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Genotypic and environmental effects on cottonseed oil, nitrogen, and gossypol contents in 18 years of regional high quality tests

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Abstract Determination of environmental influence on seed traits is critical for genetic improvement of seed quality in upland cotton (*Gossypium hirsutum* L.). The objective of this study was to analyze the relative contribution of environment and genotype (G) for seed oil, nitrogen (N), and gossypol contents using historical data from the regional high quality (RHQ) tests conducted from 1996 through 2013. The 18-year tests of RHQ were divided into six 3-year cycles with an average of about 20 genotypes and 7–10 testing locations (loc) in each cycle. Variance components of oil, N, and gossypol contents were estimated in each cycle and expressed as percentages of the total variance. Highly significant G $\times$ loc effects were identified for all seed quality traits in each cycle. For oil content, variance estimates of G to the total variance ranged from 20 to 57 % in different cycles. For N content, loc was the main source of variance with variance estimates of loc to the total variance ranging from 44 to 73 % in different cycles. In most cycles, loc and G were the main source of variance for free-gossypol content. For most seed quality traits, there was not a clear trend of changes among testing cycles for the variance estimates of G to the total variance. Broad-sense heritability for oil

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content, N content, and free-gossypol ranged from 0.79 to 0.96, 0.65 to 0.86, and 0.28 to 0.93, respectively. Highly significant G $\times$ loc interactions indicate that multiple location trials for testing seed quality traits are necessary. However, heritability estimates for these seed traits indicate stability across environments as well as the potential for genetic improvement. Significant reduction in seed index was observed in half of the testing cycles with a range of 10.4–9.52 within cycles. Correlation between seed index and oil content was positive with r values ranging from 0.23 to 0.77 in different cycles.

Keywords Cotton · Cotton seed traits · Gossypol · Nitrogen content · Oil content

Abbreviations

G	Genotype
loc	Location
N	Nitrogen
RHQ	Regional high quality

Introduction

Upland cotton (*Gossypium hirsutum* L.) is planted primarily to provide natural fibers for the textile industry. As a by-product of cotton production, cottonseed can be used in dairy cattle feeding because of its high feeding value with high fat and protein contents and neutral detergent fiber (Arieli 1998). Gossypol, a polyphenolic compound, is an anti-nutritive component in cottonseed that causes disorders in calves with undeveloped rumen and in all non-ruminants such as chicken (Bailey et al. 2000). Cottonseed can be processed to provide oil, hulls, and protein products, i.e., cottonseed meals, (Cherry and Leffler 1984). The purified cottonseed oil is considered a desirable vegetable oil for making trans-free products that can reduce unfavorable effects of vegetable oil on blood cholesterol (O'Brien and Wakelyn 2005). In addition, the crude oil refined from cottonseed is considered a potential biofuel resource (Karaosmanoglu et al. 1999; Meneghetti et al. 2007). In 2012, the U.S. produced 5.7 million tons of cottonseed, and less than half (2.5 million tons) were crushed to produce 363,000 tons of cottonseed oil and 1.1 million tons of cottonseed meal (National Cottonseed Products Association 2013). In the U.S., cottonseed ranked fourth behind soybean, corn, and canola, but ahead of sunflower seed and peanut in term of oil production in 2012 (USDA-ERS 2013).

Improving cottonseed compositions could increase cotton growers' profit and promote cotton production. According to a survey by the National Cotton Council (2012), secondary products from cottonseed contributed to 14–19 % of the total gross value in cotton production during 2009 and 2011. Because most value, at least 80 % of the gross, is from fibers, cotton breeders have focused on genetic improvement of lint yield and fiber quality. Lack of breeding efforts to improve oil and nutritional components in cottonseed may be a partial cause of the limited utilization of cottonseed.

Seed quality traits in cotton have been analyzed in a number of studies for their genetic basis and genetic variation among genotypes. It is known that two loci, Gl_2 and Gl_3, controll variation of gossypol in seeds and leaves of Upland cotton and plants of the genotype $gl_2gl_2gl_3gl_3$ are glandless, i.e., no gossypol, in seed and other plant parts (McMichael 1960; Calhoun 1997). The locus Gl_2 contributes three times as much additive variance of seed gossypol as the locus Gl_3 (Lee et al. 1968). Cotton plants of the genotype $gl_2gl_2Gl_3Gl_3$ had more glands in fruit surfaces than plants of the genotype $Gl_2Gl_2gl_3gl_3$ (Lee 1978). Calhoun (1997) identified a greater number of glands in hypocotyls in the monomeric line of gl_2GL_3, but not in the monomeric line of $\mathrm{Gl_2gl_3}$ and hypothesized that gland number in flower buds were controlled by allele of Gl_3. Gossypol, i.e., the total gossypol, consists of (+) and (−) isomers. Although the ratio of (+) to (−) isomers in Upland cotton seed is usually 3:2, most biological activities are caused by (−) gossypol (Stipanovic et al. 2005). Significant genetic variability of oil content and protein content in cotton genotypes was reported by Kohel et al. (1985) and Qayyum et al. (2010). Wu et al. (2010) determined significant additive genetic effects for protein content and dominant effects for oil content using F_3 hybrids of chromosome substitution lines and elite cultivars. This study suggests the potential of genetic improvement of oil and protein content in cotton seeds. Yu et al. (2012) detected 17 QTLs on 12 chromosomes for oil content, 22 QTLs on 12 chromosomes for protein content, and 3 QTLs on two chromosomes for gossypol content using a backcross inbred line population with a normal glanded genotype $Gl_2Gl_2Gl_3Gl_3$. Most recently, Faria et al. (2013) determined gain from selection for oil content in a F_5 generation to be 4.98 %.

Determination of environmental factors (E) and interactions between E and G is critical for genetic improvement of seed quality in cotton. The reports on environmental effects for oil content, protein content, and gossypol are scarce. According to a review by Meredith et al. (2012), a few early studies analyzed G, E, and G $\times$ E effects on oil and protein in cottonseed (Pope and Ware 1945; Turner et al. 1976). In these studies, large E effects were observed and G effect was only about 5 % for oil content. In a recent evaluation of the RHQ tests during 2001 and 2007 (Meredith et al. 2012), the effects of G for oil and protein expressed as a percentage of the total variance were 36.7 and 10.8, respectively while the effects of G for total gossypol and its (+) and (−) isomers expressed as a percentage of the total variance were 36, 47, and 29 %, respectively. An analysis of G, E, and G $\times$ E effects on seed quality traits in a long term breeding process can better determine the potential of genetic improvement. In this study, these effects were investigated using the historical data of the RHQ from 1996 through 2013. The objectives of this study were to (1) analyze the relative contribution of G and E to the total variance for seed quality traits during testing cycles, and (2) determine interrelationships of seed quality traits with lint yield and fiber quality.

Materials and methods

Data analyzed in this study were obtained from 7 to 10 testing locations in the RHQ conducted between 1996 and 2013. The goal of the RHQ program is to test high-quality cotton cultivars and elite strains across different locations in the U.S. Cotton Belt for lint yield and fiber quality to aid breeders in selecting superior genotypes across environments. The RHQ sites were located in five agric-climatic regions of the U.S. identified as Eastern, Delta, Central, Plains, and Western. Among these regions, Eastern includes locations of Belle Mina, AL, Florence, SC, Jackson, TN, and Tifton, GA; Delta includes locations of Clarkedale, AR, Keiser, AR, Portageville, MO, and Stoneville, MS; Central includes locations of Bossier City, LA and College Station, TX; Plains includes the location of Lubbock, TX; Western includes the location of Las Cruces, NM. These locations differed substantially in terms of geographic locations, temperature, and rainfall as described in a previous study (Zeng et al. 2014).

The same sets of cotton entries were evaluated at different locations each year in the RHQ tests, but different sets of entries were evaluated across years. Two to three cultivars in these sets were used as the national standard cultivars that were planted at all testing locations in a 3-year cycle. These tests from 1996 through 2013 were divided into six three-year cycles with seven to ten testing locations and 18 to 24 entries in each testing cycle (Table 1).

The field tests were described in a previous report (Meredith et al. 2012). At each location, the experimental design was a randomized complete block with four to six replications. Boll samples were obtained from 50 to 150 hand-picked bolls per plot from two replications. Plants were grown in two row plots, 12 m $\times$ 1 m. Boll samples from individual plots were ginned separately using a laboratory saw gin to determine lint percentage. Seed index was determined as the weight (g) per 100 fuzzy seeds. Lint samples of two replications from each location were submitted for measurements of fiber properties. Lint samples of 150 g each were submitted to StarLab, Inc. (Knoxville, TN) to measure fiber length and fiber strength using the high volume instrument (HVI) in the trials during 1996 and 2011. In 2012 and 2013, lint samples were submitted to Southern Regional Research Center of USDA-ARS (New Orleans, LA) for measurements of fiber properties using HVI.

Oil content was measured from fuzzy seeds by the American Oil Chemists' Society (AOCS) recommended practice Aa 4-38 (AOCS 2001). Nitrogen was measured from fuzzy seeds by the American and AOCS Method Ba 4-38 (AOCS 1976). The oil content and nitrogen content were expressed as percentages of the fuzzy seed mass. Gossypol was measured from de-hulled seeds which were dried in a forced-draft oven at 82 °C for 4 h. The method was the AOCS recommended practice Ba 8a-99 (AOCS 1998). The isomers of the (+) and (−) gossypol were determined by high performance liquid chromatography (HPLC). Seed traits of oil, nitrogen, and gossypol were measured by Eurofins Scientific[1] (Memphis, TN).

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation by the US Department of Agriculture.

Table 1 Cycles, standards, and locations of the regional high quality (RHQ) tests from 1996 through 2013

Cycles	Years	Testing locations[a]	Entries/year	Standards	PVP
1	1996–1998	Belle Mina, AL; Bossier City, LA; College Station, TX; Florence, SC; Clarkedale, AR; Portageville, MO; Stoneville, MS	19–20	Acala Maxxa Sure-Grow 125 LA 887	PVP 9000168 PVP 9400063 PVP 9100065
2	1999–2001	Belle Mina, AL; Bossier City, LA; College Station, TX; Florence, SC; Clarkedale, AR; Keiser, AR; Portageville, MO; Stoneville, MS; Tifton, GA	18–20	Acala GTO Maxxa Sure-Grow 747 NuCOTN 33B	PVP 9700072 PVP 9800118 PVP 9500109
3	2002–2004	Belle Mina, AL; Bossier City, LA; College Station, TX; Florence, SC; Clarkedale, AR; Lubbock, TX; Portageville, MO; Stoneville, MS; Tifton, GA	19–24	Acala 1517–99 DP 458 B/RR″ DP 555 BG/RR	PVP 200000181 PVP 98000206 PVP 200200047
4	2005–2007	Belle Mina, AL; Bossier City, LA; College Station, TX; Florence, SC; Keiser, AR; Las Cruces, NM; Lubbock, TX; Portageville, MO; Stoneville, MS	20–22	PHY 72 Acala ST 4892BR DP 555 BG/RR	PVP 200100115 PVP 200000253 PVP 200200047
5	2008–2010	Belle Mina, AL; Bossier City, LA; College Station, TX; Florence, SC; Jackson, TN; Keiser, AR; Las Cruces, NM; Lubbock, TX; Portageville, MO; Stoneville, MS	19–22	PHY 72 Acala DP 555 BG/RR	PVP 200100115 PVP 200200047
6	2011–2013	Belle Mina, AL; College Station, TX; Florence, SC; Keiser, AR; Las Cruces, NM; Lubbock, TX; Portageville, MO; Saint Joseph, LA; Stoneville, MS	20–21	PHY 375 WRF FM 9058F	 200700206

[a] In Cycle 2, the RHQ tests were conducted at Clarkedale, AR in 2000 and at Keiser, AR in 1999 and 2001

Variance components for lint yield and seed traits were estimated by PROC GLIMMIX in SAS (9.4) (SAS Institute 2013). Because testing entries were different among years within cycles, the variable year was considered as a blocking effect and the variables of location and genotype were nested in the variable year. The variable of replication was nested in location and year. The variables of year, location (year), genotype (year), location × genotype (year) and replication (location, year) were treated as random effects in the linear model.

Broad-sense heritability for lint yield and seed traits were estimated according to Fehr (1991) in the equation

$$H^2 = \sigma_g^2 \Big/ \left(\sigma_{year}^2 \Big/ ry + \sigma_{loc}^2 / rl + \sigma_{g\times loc}^2 \Big/ l + \sigma_g^2 \right)$$

where σ_g^2 is the variance component of genotype; σ_{year}^2 is the variance component of year; σ_{loc}^2 is the variance component of location; $\sigma_{g\times loc}^2$ is the variance component of G × loc; y is the number of year within cycles; r and l are the averaged numbers of replications and locations, respectively, across years within cycles. Means of lint yield and seed traits were calculated in each cycle and separated using Duncan multiple range test. Phenotypic correlations between yield traits and seed traits were calculated by PROC CORR in SAS using data averaged across locations and years within each testing cycle.

Results and discussion

The 18-year multiple location tests were divided into six 3-year cycles: Cycle 1, 1996–1998; Cycle 2, 1999–2001; Cycle 3, 2002–2004; Cycle 4, 2005–2007; Cycle 5, 2008–2010; Cycle 6, 2011–2013 (Table 1). Because the interests in this study were to determine the variance components of year, loc, and G and percentages of the total variance contributed by the respective components, these independent variables were included in the linear model as random effects. The variance components of G, year, loc, G × loc for seed quality traits were analyzed in each 3-year cycle (Table 2). Variance components of loc and G for all traits were highly significant in all cycles which indicate the significant difference among testing locations and testing entries for seed traits analyzed. Variance components of year for seed quality traits were significant in most cycles. For lint yield and seed yield, the random effect of year was zero in most cycles. In order to confirm these results, the variable of year for lint yield and seed yield was analyzed as a fixed effect and the F values were less than 1 in most cycles (data not shown). The variance component of G $\times$ loc was generally highly significant for yield traits and seed quality traits in all cycles.

For lint yield and seed yield, the loc was the main source of variance in all cycles (Table 2). Although the variance component of G was highly significant for yield in all cycles, the total variance contributed by G was low relative to the environmental effects. The variance estimates of G to the total variance ranged from 3.5 to 11 % for lint yield and from 0.7 to 10 % for seed yield in different cycles. In contrast, the variance components of loc to the total variance ranged from 57 to 72 % for lint yield and from 32 to 78 % for seed yield in different cycles. There was no obvious trend of changes among cycles for the percentages of the total variance contributed by G.

For the oil content, the effects of loc and G were nearly equal in cycles (Table 2). The variance estimates of G to the total variance ranged from 20 to 57 % in different cycles. The variance estimates of loc to the total variance ranged from and 18 to 47 % in different cycles while that for year ranged from 0.0 to 31 %.

For N content, the factor of loc was the main source of variance in all cycles. The variance estimates of loc to the total variance ranged from 44 to 73 % in different cycles while that for G ranged from 9.0 to 27 % within cycles. There was not a clear trend of changes in different cycles for the percentages of the total variance by G.

Year effect had small influence on plus-gossypol and minus-gossypol content in most cycles (Table 2). For the free-gossypol content, the factors of year and loc were the main source of variance in Cycle 1 and Cycle 2. In Cycle 3, Cycle 4, Cycle 5, and Cycle 6, the factors of loc and G were the main source of variance. There appears a trend of increase for the variance estimates of G to the total variance with 5–15 % in the early cycles before 2001 and 25–44 % in the late cycles after 2001 for the free-gossypol content.

Broad-sense heritability (H^2) was calculated for lint yield and seed quality traits from variance estimates (Table 3). Moderate H^2 (0.53–0.8) was observed for lint yield, and low to moderate H^2 (0.21–0.7) was observed for seed yield. Higher H^2 was observed for oil content ranging from 0.79 to 0.96 in different cycles. H^2 of N content ranged from 0.64 to 0.86 in different cycles. H^2 of plus-, minus-, and free-gossypol content ranged from 0.62 to 0.96, 0.7 to 0.88, and 0.28 to 0.93, respectively, in different cycles. Heritability estimates in oil, N, and gossypol content indicates potential of genetic improvement of these seed quality traits.

Lint yield and seed yield significantly increased from the early cycles to the late cycles (Table 4). This trend of yield increase coincided with the involvement of transgenic lines in the tests over cycles. In the first two cycles during 1996–1998 and 1999–2001, the number of transgenic lines among the testing entries ranged from 0 to 2. In the next four cycles during 2002 and 2013, the number of transgenic lines among the testing entries ranged from 3 to 13. There was a decrease of seed index from Cycle 1 (10.4 g) to the remaining cycles (9.52–10.1 g) although the reductions were not consistently significant among cycles. The non-significance among Cycle 1, Cycle 4, and Cycle 6 for seed index was due to a wide range of variation among testing locations (data not shown). There was not a clear trend of increase or decrease over cycles for the remaining seed traits although there were significant differences among cycles. These results are expected because breeding practices were mainly focused on lint yield and fiber quality during these testing cycles.

Determination of interrelationships of seed traits with yield and fiber properties will facilitate improving seed quality traits and other important traits simultaneously. Because homogeneity tests for variances among cycles were highly significant for lint yield and seed traits (data not shown), correlation coefficients of seed traits with yield and fiber properties were estimated within cycles (Table 5). Low correlations ($r = 0.31$–0.39) or non-significant correlations of lint yield and seed yield with oil content were observed. These results implied a non-existence of unfavorable linkages between yield traits and oil content and therefore, a possibility of simultaneous genetic improvement of these traits in breeding. However, the associations of yield traits with N

Table 2 Variance components for lint yield and seed traits in six cycles from 1996 through 2013 in the RHQ test

Source[a]	Lint yield × 10^{-3}	Seed yield × 10^{-4}	Oil content	N content × 10^3	Plus-gossypol × 10^3	Minus-gossypol × 10^3	Free-gossypol × 10^3
Cycle 1							
σ^2_{year}	6.1*	0.0	1.07*	0.39	–[b]	–	14***
$\sigma^2_{loc(year)}$	58***	8.7***	0.63***	32***	–	–	15**
$\sigma^2_{g(year)}$	7.4***	1.1***	1.1***	20***	–	–	7.4***
$\sigma^2_{loc\times g(year)}$	9.9***	1.1***	0.24***	7.0***	–	–	6.0***
$\sigma^2_{rep(year,loc)}$	5.4***	0.2***	0.04***	2.5***			1.1***
$\sigma^2_{residual}$	13	1.1	0.37	11	–	–	5.3
% year[c]	6.1	0.0	31	0.5			29
% loc (year)	58	78	18	44			31
% g (year)	7.4	10	32	27			15
Cycle 2							
σ^2_{year}	0.0	0.0	0.65**	7.9	0.0	0.0	112***
$\sigma^2_{loc(year)}$	83***	17***	2.0**	116**	4.8***	3.8***	28***
$\sigma^2_{g(year)}$	13***	1.4***	0.96***	16***	4.8***	2.4***	8.6***
$\sigma^2_{loc\times g(year)}$	7.9***	1.2***	0.33***	11***	8.5***	2.6***	14***
$\sigma^2_{rep(year,loc)}$	3.3***	0.37***	0.06***	3.5***	0.0	0.0	0.12***
$\sigma^2_{residual}$	13	3.2	0.81	23	3.7	2.0	10
% year	0.0	0.0	14	4.5	0.0	0.0	65
% loc (year)	69	73	42	65	22	35	16
% g (year)	11	6.0	20	9.0	22	22	5.0
Cycle 3							
σ^2_{year}	0.0	0.0	0.27**	12*	1.9*	1.6*	7.1**
$\sigma^2_{loc(year)}$	107***	22***	1.1***	114***	8.6***	6.7***	30***
$\sigma^2_{g(year)}$	11***	1.8***	1.5***	17*	17***	4.4***	34**
$\sigma^2_{loc\times g(year)}$	12***	2.4***	0.37***	10***	2.2***	1.0***	5.9***
$\sigma^2_{rep(year,loc)}$	7.2***	4.2***	0.62***	2.9***	0.14	0.07***	0.46***
$\sigma^2_{residual}$	18	4.0	0.67	26	3.8	1.8	9.5
% year	0.0	0.0	6.8	6.6	5.6	10	8.2
% loc (year)	69	64	28	63	25	43	35
% g (year)	7.1	5.2	38	9.1	51	28	39
Cycle 4							
σ^2_{year}	0.0	0.0	0.0	5.3*	2.3*	1.5*	8.1**
$\sigma^2_{loc(year)}$	144***	35***	2.3***	47***	11***	8.9***	39***
$\sigma^2_{g(year)}$	7.0***	0.80***	1.7***	13***	12***	5.6***	29***
$\sigma^2_{loc\times g(year)}$	13***	10***	0.35***	7.6***	1.9***	1.3***	4.5***
$\sigma^2_{rep(year,loc)}$	16***	2.2***	0.06***	2.5***	0.57***	0.19	1.5***
$\sigma^2_{residual}$	22	62	0.44	19	2.5	3.1	7.6

Table 2 continued

Source[a]	Lint yield $\times 10^{-3}$	Seed yield $\times 10^{-4}$	Oil content	N content $\times 10^3$	Plus-gossypol $\times 10^3$	Minus-gossypol $\times 10^3$	Free-gossypol $\times 10^3$
% year	–	–	0.0	5.6	7.7	6.7	9.0
% loc (year)	71	32	47	51	35	40	43
% g (year)	3.5	0.70	36	13	41	25	32
Cycle 5							
σ^2_{year}	0	27***	–	0.0	11***	9.9***	0.0
$\sigma^2_{loc(year)}$	164***	142***	–	80***	6.4***	7.9***	27***
$\sigma^2_{g(year)}$	16***	1.9***	–	15***	4.0***	5.3***	16***
$\sigma^2_{loc\times g(year)}$	18***	12***	–	14***	2.8***	2.6***	10***
$\sigma^2_{rep(year,loc)}$	5.1***	2.0	–	3.9***	0.30***	0.20***	1.0***
$\sigma^2_{residual}$	24	9.1	–	26	2.2	3.0	9.5
% of year	0	14		0.0	41	34	0.0-
% of loc (year)	72	73		58	24	27	42
% of g (year)	7.1	1.0		11	15	18	25
Cycle 6							
σ^2_{year}	0.0	0.0	0.0	0.0	0.02	0.42*	1.3*
$\sigma^2_{loc(year)}$	102***	20***	1.3***	141***	6.5***	4.5***	22***
$\sigma^2_{g(year)}$	7.8***	0.35***	3.0***	19***	14***	3.3***	26***
$\sigma^2_{loc\times g(year)}$	6.4***	1.7***	0.45***	9.7***	1.7***	0.98***	4.4***
$\sigma^2_{rep(year,loc)}$	0.97***	0.36***	0.05	3.3*	0.11***	0.12***	0.45***
$\sigma^2_{residual}$	58	11	0.49	21	1.9	0.83	4.8
% of year	0.0	0.0	0.0	0.0	0.10	4.1	2.2
% of loc (year)	57	60	24	73	28	45	37
% of g (year)	4.4	1.1	57	9.8	57	32	44

* Significant at $p \leq 0.05$

** Significant at $p \leq 0.01$

*** Significant at $p \leq 0.001$

[a] σ^2_{year}, the variance component of year; $\sigma^2_{loc(year)}$, the variance component of location; $\sigma^2_{g(year)}$, the variance component of genotype; $\sigma^2_{loc\times g(year)}$, variance component of location × genotype; $\sigma^2_{rep(year,loc)}$, the variance component of replication

[b] Data not available

[c] Percentage of the total variance including residual contributed by $\sigma^2_{year,}\sigma^2_{loc(year)}$ or $\sigma^2_{g(year)}$

content and free-gossypol content were generally unfavorable. Except for Cycle 5, lint yield and seed yield were negatively correlated with N content with r values ranging from −0.30 to −0.70. Lint yield and seed yield were positively correlated with free-gossypol content in four out of the six cycles with r values ranging from 0.23 to 0.39. Lint percentage was negatively correlated with oil content in three of the five cycles with r values ranging from −0.31 to −0.48. The unfavorable associations between yield traits and seed quality traits, N and free-gossypol content, implied unfavorable linkages or pleiotropic effects

Table 3 Broad-sense heritability of lint yield and seed traits in different cycles of the RHQ tests from 1996 through 2013

Cycles	Lint yield	Seed yield	Oil	N	Gossypol (+)	Gossypol (−)	Gossypol (total)
1	0.53	0.59	0.81	0.86	–[a]	–	0.63
2	0.80	0.70	0.79	0.65	0.79	0.83	0.28
3	0.71	0.67	0.91	0.64	0.94	0.85	0.91
4	0.56	0.28	0.91	0.74	0.91	0.86	0.88
5	0.73	0.21	–	0.74	0.62	0.70	0.87
6	0.69	0.32	0.96	0.68	0.96	0.88	0.93

[a] Data not available

Table 4 Means of lint yield and seed traits in different cycles of the RHQ tests from 1996 through 2013

Cycles	Lint yield (kg ha^{-1})	Seed yield (kg ha^{-1})	Lint percent (%)	Boll (wt g)	Seed index (g)	Oil (%)	N (%)	Plus-gossypol (%)	Minus-gossypol (%)	Free-gossypol (%)
1	969d	1220d	39.2ab	5.19a	10.4a	19.6ab	3.66a	–[a]	–	0.70d
2	1043c	1470c	39.3ab	4.67c	9.52b	19.6ab	3.40bc	0.755ab	0.524bc	0.960c
3	1240b	1525b	40.5ab	5.08ab	9.65b	19.2b	3.33c	0.787a	0.570ab	1.36a
4	1196b	1586c	40.0ab	4.94b	10.1a	19.3b	3.60ab	0.725b	0.496c	1.22b
5	1202b	1988ab	38.4b	5.16a	9.56b	–	3.32c	0.614d	0.599a	1.21b
6	1394a	2106a	41.4a	5.22a	10.1a	19.9a	3.39bc	0.672c	0.482c	1.16b

[a] Data not available

of genes controlling these traits. These unfavorable associations must be broken for simultaneous genetic improvement of both yield and seed quality traits. Seed index was positively correlated with oil content consistently among cycles with r values ranging from 0.23 to 0.77. The interrelationships between fiber properties and seed properties were not consistent in different cycles.

Analysis of the relative contribution of environment (E), G, and G × E will help foresee the effectiveness of selection and determine the number of environments which would be required to test for performance of cultivars. Meredith et al. (2012) reported that E, G, and G × E contributed 52, 37, and 11 %, respectively, of the total variance for plus-gossypol, minus-gossypol, and free-gossypol in the RHQ tests during 2001 and 2007. Due to the relative large effects of G in that study, they concluded that selection for gossypol traits could be effective in a small number of environments. In this study, the variance estimate for G to the total variance averaged over the plus-, the minus-, and the free-gossypol ranged from 15 to 45 % in different cycles. These results generally agree with the previous finding of Meredith et al. (2012). However, the current study employed data from a much longer duration of trials in the RHQ which allows analysis of variance components of E, G, and their interactions within different testing cycles. The results of highly significant interactions of G × loc for oil content, N content, and gossypol content indicate the requirement of multiple location trials for seed properties. Especially for N content, the environmental factor was the main source of variance in all cycles. Therefore, a sufficient number of environments are necessary for testing of seed quality traits. In most testing cycles, loc factor was a larger source of variance than the year factor for seed quality traits. These results suggest that testing of cultivars for seed traits could be conducted at multiple locations in 1 or 2 years in maximum. Another new finding in this study is the reduction of seed size in most testing cycles analyzed. This might have been caused by breeding practice for higher lint yield through extensive selection of high lint percentage which has been reported previously (Hoskinson and Stewart 1977). Although the seed crushing industry has complained about the problems of small seed for oil extraction, no reports in literature have confirmed this as a problem. The positive correlation between seed size and oil content was consistently identified in

Table 5 Pearson correlation coefficients between yield and seed traits and between fiber properties and seed traits in different cycles

	Oil	N	Gossypol
Cycle 1			
Lint yield	0.09	−0.01	0.27*
Seed yield	0.21	−0.30**	0.29**
Lint percentage	−0.31**	0.40***	0.18
Seed index	0.31**	0.30**	−0.01
Length	−0.43***	−0.33	0.11
Strength	−0.38***	−0.44***	−0.16
Cycle 2			
Lint yield	0.37**	−0.66***	0.31*
Seed yield	0.39**	−0.70***	0.39**
Lint percentage	−0.24	−0.31*	−0.28*
Seed index	0.17	0.60***	−0.72***
Length	0.11	0.20	0.02
Strength	−0.23	−0.15	0.74***
Cycle 3			
Lint yield	0.11	−0.40***	−0.07
Seed yield	0.31**	0.19	−0.16
Lint percentage	−0.39***	−0.04	0.16
Seed index	0.77***	−0.01	−0.17
Length	0.09	−0.08	−0.14
Strength	0.49***	−0.35**	0.16
Cycle 4			
Lint yield	−0.27*	−0.03	0.24*
Seed yield	−0.02	−0.20	0.22
Lint percentage	−0.48***	0.15	0.18
Seed index	0.61***	−0.30*	0.23
Length	0.34**	−0.07	−0.15
Strength	0.01	0.16	−0.41***
Cycle 5			
Lint yield	–[a]	0.40***	0.14
Seed yield	–	0.36***	−0.03
Lint percentage	–	−0.13	0.15
Seed index	–	−0.28**	0.17
Length	–	−0.17	0.21*
Strength	–	0.57***	−0.13
Cycle 6			
Lint yield	0.00	−0.48***	0.23*
Seed yield	0.03	−0.48***	0.05
Lint percentage	−0.16	−0.03	0.23*
Seed index	0.23*	0.00	−0.06
Length	0.23*	−0.68***	0.02
Strength	−0.13	−0.32**	−0.10

* Significant at $p \leq 0.05$

** Significant at $p \leq 0.01$

*** Significant at $p \leq 0.001$

[a] Data of oil content not available

different testing cycles in the current study. These results are consistent with the potential problem of small seeds in the seed crushing industry for oil extraction.

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