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RESEARCH

Genetic Analysis of Cottonseed Protein and Oil in a Diverse Cotton Germplasm

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ABSTRACT

Historically, the primary objective of cotton (*Gossypium hirsutum* L.) breeding programs was to improve the quantity and quality of cotton fiber. Because of the added value of cottonseed and its many uses, including a feed and human food source, there is interest in developing cotton breeding programs that focus improvement efforts simultaneously on cotton fiber and seed. Genetic analysis of cottonseed traits, such as protein and oil, is a prerequisite to building new joint fiber and seed cotton breeding programs. In this study, we conducted a genetic analysis of a diverse set of elite upland cotton germplasm for cottonseed protein and oil. Environment was responsible for a sizeable portion of the total variation for protein and oil, and genetics accounted for a larger portion of variation for oil than protein. Genotype × environment (G × E) interactions significantly impacted oil but not protein. Genotypic correlation analysis found a strong, negative relationship between protein and oil. Positive genotypic correlations were found for protein and several agronomic traits including lint yield; whereas, negative correlations were found between oil and lint yield along with other agronomic traits. Overall, results showed very little association between protein, oil, and fiber quality traits. These findings indicate that altering protein and oil seed composition will impact yield and yield component traits. However, alterations in seed composition should not impact fiber quality.

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Abbreviations: DP, Deltapine; FM, FiberMax; G × E, genotype × environment; ST, Stoneville; TD-NMR, time-domain nuclear magnetic resonance.

Historically, cotton breeding programs focus on improving cotton fiber and providing the textile industry with natural, spinnable fibers. The cottonseed remaining after capturing value from cotton fiber is most often used as a by-product for cattle feed, fertilizer, cooking oil, and numerous industrial applications (Campbell and Hinze, 2010). As a consequence, cotton breeding programs have not placed a large focus on improving cottonseed.

In an effort to maximize return on investments made by growers in cotton fiber production systems, there is interest to develop breeding programs that also improve cottonseed traits. Instead of cottonseed being considered only as a by-product of cotton fiber production, a joint fiber and seed cotton production system could be established that maximizes value from both cotton fiber and seed constituents. Since every 1 kg of cotton fiber produced also produces, on average, 1.6 kg of cottonseed, there exists tremendous potential to better capture additional value through cottonseed. Moreover, cottonseed has tremendous

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potential as a food source because cottonseed oil is high in unsaturated fatty acids (primarily linoleic acid) and displays higher stability under cooking applications and storage (Kothari et al., 2015).

Several prerequisites are required to develop a joint fiber and seed cotton breeding program and include the following. First, germplasm must be surveyed and phenotyped for cottonseed traits. Second, studies must be conducted to determine the inheritance and genetic architecture of cottonseed traits. Third, extensive field studies must be conducted to determine the extent of G × E interactions on cottonseed traits. Fourth, studies must be conducted to determine the impact of correlations among cottonseed, agronomic, and fiber quality traits and their impact on selection.

A limited number of studies have addressed these prerequisites, but more information is needed. Early studies used laborious and destructive methods to measure seed protein and oil and reported the presence of both genetic and environmental variation for these traits (Kohel, 1978; Kohel et al., 1985). Significant genetic variation for seed protein and oil content was reported (Kohel et al., 1985; Qayyum et al., 2010). Using chromosome substitution lines crossed with elite cultivars, Wu et al. (2010) determined significant additive effects for protein and dominant effects for oil. Using data collected in the Regional High Quality trial of the National Cotton Variety Testing program from 2001 through 2007, Meredith et al. (2012) estimated the genetic contribution of the total phenotypic variation for seed protein and oil at 36.7 and 10.8%, respectively. Also, using data collected in the Regional High Quality trial, Zeng et al. (2015) extended this analysis to evaluate trials conducted from 1996 through 2013 and determined the genetic contribution to oil content ranged from 20 to 57% across different trial cycles, while the environment contribution explained 44 to 73% across different trial cycles for protein content. They also reported highly significant genotype × location effects for both traits in each trial cycle. Several studies also reported the impact of environmental factors on cottonseed quality. Kohel and Cherry (1983) reported the impact of harvest date on both seed protein and oil. Pettigrew and Dowd (2011, 2012, 2014) demonstrated the effect of N fertility, irrigation regime, and planting date on cottonseed composition.

Recently, Horn et al. (2011) developed a new, nondestructive, low-field ^{1}H time-domain nuclear magnetic resonance (TD-NMR) method to allow for rapid measurement of seed protein and oil. Using this approach, Hinze et al. (2015) surveyed the seed protein and oil content of 2256 accessions representing 33 species (five tetraploid and 28 diploid) from the US National Cotton Germplasm Collection. They found that protein content ranged from 10 to 36%, while oil content ranged from 8 to 27%. Also using this new technique, Kothari et al. (2015) estimated the broad-sense heritability of oil content (H^2 = 0.52) and noted the amenability of plant breeding to improve oil content as a result of the presence of sufficient additive variance.

Collectively, most studies suggest a relatively strong, negative relationship between seed protein and oil (Hinze et al., 2015; Kohel and Cherry, 1983). This negative relationship is similar to what has been reported in other oilseeds (Blumenthal et al., 2008). In terms of agronomic traits, Hinze et al. (2015) and Zeng et al. (2015) reported a positive relationship between seed oil and seed size and a negative relationship between seed protein and seed size. Zeng et al. (2015) also reported a negative relationship between yield (lint yield and seed cotton yield) and seed protein. Kothari et al. (2015) reported a weak, positive relationship between seed oil and lint yield. Zeng et al. (2015) and Kothari et al. (2015) reported a negative relationship between seed oil and lint percentage. Kothari et al. (2015) reported a weak, positive relationship for seed oil with boll size and a negative relationship with lint index.

Seed protein and oil associations with fiber quality traits have not been thoroughly investigated. Kothari et al. (2015) reported positive relationships for seed oil with fiber strength, uniformity index, and fiber length. Turner et al. (1976) reported a positive relationship between seed oil and micronaire.

In the current study, our overall objective was to survey the cottonseed protein and oil content of a population of diverse, elite upland cotton germplasm lines and cultivars. Outside of collecting protein and oil data on these diverse genotypes, our specific goals were also to (i) determine the impact of G × E interactions on seed protein and oil and (ii) to determine the impact of genotypic correlations among cottonseed, agronomic, and fiber quality traits.

MATERIALS AND METHODS

Field Evaluations

Based on both breeding cycle and pedigree, 82 officially released cotton germplasm lines and cultivars were selected to represent the history of the Pee Dee cotton germplasm enhancement program (see Campbell et al. [2011] for detailed germplasm information). For each field trial, a total of two to six check cultivars were selected for comparison purposes. Two of the checks were conventional cultivars Deltapine 491 (DP 491, PVP No. 200100159) and FiberMax 958 (FM 958, PVP No. 200100208) and the remaining four included transgenic, commercial cultivars widely grown in the southeastern United States. These included cultivars Deltapine 444BR (DP 444BR, PVP No. 200300134), Deltapine 555BR (DP 555BR, PVP No. 200200047), FiberMax 960BR (FM 960BR, PVP No. 200400224), and Stoneville 5599BR (ST 5599BR, PVP No. 200300279).

As described in Campbell et al. (2012), during 2004 to 2006, a total of 11 replicated field trials were conducted across North Carolina, South Carolina, Georgia, and Mississippi. The experimental design for each trial consisted of two to four replicates arranged in an α-lattice incomplete block design. Trials in 2004 included check cultivars DP 491 and FM 958; while those in 2005 and 2006 included six check cultivars (DP 491, FM

Table 1. Description of field trials conducted over 11 location–year environments in North Carolina, South Carolina, Georgia, and Mississippi from 2004 to 2006.

Year	Location	No. checks	No. replicates	Alpha-lattice field design
2004	Blackville, SC	2	4	14 incomplete blocks of size 6
	Florence, SC	2	4	14 incomplete blocks of size 6
2005	Hartsville, SC	6	2	22 incomplete blocks of size 4
	Rocky Mount, NC	6	3	22 incomplete blocks of size 4
	Tifton, GA	6	3	22 incomplete blocks of size 4
2006	Blackville, SC	6	3	22 incomplete blocks of size 4
	Florence, SC	6	3	22 incomplete blocks of size 4
	Rocky Mount, NC	6	3	22 incomplete blocks of size 4
	Stoneville, MS	6	2	22 incomplete blocks of size 4
	Tifton, GA	6	3	22 incomplete blocks of size 4

958, DP 444BR, DP 555BR, FM 960BR, and ST 5599BR). A description of the 11 trial locations is provided in Table 1. With the exception of the Stoneville location, plots were two rows 10.6 to 15.0 m by 76 to 100 cm. At the Stoneville location, plots were single rows 10.6 m by 96.5 cm. Trial management followed the established local practices for dryland cotton production at each location.

A 25-boll sample was hand harvested from each plot before harvest and ginned on a common 10-saw laboratory gin. Fuzzy seed collected from each ginned sample was used to determine seed protein (%) and oil (%) in triplicate following the procedures described in Horn et al. (2011). This procedure involved using a rapid, nondestructive, simultaneous method TD-NMR and chemometric analysis. Seed samples (~3 g) were equilibrated to 37°C for 30 min before acquisition of data in a Bruker Minispec 20 (Bruker Corp.).

Previously collected agronomic and fiber quality trait data from each of the 11 trials was obtained. Using these data, we determined additional yield and quality component traits. These included the following (Groves and Bourland, 2010):

$$\text{Seed per boll} = \frac{\text{boll size} \times \text{100-lint percentage}}{\text{seed index}}$$

$$\text{Lint index} = \text{grams of lint per 100 seeds}$$

$$\text{Fibers per seed} = \frac{\text{lint index}/100}{\text{length} \times \text{uniformity index}/100} \times \frac{\text{micronaire}}{1{,}000{,}000}$$

$$\text{Fiber density} = \frac{\text{fibers per seed}}{35.74 + (\text{seed index} \times 6.59)}$$

Statistical Analyses

Seed protein, seed oil, seed per boll, lint index, fibers per seed, and fiber density were analyzed using a mixed-model analysis of variance and the PROC GLM module of SAS version 9.2 (SAS Institute, 2008). The RANDOM statement was included to identify random effects and make *F*-tests using the appropriate error term. Initially, individual year–location data were analyzed and homogeneity of variance tests were conducted to determine if a combined analysis of variance could be conducted for each trait. After confirming homogenous error variance, a combined analysis of variance was employed. For ease of analysis, the replicate and incomplete block terms were combined to form a single block term; the block term was considered a random effect. Each year–location trial was considered a single environment; environment was considered a random effect. Genotypes were considered fixed effects. Analysis of variance for agronomic and fiber quality traits were previously reported (Campbell et al., 2012).

The importance of G × E interactions was first evaluated by comparing each source of variation's contribution to the adjusted total sums of squares calculated from the combined analysis of variance. Total sums of squares were adjusted by removing sums of squares for blocks, replications, and pooled error. For both traits, the percentage sums of squares for environment, genotype, and G × E interactions was calculated relative to the adjusted total sums of squares.

To evaluate genotype stability and dissect G × E interactions, stability parameters (Eberhart and Russell, 1966) were estimated, with the 11 environments, by regressing genotype means on an environmental index. The environmental index was estimated as the mean of all genotypes at a specific environment minus the grand mean over all environments. The regression coefficient (b_i) and deviations from regression (s^2d) were used to compare environmental responses of genotypes. A *t*-test was conducted to determine if $b_i = 1$ for each genotype.

The relationship between seed protein and oil with agronomic and fiber quality traits was determined by estimating genotypic correlations. Genotypic correlations and their standard errors were calculated using SAS v.9.2 (SAS Institute, 2008) as described by Holland (2006).

RESULTS AND DISCUSSION

Genotype Performance, Genotype × Environment Interactions, and Genotype Stability

Significant variation was detected among genotypes for seed protein, seed oil, seed per boll, lint index, fibers per seed, and fiber density (Table 2). Figure 1 shows seed protein and oil histograms across genotypes. Both traits followed a normal distribution, and several genotypes were one or more least significant difference (LSD) values above or below the overall mean. Overall, eight genotypes (PD 7723, Earlistaple 7, PD 93034, PD 94042, FM 960BR, DP 491, DP 555BR, and DP 444BR) displayed high seed protein. PD-2, PD 5363, and PD 9363 displayed low seed protein. ST 5599BR had the highest seed oil, whereas PD 94042 had the lowest. DP 444BR also displayed high seed oil, and DP 555BR, PD 7723, and DP 491 displayed low seed oil.

Genotype × environment interactions were significant only for seed oil (Table 2). Figure 2 shows the percentage sums of squares remaining for environment, genotype, and G × E interactions for both seed protein and oil. On average, the environment sums of squares accounted for

Table 2. Analysis of variance for seed protein, seed oil, and yield component traits of Pee Dee germplasm lines and check cultivars combined over eleven location–year environments in North Carolina, South Carolina, Georgia, and Mississippi from 2004 to 2006.

		Mean squares					
Source	df	Percentage protein	Percentage oil	Seed per boll	Lint index	Fibers per seed	Fiber density
Environment (E)	10	622.8**	159.8**	695.4**	79.2**	2,127,209**	197.9**
Block (E)	667	13.0**	1.6**	17.5**	0.4**	6,343**	0.4**
Genotype (G)	86	4.5*	7.1**	26.2**	1.8**	12,586**	1.6**
G × E	848	3.4	0.9**	10.5	0.3	3,122	0.2
Pooled error	1402	3.4	0.6	9.9	0.3	3,525	0.2
Mean		22.2	19.3	31.9	6.7	632	5.8
CV		8.3	4.0	9.9	7.9	9	8.1

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

the largest amount of total variation for each trait (66% for seed protein and 55% for seed oil). The percentage sums of squares accounted for by genotype was 4% for seed protein and 21% for seed oil. Although not significant according to the analysis of variance presented in Table 2, G × E interactions accounted for 30% of the total variation for seed protein. Genotype × environment interactions accounted for 24% of the total variation for seed oil. Zeng et al. (2015) reported a large environmental effect on seed protein and oil while also reporting significant genotype × location interactions for both traits. Pettigrew and Dowd (2011, 2012, 2014) reported the effects of environmental influences and production practices on seed composition such as irrigation, fertilization, and planting date.

To better dissect the significant G × E interaction for seed oil, stability parameters were calculated for each genotype. Genotypes with slope (b_i) not equal to one were considered environmentally sensitive and impacted by G × E interactions (Eberhart and Russell, 1966). A genotype was considered stable when b_i was equal to one. Most of the genotypes had slopes not different than one, indicating they responded similarly across a low-to-high gradient of environmental indices for seed oil. However, a total of nine genotypes had slopes not equal to one (Fig. 3). Six of the nine genotypes had slopes >1 including AC 241, PD 5256, PD 6142, PD 9223, PD 93019, and PD 9364. The remaining three genotypes had slopes <1 including DP 491, PD 0683, and PD 0723. In total, these nine lines responded dissimilarly across a low-to-high gradient of environmental indices for seed oil. Figure 3 shows that G × E interactions for seed oil were a result of crossover interactions in which genotypes changed rank for seed oil across environments.

Genotypic Correlations

Genotypic correlations and their standard errors were calculated to determine the genetic relationships among seed composition traits (protein and oil) and agronomic and fiber quality traits (Table 3). Protein and oil were highly, negatively correlated (−0.88). Figure 4 provides a two-way graphical representation of seed protein and oil. PD 94042, DP 491, and DP 555BR had high protein and low oil. PD-2 displayed low protein and high oil. FM 960BR and DP 444BR had both high seed protein and oil. None of the genotypes displayed both low protein and low oil. This is not surprising, as poor seed viability and reduced seedling vigor have been associated with low seed protein and oil (Chapman et al., 2008; Snider et al., 2014). Although the negative genotypic correlation between protein and oil is a major impediment toward simultaneously increasing both traits, Figure 4 demonstrates the presence of high protein and oil genotypes.

Protein content was highly correlated with lint percentage (0.68) and moderately correlated with lint yield (0.42), micronaire (0.37), fineness (0.35), seed index (−0.43), lint index (0.48), and fiber density (0.49). Collectively, these component trait correlations indicate that increased lint yield is associated with increased seed protein. The negative correlation with seed index and positive correlations with lint index and fiber density are not surprising, as they highlight the allocation of seed protein towards developing fibers rather than seed constituents. Oil was moderately correlated with lint percentage (−0.28), micronaire (−0.32), strength (0.27), fineness (−0.26), seed index (0.42), seed per boll (−0.44), and fibers per seed (0.32). These findings indicate that increased seed oil can be favorably allocated to both seed and fiber sinks. Apparently, seed oil was allocated to both seed mass (seed index) and fiber initials on the seed surface.

Considering protein and oil together, correlations with lint percentage, micronaire, fineness, and seed index were moderate to high and in opposite directions. These data suggest the complexity of trait associations facing breeders interested in simultaneously improving seed protein, seed oil, and lint yield. Apparently, aside from micronaire and fineness, seed protein and oil do not appear to display strong associations with typically measured fiber quality traits. This indicates that selecting for high or low levels of seed protein and oil would not negatively impact most fiber quality traits (except micronaire and fineness).

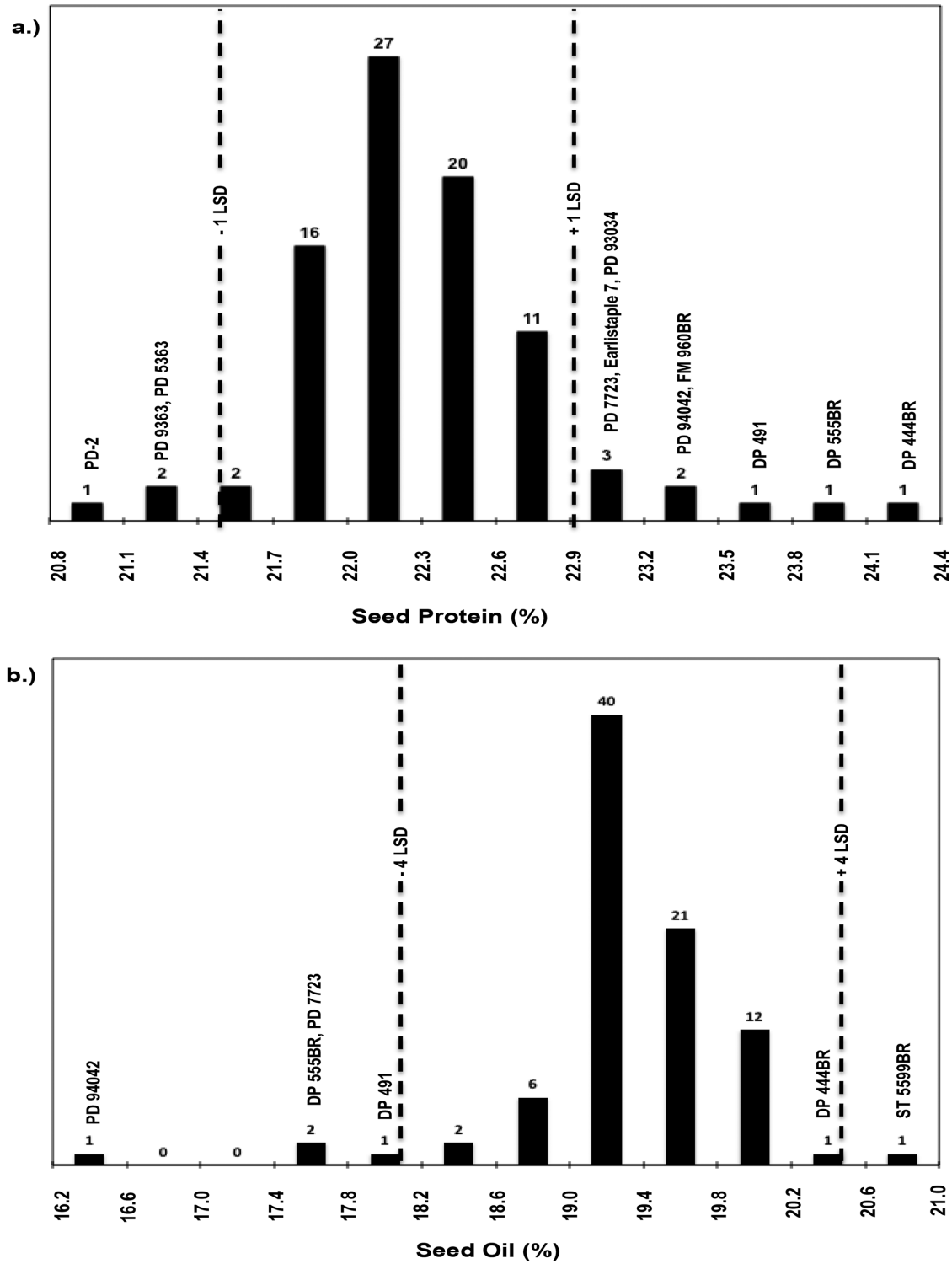

Fig. 1. Distribution of germplasm values including the least significant difference (LSD, $p < 0.05$) from the mean for (a) seed protein and (b) seed oil.

CONCLUSIONS

This study demonstrates the impact of G × E interactions and genotypic correlations among cotton seed traits and agronomic and fiber quality traits for a long-term germplasm enhancement program focused on simultaneously improving lint yield and fiber quality. To our knowledge, seed protein and oil were not used as selection criteria since the germplasm enhancement program began ca. 1935. Hence, this dataset offers the unique opportunity to assess seed composition traits void of any affects of direct selection practiced on those traits.

Overall, our results suggest genetic variation is present for both seed protein and oil among the Pee Dee germplasm. Similar to reports of previous studies, in this study, a large part of the total variation for seed protein and oil was due to environment. Genotype × environment

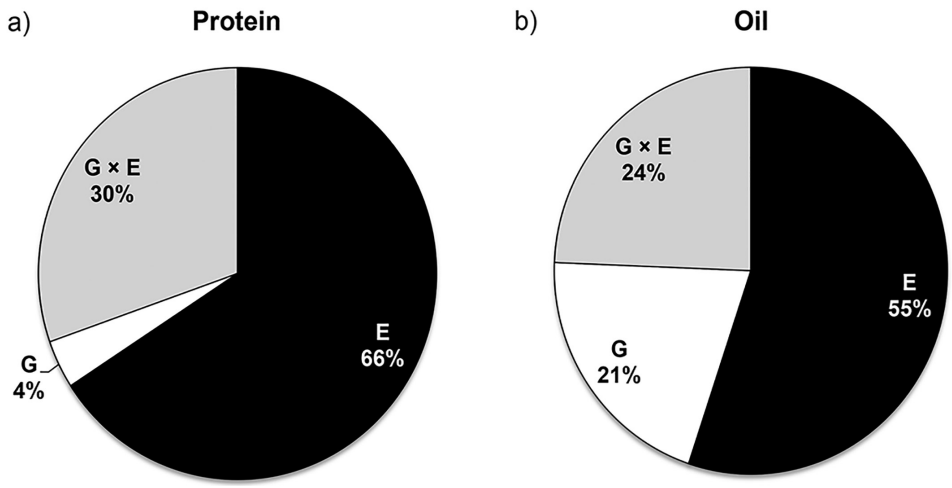

Fig. 2. Percentage of total variation explained by environment (E), genetics (G), and genotype × environment interactions for (a) seed protein and (b) seed oil.

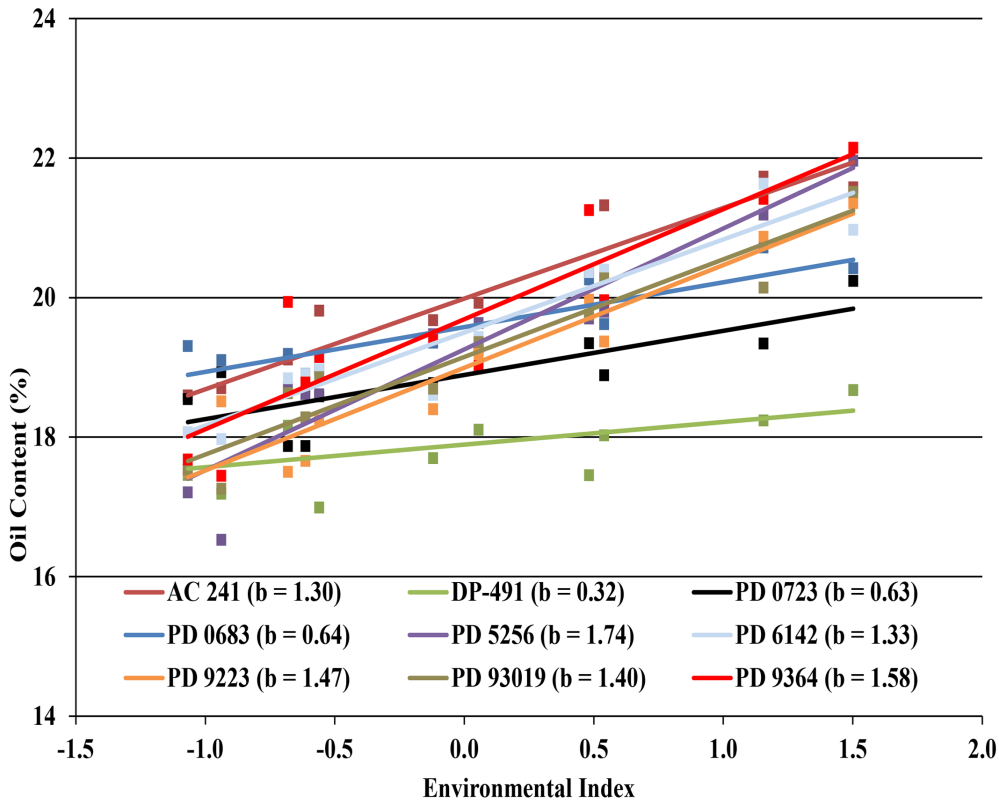

Fig. 3. Regression of oil content on environmental index for genotypes with slopes significantly different than one.

interactions were significant for seed oil and explained a sizeable portion of the total variation; however, G × E interactions were not significant for seed protein. Further, stability analysis allowed for the identification of specific genotypes that were particularly sensitive for seed oil content across the environments evaluated in this study.

To our knowledge, this study is one of the first to evaluate the relationships of seed protein and oil with agronomic and fiber quality traits using genotypic correlation analysis. The genotypic correlation between seed protein and oil was estimated as −0.88 compared with a phenotypic correlation of −0.22 (data not shown). This difference between correlation estimates is important to consider, as estimates of phenotypic correlations are useful for determining relationships between phenotypic values of different traits, but they do not reflect expected correlated changes that may occur because of selection on one of the traits (Holland, 2006). Nonetheless, the negative correlation between protein and oil was similar to reports from previous studies (Hinze et al., 2015; Zeng et al., 2015).

Results of the genotypic correlation analyses raise important biological questions relevant to future breeding

Table 3. Genotypic correlations and their standard errors for protein and oil with agronomic and fiber quality traits.

	Protein content	Oil content		Protein content	Oil content
Oil content	−0.88 ± 0.16	–	Boll weight	0.02 ± 0.22	0.08 ± 0.13
Seed cotton yield	0.10 ± 1.38	−0.12 ± 0.59	Seed index	−0.43 ± 0.18	0.42 ± 0.10
Lint percentage	0.68 ± 0.16	−0.28 ± 0.11	Seed per boll	0.24 ± 0.21	−0.44 ± 0.11
Lint yield	0.42 ± 0.21	−0.21 ± 0.13	Bolls square meter	0.13 ± 0.24	−0.16 ± 0.14
Micronaire	0.37 ± 0.19	−0.32 ± 0.11	Lint index	0.48 ± 0.19	0.12 ± 0.12
Length	−0.06 ± 0.19	0.15 ± 0.12	Fibers per seed	0.24 ± 0.21	0.32 ± 0.12
Uniformity index	−0.09 ± 0.21	0.12 ± 0.12	Fiber density	0.49 ± 0.18	−0.06 ± 0.12
Strength	−0.14 ± 0.19	0.27 ± 0.11			
Fineness	0.35 ± 0.21	−0.26 ± 0.12			

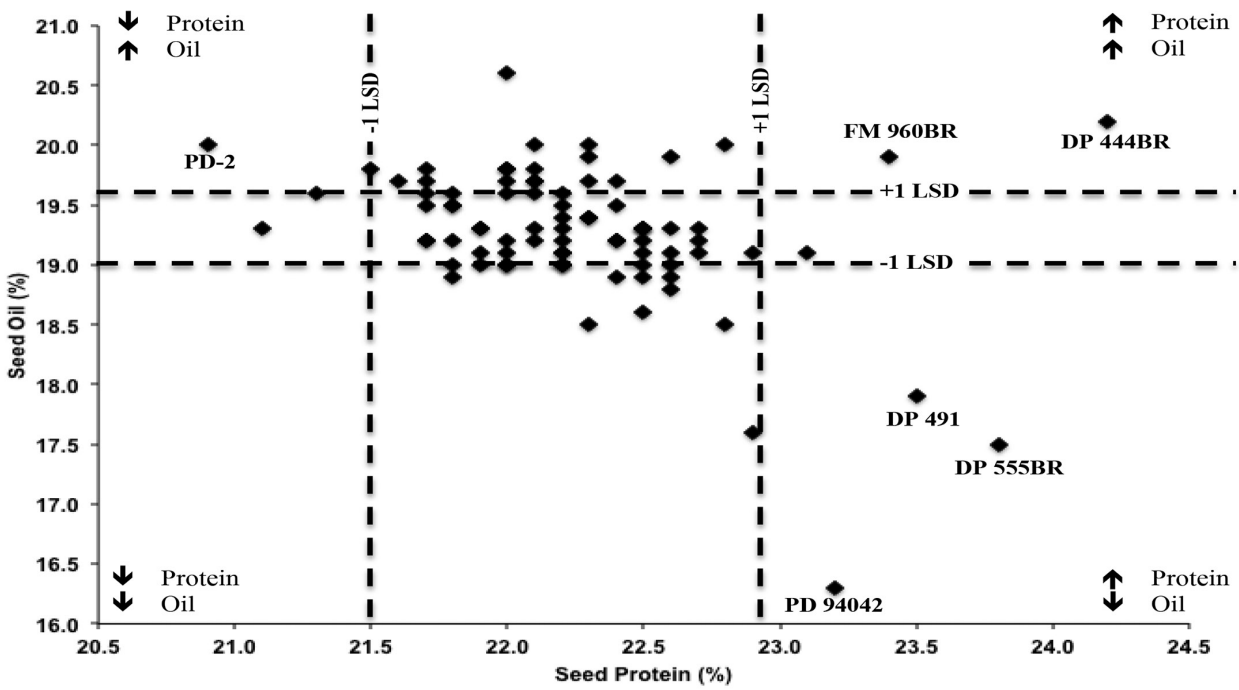

Fig. 4. Distribution of genotypes, including the least significant difference (LSD, $p < 0.05$) from the mean, for seed protein and oil.

efforts focused on improving both fiber and seed constituents for a future joint fiber and seed cotton production system. Although Pandey and Thejappa (1975) noted that any increase in oil content in cottonseed will result in a relative reduction in protein or vice versa, the current study suggests this trend does not always hold. In this study, we detected two high-protein, high-oil genotypes (FM 960BR and DP 444BR). Interestingly, both of these genotypes (used as checks in this study) were previously grown as commercial cultivars in the 2000s; they demonstrate the realization of commercially acceptable (lint yield and quality) high-protein, high-oil cultivars. It is not known whether seed protein or seed oil were used as selection criteria in their development.

Fiber traits such as lint percentage, lint yield, lint index, fibers per seed, and fiber density were positively correlated with seed protein; whereas seed size (seed index) was negatively correlated with seed protein. This suggests that increased seed protein may be critical for developing fiber sinks. On the other hand, negative correlations with seed oil were found for lint percentage and lint yield; whereas seed size (seed index) was positively correlated with seed oil. This suggests that increased seed oil is primarily important for developing seed sinks. This may support the hypothesis that lower seed oil results in greater availability of carbohydrate precursors for cellulose biosynthesis (Chapman et al., 2008). Pettigrew and Dowd (2012) also noted that larger seed mass genotypes might command more of the plant's photosynthesized carbon, thus leading to greater oil production in the seed. On the contrary, small seed mass genotypes may allocate more photosynthesized C toward developing fibers resulting in lower seed oil content (Pettigrew and Dowd, 2012).

Overall, the positive correlations of seed protein with lint percentage, lint yield, lint index, and fiber density indicate that seed protein and lint yield can be simultaneously improved. Moreover, with the exception of micronaire and fineness, fiber quality traits show little association with seed protein. However, the trait associations become more complex and difficult when also considering

seed oil. The negative association of seed oil with lint percentage, which has been documented as an important component of improving lint yield (Campbell et al., 2014; Campbell and Myers, 2015), indicates difficulty in simultaneously improving lint yield and seed oil. As noted in Kothari et al. (2015), future joint fiber and seed cotton breeding programs must understand and take into consideration these trait correlations and their impact on selection for increased protein, oil, lint yield, and fiber quality. Future studies should address the allocation of photosynthesized C available to the plant and its distribution to seed and fiber sinks. A better understanding of C allocation among these cotton sinks could allow for increases in fiber production and enhanced cottonseed quality.

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