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Inheritance and Variability of (+)-Gossypol Percent in Seed from Cotton Hybrids and Correlation with Agronomic Traits

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ABSTRACT

Cottonseed cannot be used directly as a feed for non-ruminant animals because it contains the toxin gossypol. However, gossypol occurs in two forms (+)-gossypol and (-)-gossypol. Only the latter shows a high level of toxicity. The objective of our research was to develop breeding germplasm with a high percentage of (+)-gossypol in seed that will serve as a new source of plant protein that can be safely used as a feed for non-ruminant animals. To develop cottonseed that contains a high percent of (+)-gossypol, we determined the inheritance and variability of this trait in hybrids derived from U.S. accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 that exhibit the high percent (+)-gossypol seed trait and Uzbek varieties S-6524, S-6530 and S-6532, and lines L-10/04, and L-16/04. In field and greenhouse studies we found an intermediate inheritance of (+)-gossypol percent in petals and seeds among F_1 hybrids and positive and negative transgresses in F_2-F_3 hybrids. The (+)-gossypol contents in flower petals were positively correlated (r=0.69-0.91) with (+)-gossypol content in seed of F_2 hybrid populations. The percent (+)-gossypol in flower petals can be used as an efficient predictor of percent (+)-gossypol in cottonseed. Low correlations were observed between percent of (+)-gossypol in seed and total gossypol in seed, and with agronomic qualities including weight of 1,000 seeds, weight of bolls, seed yield, fiber length and fiber output. The low correlation values between percent of (+)-gossypol in seed with total gossypol and the other agronomic traits indicate that the high percent (+)-gossypol seed trait is inherited independently of these other agronomic traits. This should allow the selection of progenies with a high percent of (+)-gossypol in the seeds in combination with desirable agronomic traits.

Keywords: accessions, backcross progeny, cottonseed, *Gossypium hirsutum* L., flower petals, variety
Abbreviations: HPLC, High Performance Liquid Chromatography; **USDA**, United States Department of Agriculture

INTRODUCTION

Cottonseed is an important source of vegetable protein. The USDA Foreign Agricultural Service estimates that Uzbekistan produced 1.8 M metric tons of cottonseed in 2009 (Agro Stats, 2009). This represents an inexpensive source of protein that could be used to produce animal protein within Uzbekistan. However, the use of cottonseed protein in animal nutrition is limited because of the presence of gossypol which occurs naturally in the seed, and thus the seed is considered to be toxic to ruminant and non-ruminant animals.

Gossypol is biosynthesized by the free radical coupling of two molecules of hemigossypol to provide two enantiomers, (+)- and (-)-gossypol (Liu *et al*. 2008). Most of the toxicity of gossypol resides in the (-)-enantiomer (Wu *et al*. 1986). The ratio of (+)- to (-)-gossypol has been reported to vary between a high of 98:2 and a low of 31:69 in seeds (Cass *et al*. 1991; Stipanovic *et al*. 2005). Interestingly, a high percentage of (+)-gossypol has been found in the seed of several wild cotton species [(i.e., *Gossypium hirsutum* var. *marie-galante*, 97%; *G. mustelinum*, 94%; *G. anomalum*, 98%; *G. capitis-viridis*, 96%; and *G. gossypioides*, 94%); Stipanovic *et al*. 2005]. Hybrids prepared by crosses between commercial cottons grown in the U.S. and *G. hirsutum* var. *marie-galante* have been developed (Bell *et al.* 2000). Preliminary experiments established that cottonseed containing a high level of (+)-gossypol can replace soybean meal in broiler diets (Bailey *et al*. 2000).

Bell *et al*. (2000) indicated that two dominant genes are responsible for controlling (+)-gossypol expression in seed; the first is most highly expressed in flower petals and the second is equally expressed in seed. Their results suggest that analysis for (+)-gossypol in flower petals may be used to predict the level of (+)-gossypol in seeds, and thus accelerate the hybridization and selection process. Using this technique, we studied the inheritance, variability and correlation of (+)-gossypol in petals and seeds, and examined their relationship with some agronomic traits of U.S. cotton accessions, and F_1-F_3 hybrids developed from these accessions and local varieties.

The primary objective of our research was to determine the character of inheritance and variability of (+)-gossypol in seed and petals, and the correlation between important agronomic traits of ecologically remote hybrids with the ultimate goal of developing breeding germplasm with a high percentage of (+)-gossypol in seed that will serve as a new source of plant protein that can be safely used as a feed for poultry and other non-ruminant animals.

MATERIALS AND METHODS

Agronomics

The American accessions of cotton used in this study were BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 which exhibit a high percent of (+)-gossypol (Namazov *et al.* 2008) in seeds (93.9% and 93.3%, respectively) and petals (98.8% and 98.3%, respectively). These were crossed with Uzbekistan cultivars 'S-6524', 'S-6530', 'S-6532', and lines 'L-10/04', and 'L-16/04'. The F_1-F_3 hybrids were derived

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from these accessions, cultivars and lines. Individual plants were selected and flower petals and seed were analyzed to determine the total and percent (+)- and (-)-gossypol. Testing of different cotton breeding materials were conducted according to the methodology accepted in the Uzbek Scientific Research Institute of Cotton Breeding and Seed Production (Belousov *et al.* 1973). Statistical analyzes were conducted according to Dospekhov (1985). Correlation coefficients of hybrids also were determined according to the following formulae (Dospekhov 1985):

$$r = \frac{\sum XY - (\sum X \bullet \sum Y)/n}{\sqrt{(\sum X^2 - (\sum X)^2/n) \bullet (\sum Y^2 - (\sum Y)^2/n)}}$$

$$m = \sqrt{\frac{1-r^2}{n-2}}$$

$$tr = \frac{r}{Sr}$$

where: r – correlation coefficient; *X,Y* – data of quantitative traits; *m* – error of correlation coefficient; *n* – number of observations; *tr* – criteria of significances of correlation.

Coefficient of dominance was identified by the formula of Wright (Beil and Atkins 1965):

$h_p = (F_1\text{-}MP)/P\text{-}MP$

where: h_p – dominance; F_1 – medium value of F_1 hybrids; P – medium value of the best parent; MP – medium value of both parental forms.

Estimation of dominance of F_1 hybrids conducted according to the criteria:

Absence of dominance - $h_p = 0$
Partial dominance - $0 < h_p < 1$
Absolute dominance - $h_p = 1$
Heterosis- $h_p > 1$

Experiments were conducted in the greenhouse from winter to spring and in the field during spring to fall. The temperature in the greenhouse varied according to the following parameters: from planting to bud formation with daytime highs of 34-36°C and nighttime lows of 18-20°C; flowerings and fruiting with daytime highs of 26-28°C, and nighttime lows of 18-20°C; during the maturing phase the daytime highs were 34-36°C, and nighttime lows were 25-28°C. The experimental field plots have typical 'serozem' (grey-type) soils with small residual humus (up to 1%) and deep ground water level (7-8 m). The long-term precipitation per year averages 360 mm^3, which occurs mainly during the autumn-winter-spring period. Field plots were laid out in a complete randomized block design. Seeds of the initial parental forms and hybrids were planted at a depth of 4-5 cm, row spacing was 60 cm, spacing between the plants was 25 cm with a single plant per hole. During the growing season, the plants were irrigated 3-4 times on a regular basis as needed. Plants were fertilized as follows: N-240 kg/ha, P_2O_5-160 kg/ha, K_2O-120 kg/ha.

Gossypol analyses

The total and percent (+)- and (-)-gossypol was determined by HPLC analysis of their d-alaninol derivatives (Stipanovic *et al.* 2005). Twenty seeds from a cotton plant were manually de-hulled, and then ground into a meal with an agate mortar and pestle. The meal was then stored at –20°C until the analysis were carried out. The gossypol derivatization procedure was carried out using 19-21 mg of meal in a screw-cap test tube. The reaction mixture was diluted with 5.0 mL of acetonitrile and centrifuged for 3 to 5 min. A portion of the resulting clear supernatant was transferred to a vial for HPLC analysis.

HPLC analysis was performed within 8 h of obtaining the supernatant samples from either seed meal. The column was a GL Sciences Inertsil ODS-3 (3.0 × 150 mm). The mobile phase was an 80: 20 mixture of acetonitrile and 10 mM KH_2PO_4 (the latter adjusted to pH 3.0 with concentrated H_3PO_4) and was run at a flow rate of 0.60 mL/min. The chromatogram signal was monitored at 254 nm while UV spectra were recorded over 220-240 nm. The injection volume was 5 or 10 µL depending on the concentration of gossypol in the sample. The gossypol-amino-propanol complexes appear at 3.2 and 4.8 min for the (+)- and (-)- enantiomers, respectively. These peaks were integrated and the peak area percentages calculated. Since the UV spectra for the (+)- and (-)-gossypol adducts are identical, the ratio of the peak area percentages of these compounds are considered to be equivalent to the enantiomeric ratio.

Table 1 Inheritance of (+)-gossypol in flower petals and seeds in F_1 hybrids.

Cultivars and hybrids	Flower petals		Seeds	
	(+) gossypol	hp	(+) gossypol	hp
BC3S1-47-8-1-17	98.8		93.9	
BC3S1-1-6-3-15	98.3		93.3	
S-6524	76.5		69.5	
S-6530	72.2		67.2	
S-6532	79.8		70.9	
L-10/04	76.2		69.5	
L-16/04	70.6		60.1	
F1S-6524 x BC3S1-47-8-1-17	90.3	0.2	77.3	-0.4
F1S-6530 x BC3S1-47-8-1-17	89.0	0.3	76.8	-0.3
F1S-6532 x BC3S1-47-8-1-17	90.3	0.1	80.4	-0.2
F1L-10/04 x BC3S1-47-8-1-17	85.6	-0.2	76.1	-0.4
F1L-16/04 x BC3S1-47-8-1-17	88.0	0.2	80.6	0.2
F1S-6524 x BC3S1-1-6-3-15	90.7	0.3	62.4	-1.6
F1S-6530 x BC3S1-1-6-3-15	98.1	1.0	83.2	0.2
F1S-6532 x BC3S1-1-6-3-15	91.3	0.2	86.7	0.4
F1L-10/04 x BC3S1-1-6-3-15	87.7	0.04	78.0	-0.3
F1L-16/04 x BC3S1-1-6-3-15	89.0	0.3	69.3	-0.4
$LSD_{0.5}$	5.99		6.64	

RESULTS AND DISCUSSION

The U.S. accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 were found to have the highest percent of (+)-gossypol (>93%) in seeds (**Table 1**), while the percent (+)-gossypol in the local cultivars varied between 47.9-70.0 (Namazov *et al.* 2007, 2008; Uzbekov *et al.* 2009; Namazov *et al.* 2010). Among the local cultivars, the highest percentage of (+)-gossypol in seeds were in cultivars S-6524, S-6530 and S-6532. These cultivars and glandless lines L-10/04, and L-16/04 were hybridized with the U.S. accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15. The results of these crosses are shown in **Table 1**; the percentage of +)-gossypol in petals is inherited mainly as an intermediate type in F_1 hybrids with parameters of hp=-0.2 (F_1L-10/04 × BC_3S_1-47-8-1-17) and hp=1.0 (F_1S-6530 × BC_3S_1-1-6-3-15). The average percent of (+)-gossypol in petals ranged from 85.6 % (hybrids F_1L-10/04 × BC_3S_1-47-8-1-17 and F_1L-10/04 × BC_3S_1-1-6-3-15) up to 98% (F_1S-6530 × BC_3S_1-1-6-3-15). The level of (+)-gossypol in flower petals of the F_1 hybrids developed from crosses with the cultivars and glandless lines 'L-10/04' and 'L-16/04' were similar.

In regard to seed, the analysis of initial cultivars, lines and hybrids showed the tendency to have a lower average percent of (+)-gossypol in seed than in flower petals. The largest reduction in percent (+)-gossypol in seed compared to petals was observed in hybrids F_1S-6524 × BC_3S_1-1-6-3-15 and F_1L-16/04 × BC_3S_1-1-6-3-15 (28.3 and 19.7%, respectively). The inheritance of percent (+)-gossypol in seeds of hybrids was also of an intermediate type.

The percentage of (+)-and (-)-gossypol in flower petals of U.S. accessions was a comparatively stabile trait in the U.S. accessions, but it had wide variability in the F_2 hybrids (**Table 2**). Hybrid plants showed a distribution of (+)-gossypol percent in petals that varied between 60.0 and 96.0% depending on the hybrid pairs. The highest variability of this trait (16.2%) was observed in the hybrid F_2S-6530 × BC_3S_1-1-6-3-15, and lowest variability (4.9%) with the F_2S-6524 x BC_3S_1-1-6-3-15 with pair average contents of (+)-gossypol equal to 77.3 and 89.8%, respectively. The popula-

Table 2 Variation of percent (+)-gossypol in flower petals of U.S. accessions and F_2 hybrids.

Cultivars and hybrids	K = 5.0											M±m %	σ	V%
	n	60.1-64.0	64.1-68.0	68.1-72.0	72.1-76.0	76.1-80.0	80.1-84.0	84.1-88.0	88.1-92.0	92.1-96.0	>96.1			
BC_3S_1-47-8-1-17	20									2	18	98.05 ± 0.25	1.10	1.12
BC_3S_1-1-6-3-15	20										20	98.75 ± 0.10	0.44	0.45
F_2S-6524 x BC_3S_1-47-8-1-17	40			3	7	4	3	6	3	5	9	85.76 ± 1.54	9.73	11.34
F_2S-6530 x BC_3S_1-47-8-1-17	41	1	1	1	4	1	1	1	2	7	22	91.18 ± 1.59	10.21	11.20
F_2S-6532 x BC_3S_1-47-8-1-17	38	2	1	3	2	3	4	8	6	6	3	83.76 ± 1.63	10.06	12.01
F_2S-6524 x BC_3S_1-1-6-3-15	41					2	3	10	9	14	3	89.84 ± 0.76	4.88	5.43
F_2S-6530 x BC_3S_1-1-6-3-15	40	12	4	2		1	3	2	4	8	4	77.28 ± 2.56	16.16	20.91
F_2S-6532 x BC_3S_1-1-6-3-15	40			2	3	2	4	6	11	2	10	88.18 ± 1.31	8.31	9.43

Table 3 Variation of percent (+)-gossypol in seeds of U.S. accessions and F_2 hybrids.

Cultivars and hybrids	K= 5.0											M±m %	σ	V%
	n	60.1-64.0	64.1-68.0	68.1-72.0	72.1-76.0	76.1-80.0	80.1-84.0	84.1-88.0	88.1-92.0	92.1-96.0	>96.1-			
BC_3S_1-47-8-1-17	20								5	15		93.35 ± 0.30	1.35	1.44
BC_3S_1-1-6-3-15	20						1		3	16		93.25 ± 0.73	3.24	3.48
F_2S-6524 x BC_3S_1-47-8-1-17	40	11	2	3	3	4	7	1	2	7		76.15 ± 1.94	12.27	16.12
F_2S-6530 x BC_3S_1-47-8-1-17	41	4	2	1	2	2	1	1	13	15		85.41 ± 1.95	12.48	14.62
F_2S-6532 x BC_3S_1-47-8-1-17	38	9	4	7	3	2	2	1	5	5		74.92 ± 1.97	12.17	16.24
F_2S-6524 x BC_3S_1-1-6-3-15	41	8	7	7		3		2	9	5		74.84 ± 2.59	16.60	22.17
F_2S-6530 x BC_3S_1-1-6-3-15	40	1	3	5	7	6	3	3	3	8	1	80.66 ± 1.61	10.21	12.66
F_2S-6532 x BC_3S_1-1-6-3-15	40	3	4	5	5	5	2	4	12			79.13 ± 1.52	9.59	12.13

Table 4 Variation of percent (+)-gossypol in flower petals of F_3 hybrids.

Hybrids	K = 5.0								M±m %	σ	V%
	n	77.1-81.0	81.1-85.0	85.1-89.0	89.1-93.0	93.1-97.0	97.1-98.0	>98.0			
F_3S-6524 x BC_3S_1-47-8-1-17	59		1	1	5	16	12	24	96.29 ± 0.43	3.30	3.43
F_3S-6530 x BC_3S_1-47-8-1-17	114		5	3	8	22	30	46	96.63 ± 0.32	3.45	3.57
F_3S-6532 x BC_3S_1-47-8-1-17	18					1	5	12	98.06 ± 0.17	0.74	0.75
F_3S-6524 x BC_3S_1-1-6-3-15	17				1	2	2	12	97.78 ± 0.44	1.81	1.85
F_3S-6530 x BC_3S_1-1-6-3-15	39		1	2	2	20	9	5	95.54 ± 0.50	3.14	3.29
F_3S-6532 x BC_3S_1-1-6-3-15	12	1		1	2	5	-	3	92.96 ± 1.68	5.81	6.25

tion of hybrid F_2S-6530 × BC_3S_1-47-8-1-17 segregated to give comparatively more plants with a high percent (>96.1) of the trait in flower petals that affected the average percent of (+)-gossypol (91.2%) in petals. However, some F_2 hybrids showed a negative transgression with segregation of plants containing less (+)-gossypol in petals (60.1-64.0%) than in the parents.

Analysis of the seeds of the F_2 hybrids showed a larger variation of (+)-gossypol content in the segregating F_2 populations than in the initial U.S. accessions and local cultivars (**Table 3**). The U.S. accessions BC_3S_1-47-8-1-17 and BC_3S1-1-6-3-15 distributed into classes that varied between 80.1-84.0 to 92.1-96.0% with an average percentage of (+)-gossypol in seeds of 93.0% or higher. The percent of (+)-gossypol in seeds of F_2 plants varied from 53.0% up to 96.6%. Most of the plants with high (+)-gossypol in seeds were observed among hybrid populations derived from F_2S-6530 x BC_3S_1-47-8-1-17 and F_2S-6530 × BC_3S_1-1-6-3-15 which showed the highest mean percent of (+)-gossypol with respective values of 85.4 and 80.7%. The comparatively high levels of (+)-gossypol in the seed of hybrid F_2S-6530 × BC_3S_1-47-8-1-17 may explain the appearance of relative more plants (28 from a total of 41 plants) with the percentage of (+)-gossypol at 88% and higher. Among the segregating populations we found both negative (<60.1%) and positive (>96.1%) transgressive segregation of plants with respect to percent (+)-gossypol in seeds. We observed a low average value of (+)-gossypol (74.8%) in F_2S-6524 × BC_3S_1-1-6-3-15 crosses, though this hybrid showed a relative high dispersion (σ=16.6%) and wide variability (V=22.2%) of this trait. Low dispersions were observed in both U.S. accessions and with the hybrid F_2S-6532 × BC_3S_1-1-6-3-15 (1.35, 3.24 and 9.6%, respectively). Selected F_2 plants with a high percentage of (+)-gossypol in seeds were planted in the greenhouse for further analysis of (+)-gossypol in flower petals and seeds of F_3 plants.

Flower petals of F_3 progenies showed (**Table 4**) that selection of plants with the high percent of (+)-gossypol found in F_2 was an effective selection tool and all investigated F_3 plants had >80% (+)-gossypol in petals. The average level of (+)-gossypol in F_3 plants ranged from 93.1% (F_3S-6532 × BC_3S_1-1-6-3-15) up to 96.9% (F_3S-6532 × BC_3S_1-47-8-1-17). Plants of this generation were distributed into classes with percent (+)-gossypol in flower petals that ranged from 81.1-85.0% up to 99% or higher. In general, the hybrid plants appeared in the classes of 93.1-96.0% and 96.1-99.0%. A comparatively wide variability of (+)-gossypol in flower petals (σ = 5.1%) was observed in the hybrid F_3S-6532 × BC_3S_1-1-6-3-15 with the lowest percent of (+)-gossypol that averaged 93.1%. The percentages of (+)-gossypol in flower petals of the remaining hybrids were 95.1 and higher.

The average (+)-gossypol percent in seeds of F_3 hybrids was higher than in F_2 plants (**Table 5**). This was probably the result of selecting F_2 plants for selfing from classes with 87.1-90.0% and 90.1-93.0% (+)-gossypol in seed. Among the hybrids F_3S-6530 × BC_3S_1-47-8-1-17 there appeared negative recombinants with (+)-gossypol percent of 66.0% and lower. Hybrids F_3S-6524 × BC_3S_1-47-8-1-17; F_3S-6530 × BC_3S_1-47-8-1-17 and F_3S-6532 × BC_3S_1-47-8-1-17 showed an average (+)-gossypol level of 89.4, 89.2 and 89.0%, respectively. Dispersion of the (+)-gossypol trait in hybrids showed σ parameters that ranged from 3.83 to 5.41. The exception was F_3S-6524 × BC_3S_1-47-8-1-17, which showed high dispersion parameters (σ = 11.9%). These results show the comparative stability of the (+)-gossypol trait in the F_3 generation. All plants with a high percent of (+)-gossypol (>93%) were selected and planted in the greenhouse for seed increase and to study their agronomic traits.

Table 5 Variation of percent (+)-gossypol in seeds of F_3 hybrids.

Hybrids	**K= 5.0**											**M±m %**	**σ**	**V%**
	n	**66.1-69.0**	**69.1-72.0**	**72.1-75.0**	**75.1-78.0**	**78.1-81.0**	**81.1-84.0**	**84.1-87.0**	**87.1-9.0**	**90.1-93.0**	**93.1-96.0**			
F_3S-6524 x BC_3S_1-47-8-1-17	63				1	1	2	7	28	24		89.4 ± 1.9	11.9	13.3
F_3S-6530 x BC_3S_1-47-8-1-17	107	1	2	2	3	3	3	3	28	58	4	89.2 ± 0.5	5.33	5.98
F_3S-6532 x BC_3S_1-47-8-1-17	30				1	1	2	5	7	12	2	89.0 ± 0.8	4.28	4.81
F_3S-6524 x BC_3S_1-1-6-3-15	30					4	3	6	12	5		86.6 ± 0.7	3.83	4.42
F_3S-6530 x BC_3S_1-1-6-3-15	64		1	1	3	2	4	24	23	6		85.9 ± 0.5	4.24	4.93
F_3S-6532 x BC_3S_1-1-6-3-15	30		1	1	2	3	4	7	9	3		84.5 ± 0.9	5.41	6.41

Table 6 Correlations of (+)-gossypol percent in seeds with some traits of U.S. accessions and hybrids.

Cultivars and hybrids	**(+) Gossypol in flower petals**	**Total gossypol in flower petals**	**Weight of 1000 seeds**	**Weight of boll**	**Fiber length**
BC_3S_1-47-8-1-17	0.56	0.21	-0.43	-0.06	0.15
BC_3S_1-1-6-3-15	0.37	-0.03	-0.17	-0.33	0.23
F_2S-6524 x BC_3S_1-47-8-1-17	0.22	0.08	-0.25	0.21	0.12
F_3S-6524 x BC_3S_1-47-8-1-17	0.10	-0.26	-	-	-
F_2S-6530 x BC_3S_1-47-8-1-17	0.91	-0.03	-0.41	-0.21	-0.54
F_3S-6530 x BC_3S_1-47-8-1-17	0.61	0.36	-	-	-
F_2S-6532 x BC_3S_1-47-8-1-17	0.19	0.06	-0.45	-0.31	-0.07
F_3S-6532 x BC_3S_1-47-8-1-17	-0.41	0.25	-	-	-
F_2S-6524 x BC_3S_1-1-6-3-15	0.08	-0.17	-0.19	-0.29	0.11
F_3S-6524 x BC_3S_1-1-6-3-15	0.87	-0.02	-	-	-
F_2S-6530 x BC_3S_1-1-6-3-15	0.14	-0.35	-0.36	0.05	0.01
F_3S-6530 x BC_3S_1-1-6-3-15	-0.06	-0.25	-	-	-
F_2S-6532 x BC_3S_1-1-6-3-15	0.69	-0.01	-0.26	-0.15	0.06
F_3S-6532 x BC_3S_1-1-6-3-15	0.91	-0.70			

Correlation of (+)-gossypol and other traits

We investigated the correlations of seed (+)-gossypol percent with other agronomic traits to determine if the high (+)-gossypol seed trait could be combined with good agronomical parameters (**Table 6**). Our main focus was on the correlation of (+)-gossypol in flower petals and seeds, but we also determined correlations between seed (+)- and (-)-gossypol with such attributes as weight of 1,000 seeds, weight of bolls, productivity, fiber output and fiber length.

We found an average positive correlation between (+)-gossypol content in flower petals and seeds of the U.S. accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 ($r = 0.56$ and $r = 0.37$, respectively). F_2 hybrids showed a different correlation of these traits. For example, the correlation was positive and significant for hybrids F_2S-6530 × BC_3S_1-47-8-1-17 and for F_2S-6524 × BC_3S_1-1-6-3-15 ($r = 0.91$ and $r = 0.69$, respectively) but weak and not significant for F_2S-6524 × BC_3S_1-1-6-3-15 ($r = 0.08$). Thus, these correlations for (+)-gossypol indicate an independent inheritance of genes controlling (+)-gossypol content in petals and seeds that depends on genotypes and recombination of initial traits, characteristic for ecologic-geographically remote hybridization. We observed the tendency that plants exhibiting high (+)-gossypol in flower petals expressed a comparative high percentage in F_2 hybrids. This confirms the earlier report by Bell *et al.* (2000) concerning the possibility of using the percent of (+)-gossypol in flower petals for predicting parents that would provide a higher percent of (+)-gossypol in the seed of progeny, and use this to select plants for further breeding.

Analyses of F_3 plants showed correlations between percent of (+)-gossypol in flower petals and seeds ranged from $r = -0.38$ (F_3S-6532 × BC_3S_1-47-8-1-17) up to $r = 0.91$ (F_3S-6532 × BC_3S_1-1-6-3-15). The average percent of (+)-gossypol in flower petals and seeds in the first generation was comparatively higher (97.9 and 88.1%, respectively) than in the second generation (93.8 and 84.3%). This indicates that the coefficient of correlation does not always provide an efficient selecting tool to provide positive recombinants and the independent inheritance of genes controlling (+)-gossypol content in petals and seeds.

Our research showed that the correlation in the U.S. accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 between the total gossypol levels in petals and seeds was weak and negative (correlation parameters $r = -0.10$ and $r = -0.17$, respectively). However, hybrids developed through backcrossing with these accessions showed both negative and positive correlation values. For example, among the hybrids only F_2S-6530 × BC_3S_1-1-6-3-15 showed a negative and non-significant correlation between the total gossypol values in petals and seeds ($r = -0.28$). The remaining hybrids expressed low and positive coefficients of correlation between these traits ($r = 0.02$, F_2S-6532 × BC_3S_1-1-6-3-15 and $r = 0.26$, F_2S-6524 × BC_3S_1-1-6-3-15). Thus, the inheritance of total and (+)-gossypol in petals and seeds is inherited independently and probably depends on the genotypes of initial ecologic-geographic parents used for hybridization.

The correlation between percent (+)-gossypol in seed and weight of 1,000 seeds were negative for F_2 hybrids and U.S. accessions. The largest negative correlations ranged from $r = -0.36$ to $r = -0.45$ for hybrids F_2S-6532 x BC_3S_1-47-8-1-17, F_2S-6530 × BC_3S_1-47-8-1-17 and F_2S-6530 × BC_3S_1-1-6-3-15, and accession BC_3S_1-47-8-1-17. This indicates that seeds with a high percent of (+)-gossypol have smaller seed. However, there is a probability of occurrence of recombinants that are positive and have larger seed. Thus, a large population must be examined to identify those plants with larger seed.

A low level of total gossypol would also be advantageous for animal feeding. Thus, we also investigated the interrelation of the total gossypol with the weight of 1,000 seeds. Initial U.S. accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15, and also the hybrid combination F_2S-6532 × BC_3S_1-1-6-3-15 showed a weak positive correlation with respective values of $r = 0.11$, $r = 0.07$ and $r = 0.08$. However, this correlation is very weak and thus additional crosses are expected to exhibit a range of values for these attributes.

Correlation analysis of the high (+)-gossypol seed percent and boll weight of the U.S. accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 show a weak and average negative correlation ($r = -0.06$ and $r = -0.33$, respectively). In contrast, some hybrids showed a positive correlation (i.e., F_2S-6524 × BC_3S_1-47-8-1-17 and F_2S-6530 × BC_3S_1-1-6-3-15), while the remaining showed negative correlations; this is probably the result of initial genotypes on recombination of attributes.

The U.S. accessions show a weak positive correlation between total gossypol levels and boll weight (r = 0.29 and r = 0.25). Correlations of these traits in the hybrids were weak and negative (r = -0.10) or weak and positive (r = 0.19). This suggests it will be possible to develop large bolls with low levels of total seed gossypol.

We also examined the correlation between total and (+)-gossypol with the economically important parameters of fiber quality and strength. Correlations of total and (+)-gossypol with fiber length were weak and either negative or positive. This provided us the opportunity to select the positive recombinants. The U.S. accessions exhibited rather low parameters of fiber length 31.9 and 32.1 mm, respectively for BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 with the respective low positive correlations (r = 0.15 and r =0.23). However, the hybrids showed various values of length. Among the investigated hybrids the highest parameters of (+)-gossypol (85.3%) and lengths (32.8 mm) were found in the combination F_2S-6530 × BC_3S_1-47-8-1-17, but the correlation of these attributes was weakly negative. Notably, the combination F_2S-6530 × BC_3S_1-1-6-3-15, which had the highest fiber length (34.0 mm), showed a weak positive correlation with (+)-gossypol seed percent. As a whole, hybrids showed mostly positive correlations between (+)-gossypol with fiber length, though weakly, which bodes well for the possibility to select recombinants with good fiber strength.

CONCLUSION

This study demonstrates an intermediate inheritance of the high (+)-gossypol percent in petals and seeds among ecologically remote F_1 hybrids and wide variability in F_2-F_3 hybrids with positive and negative recombinant segregation. This indicates that the percent (+)-gossypol in flower petals can be used as an efficient predictor of the percent (+)-gossypol in seed. The low correlation values between the high percent of (+)-gossypol in seed with total gossypol and the other agronomic traits indicates the high percent (+)-gossypol seed trait is inherited independently of these other agronomic traits. This should allow the selection of progeny with a high percent of (+)-gossypol in the seeds in combination with desirable agronomic traits.

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