) were detected for both crude oil and protein components. Greater crude oil concentrations were found in FM 800BR, FM 960BR, and ST 5599BR than were found in the other varieties. DPL 555BR had statistically the lowest crude oil concentration of all the varieties. In a reverse of the crude oil trend, DPL 555BR had the highest protein concentration, whereas FM 960BR had the lowest protein concentration. Variety differences in crude oil level closely mirrored seed mass variety trends, but the protein variety trend was almost the antithesis of the seed mass trend. These trends are consistent with the strong negative correlations that have been observed between cottonseed protein (i.e., nitrogen) and seed crude oil levels (Pandey and Thejappa, 1975; Stansbury et al., 1956). Despite varietal differences in both traits and pronounced irrigation differences, no significant variety × irrigation interactions were detected for either component.

FM 960BR, ST 4892BR, and ST 5599BR had higher seed concentrations of total soluble carbohydrates, raffinose (the predominant soluble carbohydrate), and sucrose than did the other varieties (Table 3). Seed stachyose concentrations were greatest in FM 800BR, FM 960BR, and ST 4892BR. Although no significant variety × irrigation interactions were detected for total soluble carbohydrates or raffinose content, the minor seed carbohydrate constituents did exhibit significant variety × irrigation interactions. The decline in seed sucrose concentration that was caused by irrigation and seen in all varieties was

Table 3. Effect of varying varieties and irrigation regimes on various cottonseed carbohydrate concentrations.

Variety	Irrigation Regime	Total Soluble Carbohydrates	Sucrose	Raffinose	Stachyose
		$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$
DPL 444BR	**Dryland**	**59.3**	**9.5**	**40.1**	**9.8**
DPL 555BR		**60.5**	**9.6**	**41.6**	**9.4**
FM 800BR		**63.7**	**9.2**	**43.8**	**10.8**
FM 960BR		**66.2**	**10.3**	**45.6**	**10.2**
ST 4892BR		**67.6**	**13.0**	**44.1**	**10.4**
ST 5599BR		**65.9**	**11.5**	**45.0**	**9.4**
DPL 444BR	**Irrigated**	**63.3**	**8.9**	**44.8**	**9.5**
DPL 555BR		**63.4**	**9.2**	**46.2**	**8.0**
FM 800BR		**66.2**	**8.8**	**47.4**	**10.0**
FM 960BR		**70.1**	**10.0**	**50.1**	**10.1**
ST 4892BR		**68.7**	**11.9**	**47.1**	**9.7**
ST 5599BR		**68.4**	**9.7**	**49.6**	**9.2**
LSD 0.05[z]		**1.5**	**0.5**	**1.3**	**0.3**
	LSD 0.05[y]	**1.5**	**0.7**	**1.4**	**0.3**
$P > F$ [x]		**0.07**	**0.01**	**0.31**	**0.01**
DPL 444BR	**Irrigation Difference**	**4.0**	**-0.6**	**4.7**	**-0.3**
DPL 555BR		**2.9**	**-0.4**	**4.6**	**-1.4**
FM 800BR		**2.5**	**-0.4**	**3.6**	**-0.8**
FM 960BR		**3.9**	**-0.3**	**4.5**	**-0.1**
ST 4892BR		**1.1**	**-1.1**	**3.0**	**-0.7**
ST 5599BR		**2.5**	**-1.8**	**4.6**	**-0.2**
LSD 0.05[w]		**2.1**	**1.0**	**2.0**	**0.4**

[z] LSD for comparing varieties within irrigation regimes.

[y] LSD for comparing irrigation regimes within varieties.

[x] Probability level for significance of the interaction between irrigation regimes and varieties.

[w] LSD for comparing the differences between the dryland and irrigated regimes among the varieties.

greater for ST 5599BR than for any of the other varieties, except for ST 4892BR, which had an intermediate level of decline. Although irrigation decreased seed stachyose concentrations in all varieties, DPL 555BR exhibited the greatest stachyose decline when compared with the other varieties.

Genetic variation was detected in all the saturated fatty acid fractions, and a significant variety × irrigation interaction was also detected for all saturated fatty acid fractions, with the exception of the longer chain length behenic and lignoceric acids (Table 4). The two FiberMax varieties had the highest percentage of total saturated fatty acids (≈ 30%), and ST 5599BR had the lowest percentage of total saturated fatty acid (≈ 26%) compared with the other varieties. With the exception of lignoceric acid, which is a minor acid that contributes only a small amount to the total saturated fatty acids, ST 5599BR had the lowest percentage for any of the individual saturated fatty acid components, that is, declines in several fatty acids contributed to the reduced level of saturated fatty acids for this variety. In addition, ST 5599BR also exhibited the smallest irrigation induced decline in saturated fatty acid levels of any of the varieties studied except for FM 960BR. All the other varieties responded similarly to irrigation. Palmitic acid (the dominant saturated fatty acid in cottonseed), displayed a similar variety and variety × irrigation response to that observed for total saturated fatty acids. ST 5599BR also exhibited the smallest response to irrigation for myristic acid, with all the other varieties demonstrating a similar level of decline in response to irrigation. Arachidic acid levels declined more in response to irrigation for the two Deltapine varieties than for the two Stoneville varieties. Irrigation reduced the level of stearic acid more in DPL 555BR than it did in DPL 444BR or FM 800BR. The other varieties did not differ in their irrigation response for stearic acid.

Table 4. Effect of varying varieties and irrigation regimes on various cottonseed saturated fatty acid concentrations.

Variety	Irrigation Regime	Saturated Fatty Acids	Myristic Acid 14:0	Palmitic Acid 16:0	Stearic Acid 18:0	Arachidic Acid 20:0	Behenic Acid 22:0	Lignoceric Acid 24:0
		%[z]	%	%	%	%	%	%
DPL 444BR	Dryland	30.2	0.987	26.0	2.56	0.319	0.146	0.130
DPL 555BR		27.8	0.767	23.7	2.76	0.283	0.129	0.113
FM 800BR		30.9	0.998	26.7	2.53	0.321	0.154	0.108
FM 960BR		30.8	0.914	26.5	2.68	0.350	0.166	0.120
ST 4892BR		30.3	1.002	26.2	2.55	0.312	0.149	0.122
ST 5599BR		26.3	0.651	22.5	2.54	0.263	0.120	0.109
DPL 444BR	Irrigated	29.4	0.852	25.4	2.56	0.292	0.130	0.113
DPL 555BR		26.9	0.645	23.1	2.66	0.253	0.112	0.095
FM 800BR		30.0	0.857	26.0	2.53	0.300	0.137	0.098
FM 960BR		30.2	0.796	26.1	2.64	0.325	0.152	0.109
ST 4892BR		29.5	0.872	25.5	2.49	0.296	0.140	0.108
ST 5599BR		26.0	0.578	22.4	2.49	0.247	0.108	0.099
LSD 0.05[y]		0.2	0.026	0.2	0.03	0.006	0.005	0.006
	LSD 0.05[x]	0.3	0.030	0.2	0.05	0.007	0.005	0.006
P > F [w]		0.01	0.01	0.01	0.01	0.01	0.18	0.21
DPL 444BR	Irrigation Difference	-0.8	-0.135	-0.6	0	-0.027	-0.016	-0.017
DPL 555BR		-0.9	-0.122	-0.6	-0.10	-0.030	-0.017	-0.018
FM 800BR		-0.9	-0.141	-0.7	0	-0.021	-0.017	-0.010
FM 960BR		-0.6	-0.118	-0.4	-0.04	-0.025	-0.014	-0.011
ST 4892BR		-0.8	-0.130	-0.7	-0.06	-0.016	-0.009	-0.014
ST 5599BR		-0.3	-0.073	-0.1	-0.05	-0.016	-0.012	-0.010
LSD 0.05[v]		0.4	0.04	0.3	0.07	0.010	0.007	0.008

[z] **Percentage of the total fatty acid fraction.**

[y] **LSD for comparing varieties within irrigation regimes.**

[x] **LSD for comparing irrigation regimes within varieties.**

[w] **Probability level for significance of the interaction between irrigation regimes and varieties.**

[v] **LSD for comparing the differences between the dryland and irrigated regimes among the varieties.**

Stansbury et al. (1953) were the first to note that both variety and environment influenced cottonseed oil's iodine value, a measure of the level of unsaturated fatty acids. In this study, variations were apparent in the levels of individual and total unsaturated fatty acids (Table 5). Because cyclopropenoid fatty acids are only modest contributors to cottonseed oil fatty acid profiles, genetic variation in unsaturated fatty acids often contrasted with observed variation in saturated fatty acids (Table 5). For example, ST 5599BR, which had the lowest level of total saturated fatty acids, had the highest observed level of total unsaturated fatty acids. The two FiberMax varieties had the smallest percentage of unsaturated fatty acids, which also contrasted with their high saturated fatty acid levels. Despite having the highest percentage of total unsaturated fatty acids, ST 5599BR also demonstrated the smallest increase in unsaturated fatty acids in response to irrigation of any of the varieties in the study. Of the individual unsaturated fatty acids, ST 5599BR had the highest percentage of any of the varieties for mono-unsaturated fatty acids (i.e., palmitoleic, vaccenic, and oleic acids), whereas DPL 555BR had the highest percentage of poly-unsaturated fatty acids (linoleic and α-linolenic acids). Contrasting its composition of poly-unsaturated acids, DPL 555BR had the lowest percentage of mono-unsaturated acids compared with the other varieties. Among these varieties, FM 960BR had the lowest percentage of poly-unsaturated fatty acids. All varieties increased linoleic acid level (the predominate unsaturated fatty acid) in response to irrigation, but this response was significantly greater for ST 5599BR than it was for ST 4892BR. All other varieties had a similar irrigation response in linoleic acid production. Irrigation reduced oleic acid concentration in all varieties, but this reduction was greatest in ST 5599BR. In general, irrigation decreased the percentage of palmitoleic and vaccenic acids, but this decrease was smaller for DPL 555BR than it was for any of the other varieties. In fact, the palmitoleic acid level of DPL 555BR slightly increased in response to irrigation, whereas the palmitoleic acid in the seed of all the other varieties decreased in response to irrigation.

Table 5. Effect of varying varieties and irrigation regimes on various cottonseed unsaturated fatty acid concentrations.

Variety	Irrigation Regime	Unsaturated Fatty Acids	Palmitoleic Acid 16:1	Vaccenic Acid 18:1(n-7)	Oleic Acid 18:1(n-9)	Linoleic Acid 18:2	Linolenic Acid 18:3
		%[z]	%	%	%	%	%
DPL 444BR	Dryland	69.4	0.593	0.806	15.2	52.9	0.155
DPL 555BR		71.8	0.474	0.709	15.0	55.7	0.160
FM 800BR		68.7	0.639	0.797	16.0	51.3	0.139
FM 960BR		68.8	0.597	0.780	18.3	49.2	0.127
ST 4892BR		69.3	0.618	0.852	16.3	51.6	0.154
ST 5599BR		73.3	0.666	0.875	19.0	52.8	0.143
DPL 444BR	Irrigated	70.2	0.562	0.737	13.7	55.2	0.145
DPL 555BR		72.6	0.478	0.680	13.5	57.9	0.156
FM 800BR		69.6	0.599	0.739	14.5	53.8	0.130
FM 960BR		69.3	0.570	0.712	16.7	51.4	0.121
ST 4892BR		70.1	0.596	0.778	15.2	53.7	0.146
ST 5599BR		73.5	0.619	0.782	16.4	55.8	0.132
LSD 0.05[y]		0.2	0.014	0.014	0.3	0.4	0.007
	LSD 0.05[x]	0.3	0.015	0.020	0.4	0.6	0.007
P > F [w]		0.01	0.01	0.01	0.01	0.04	0.77
DPL 444BR	Irrigation Difference	0.8	-0.031	-0.069	-1.5	2.3	-0.010
DPL 555BR		0.8	0.004	-0.029	-1.5	2.2	-0.004
FM 800BR		0.9	-0.040	-0.058	-1.5	2.5	-0.009
FM 960BR		0.5	-0.027	-0.068	-1.6	2.2	-0.006
ST 4892BR		0.8	-0.022	-0.074	-1.1	2.1	-0.008
ST 5599BR		0.2	-0.047	-0.093	-2.6	3.0	-0.011
LSD 0.05[v]		0.4	0.021	0.028	0.6	0.8	0.010

[z] Percentage of the total fatty acid fraction.

[y] LSD for comparing varieties within irrigation regimes.

[x] LSD for comparing irrigation regimes within varieties.

[w] Probability level for significance of the interaction between irrigation regimes and varieties.

[v] LSD for comparing the differences between the dryland and irrigated regimes among the varieties.

Cyclopropenoid fatty acids are undesirable components of cottonseed oil. The levels observed (≈ 0.8% total) was typical of levels observed in prior reports (Lawhon et al., 1977). In this study, the variety exhibiting the highest percentage of cyclopropenoid fatty acids was ST 5599BR (Table 6). Correspondingly, this variety also exhibited the greatest levels of the individual cyclopropenoid acids (malvalic acid and sterculic acid) (Table 6). For all varieties, both individual and total cyclopropenoid acid levels increased with irrigation. However, the level of total cyclopropenoid acids and malvalic acid increased more in ST 5599BR and DPL 555BR than it did in DPL 444BR. DPL 444BR had the smallest sterculic acid increase in response to irrigation than did any of the other varieties, except for ST 4892BR.

Table 6. Effect of varying varieties and irrigation regimes on various cottonseed cyclopropenoid fatty acid concentrations.

Variety	Irrigation Regime	Cyclopropenoid Fatty Acids	Malvalic Acid cpe18:1	Sterculic Acid cpe19:1
		%[z]	%	%
DPL 444BR	Dryland	0.696	0.404	0.292
DPL 555BR		0.664	0.370	0.294
FM 800BR		0.643	0.371	0.272
FM 960BR		0.715	0.399	0.316
ST 4892BR		0.641	0.359	0.283
ST 5599BR		0.784	0.444	0.340
DPL 444BR	Irrigated	0.740	0.441	0.300
DPL 555BR		0.782	0.456	0.326
FM 800BR		0.748	0.443	0.305
FM 960BR		0.822	0.474	0.348
ST 4892BR		0.737	0.430	0.308
ST 5599BR		0.916	0.542	0.374
LSD 0.05[y]		0.034	0.023	0.011
	LSD 0.05[x]	0.046	0.032	0.014
$P > F$ [w]		0.01	0.01	0.01
DPL 444BR	Irrigation Difference	0.044	0.037	0.008
DPL 555BR		0.118	0.086	0.032
FM 800BR		0.105	0.072	0.033
FM 960BR		0.107	0.075	0.032
ST 4892BR		0.096	0.071	0.025
ST 5599BR		0.132	0.098	0.034
LSD 0.05[v]		0.065	0.045	0.020

[z] Percentage of the total fatty acid fraction.

[y] LSD for comparing varieties within irrigation regimes.

[x] LSD for comparing irrigation regimes within varieties.

[w] Probability level for significance of the interaction between irrigation regimes and varieties.

[v] LSD for comparing the differences between the dryland and irrigated regimes among the varieties.

The oleic desaturation ratio (ODR) describes the efficiency of the reaction to add a second double bond to convert oleic acid to linoleic acid. ODR was greatest in DPL 555BR and followed closely by DPL 444BR (Table 7). FM 960BR had the lowest ODR of the varieties. Both Deltapine varieties and ST 4892BR had the largest linoleic desaturation ratio (LDR) which estimates the efficiency of the conversion of linoleic acid into linolenic acid. ST 5599BR had the lowest C16/C18 ratio of any of the varieties with FM 800BR trending to have the highest C16/C18 ratio. FM 960BR had the highest level of long chain (> 18 carbon atoms) fatty acids. ST 5599BR had fewer of these long chain fatty acids than the other varieties. Irrigation increased the ODR in all varieties, but the increase was greater in ST 5599BR than it was in any of the other varieties. The ratio of C16/C18 fatty acids was decreased by irrigation for all varieties, but that decrease was less for FM 960BR and ST 5599BR than it was for other varieties. Irrigation reduced the production of fatty acids with acyl chains longer than 18 carbon atoms for all varieties, but the reduction was smallest in the two Stoneville varieties and largest in the two Deltapine varieties.

As has been previously described in the annual National Cotton Variety Trials, considerable variability exists among cotton varieties for the various seed composition traits (USDA, National Cotton Variety Trials, 2009). Some of these variations can be related to the average individual seed mass of the particular variety. For example, there seemed to be a clear positive relationship between seed crude oil content and seed mass. With oil levels ultimately dependent upon the plant's ability to assimilate carbon through photosynthesis and partition the fixed carbon to appropriate sinks, we speculate that a stronger sink demand (i.e., larger seed mass) might command more of the photosynthesized carbon, potentially leading to increased oil production at that sink. Similarly, the two varieties with the smallest seed mass, DPL 444BR and DPL 555BR, also possessed the lowest levels of seed soluble carbohydrate reserves, components that are also dependent upon carbon fixation and translocation. On the other hand, with only a finite amount of nitrogen available for plant growth and production, the seed protein concentrations can be diluted within larger seed containing more dry matter. This dilution phenomenon could help explain reduced protein concentrations in large seeded varieties such as ST 5599BR and elevated protein concentrations in small seed variety, for example, DPL 555BR. Protein dilution has also been apparent in comparing irrigated and dryland plant seeds (Pettigrew and Dowd, 2011).

Table 7. Effect of varying varieties and irrigation regimes on various calculated cottonseed fatty acid components.

Variety	Irrigation Regime	Oleic Desaturation Ratio (ODR)	Linoleic Desaturation Ratio (LDR)	C16 / C18 Fatty Acid Ratio	Total Fatty Acids > C18
					%[z]
DPL 444BR	Dryland	0.777	0.0029	0.376	0.595
DPL 555BR		0.788	0.0029	0.329	0.526
FM 800BR		0.762	0.0027	0.391	0.583
FM 960BR		0.729	0.0025	0.386	0.637
ST 4892BR		0.760	0.0030	0.379	0.583
ST 5599BR		0.736	0.0027	0.311	0.492
DPL 444BR	Irrigated	0.801	0.0026	0.363	0.535
DPL 555BR		0.811	0.0027	0.318	0.460
FM 800BR		0.788	0.0024	0.375	0.532
FM 960BR		0.755	0.0023	0.377	0.586
ST 4892BR		0.780	0.0027	0.366	0.544
ST 5599BR		0.773	0.0024	0.308	0.454
LSD 0.05[y]		0.004	0.0001	0.004	0.013
	LSD 0.05[x]	0.007	0.0001	0.005	0.015
P > *F*[w]		0.01	0.80	0.01	0.02
DPL 444BR	Irrigation Difference	0.024	-0.0003	-0.013	-0.06
DPL 555BR		0.023	-0.0002	-0.011	-0.066
FM 800BR		0.026	-0.0003	-0.016	-0.051
FM 960BR		0.026	-0.0002	-0.009	-0.051
ST 4892BR		0.020	-0.0003	-0.013	-0.039
ST 5599BR		0.037	-0.0003	-0.003	-0.038
LSD 0.05[v]		0.010	0.0001	0.007	0.021

[z] Percentage of the total fatty acid fraction.

[y] LSD for comparing varieties within irrigation regimes.

[x] LSD for comparing irrigation regimes within varieties.

[w] Probability level for significance of the interaction between irrigation regimes and varieties.

[v] LSD for comparing the differences between the dryland and irrigated regimes among the varieties.

Genetic variability was also detected among fatty acid distribution profiles. DPL 555BR and ST 5599BR appear to allocate more of their carbon to unsaturated fatty acid production rather than saturated fatty acid production compared with the other varieties studied. Coincidentally, those two varieties also happened to be the latest maturing varieties within the group, although it would be premature to associate cause and effect between crop maturity and the fatty acid distribution profile. Within the unsaturated fatty acid pool, DPL 555BR also tended to allocate more of its carbon toward the poly-unsaturated fatty acids rather than mono-unsaturated fatty acids when compared with other varieties.

Although variety was a predominate source of variation for many of these seed composition traits, statistically significant variety × irrigation interactions for some seed traits indicate that some interesting differences exist in the way that varieties respond to irrigation in regards to composition of the seed. For many of the components measured, the ST 5599BR response to irrigation was different than the response of the other varieties. For instance, the irrigation induced shift of carbon allocation from saturated fatty acids to unsaturated fatty acids was lower for ST 5599BR than for any of the other varieties. This different ST 5599BR response to irrigation in total saturated fatty acids is primarily due to the effect that particular genetic background had on the largest single fraction of saturated fatty acid, that is, palmitic acid. The reduced irrigation response of ST 5599BR in producing unsaturated fatty acids was primarily due to the offsetting effects it had in producing both the largest irrigation decline in oleic acid and the largest irrigation increase in linoleic acid (the two largest fractions of unsaturated fatty acids). This alteration in the production of oleic and linoleic acids by irrigation for ST 5599BR, also caused it to exhibit the largest increase in ODR ratio in response to irrigation. This response implies that irrigation increased enzymatic activity of the desaturate II enzyme in ST 5599BR more than it did in any of the other varieties. Although some of the other varieties also occasionally exhibited a different irrigation responses compared with the bulk of the varieties, none were as consistently and dramatically different in response as was ST 5599BR.

Genetics, irrigation, and planting dates can all affect cottonseed composition individually as sources of variation, but there can also be important interactions between varieties and irrigation regimes to impact cottonseed composition. Although in general, seed composition of all the varieties responded in a similar direction when irrigation was applied, the degree of the irrigation response from ST 5599BR was usually different than that observed from most of the other varieties. Lint production will always be the predominate source of income for cotton producers. However, the value for cottonseed and cottonseed products might continue to appreciate as various industries recognize the value of these products and thereby increase the demand for these products. Under that scenario, breeders and producers would have more incentive to produce and grow varieties with altered and improved seed composition. To accomplish that objective, it would be useful to understand how the seed composition traits of various genetic backgrounds respond to different growth environments. This study described the degree to which one genetic background, ST 5599BR, altered its seed composition in response to one of the many environmental factors (irrigation) differently than some of the other varieties. This information could partially be used to prescribe variety and management practice combinations to help produce desired seed composition traits.

DISCLAIMER

Trade names are necessary to report factually on available data, however, the USDA neither guarantees nor warrants the standard to the product or service, and the use of the name by USDA implies no approval of the product or service to the exclusion of others that might also be suitable.

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