

# Genetic Aspects of Total and Percent (+)-Gossypol in Cotton Hybrids

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## Introduction

Cottonseed is a major source of vegetative oil in Uzbekistan and the meal (CSM) that results after the oil is removed provides a high protein concentrate that represents a product for feeding animals. However, most commercial Uzbek varieties have high levels of total gossypol in the seed; this limits its utilization. Toxicity of cotton seed gossypol to animals has been reported.

In the vegetative and generative parts of the cotton plant, gossypol occurs as a mixture of enantiomers, which are termed (+)- and (-)-gossypol (Huang et al., 1987). The ratio of these enantiomers varies from 98:2 to 31:69 in seed (Cass et al., 1991; Percy et al., 1996; Stipanovic et al., 2005).

*Gossypium hirsutum* var. *marie galante* was found to have high levels of (+)-gossypol in the seeds (Bell et al., 2000; Stipanovic et al., 2005). Bell et al. backcrossed this trait into the cultivar "Tamcot CAMD-E" and developed initial materials with more than 95% (+)-gossypol in seeds, some of them were transferred for scientific purposes to UzSRICCBSP.

The objective of our research is to transfer the high (+)-gossypol seed trait from American accessions into local Uzbek cultivars and develop breeding material with a high percent of (+)-gossypol in the seed and retain important agronomic traits essential for cotton production in Uzbekistan. Since our initial investigation (Namazov et al., 2007), we found that cultivars S-6524, S-6530, S-6532 and low gossypol lines L-10/04, L-16/04 have comparative high percent of (+)-gossypol in seed (76.5; 72.2; 79.8; 76.2 and 70.6%, respectively). These cultivars also have high fiber quality and good agronomic traits. All of these cultivars were involved in hybridization with the US accessions BC<sub>3</sub>S<sub>1</sub>-47-8-1-17 and #19 BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 and have been used to develop new materials with high (+)-gossypol in seeds.

## Results & Discussion

Results (Table 1) show that unlike Uzbek cultivars, the American accessions have over 93% (+)-gossypol in seeds. Among the Uzbek cultivars/lines, those with the highest levels of (+)-gossypol were S-6532 (79,8%), S-6524 (76,5%) and line L-10/04 (76,2%); the line with the lowest percent was L-16/04 (70,6%).

Analysis of inheritance of (+)-gossypol in seeds in ecologically remote hybrids of F<sub>1</sub> (Table 1) show an intermediate inheritance of the trait with deviation aside to the parent with the high (+)-gossypol. Among these hybrids, only in the F<sub>1</sub>S-6530 x BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 progeny did we find a positive dominance which provided 98.1% (+)-gossypol in the seed.

For total gossypol, among the initial parental forms, progeny from local cultivars S-6524 and S-6530 had a low percentage of total gossypol (1.0% and 1.1%, respectively); however, progeny from the glandless lines L-10/04 and L-16/04 had a relatively high percentage of total gossypol (2.0% and 2.4%, respectively). Our research shows that total gossypol is inherited mainly as negative heterosis, i.e. domination of parent with low level of total gossypol.

Analysis of percent (+)- and total gossypol in segregation F<sub>2</sub> hybrid seeds and initial USA accessions with a high level of (+)-gossypol showed large variations in these parameters. The highest mean percent of (+)-gossypol (85.4%), was found in the hybrid combination F<sub>2</sub>BC<sub>3</sub>S<sub>1</sub>-47-8-1-17 x C-6530. That is, relatively more plants (70% of total) exhibited >88% (+)-gossypol in seed (Figure 1). Also, 84.2 % of the F<sub>2</sub>L-10/04 x BC<sub>3</sub>S<sub>1</sub>-47-8-1-17 cross had a high average percent of (+)-gossypol, while 74.8% of the hybrid F<sub>2</sub>BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 x C-6524 had a comparatively low percentage of (+)-gossypol, though the progeny from the latter showed relative high trait dispersion (σ=16,6%) and wide variability (V=22,1%). The dispersion was much less for the initial US accessions BC<sub>3</sub>S<sub>1</sub>-47-8-1-17, BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 and hybrid F<sub>2</sub>BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 x C-6532 (1.35%; 3.24% and 9.6%, respectively). Furthermore, the percent (+)-gossypol in seeds were 4 % lower than the average percentage of this trait in flower petals; among the segregating combinations, the percent of (+)-gossypol in seed in F<sub>2</sub> plants varied from 53.0 % up to 96.6 %.

Negative (<53.0 %) and positive (+)-gossypol (>96.0%) transgressive segregation was observed for (+)-gossypol in seeds from F<sub>2</sub>L-10/04 x BC<sub>3</sub>S<sub>1</sub>-47-8-1-17. Among the segregated plants of F<sub>2</sub>L-16/04 x BC<sub>3</sub>S<sub>1</sub>-47-8-1-17 and F<sub>2</sub>S-6530 x BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 there also appeared recombinants with (+)-gossypol level >96.0%.

Selected F<sub>2</sub> plants with the a high percent of (+)-gossypol in seeds were planted in the greenhouse for further study. The analysis of flower petals from plants of the third generations (Figure-2) showed that selection of plants with the high percent (+)-gossypol at the F<sub>2</sub> level was efficacious in regards to the percent (+)-gossypol in seed with all investigated F<sub>3</sub> plants exhibiting >80 % (+)-gossypol in petals. Plants of this generation were distributed in classes with values for the (+)-gossypol seed attribute that varied from 81.1-85.0% up to >98%. The petals of plants of the hybrid combinations appeared in a class of >98.0%. The highest percent of (+)-gossypol in flower petals (98.1%) was found in combination BC<sub>3</sub>S<sub>1</sub>-47-8-1-17 X C-6530 and lower percentages (95.5%) were found in the combination BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 X C-6530. The combination BC<sub>3</sub>S<sub>1</sub>-47-8-1-17 X C-6530 and BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 X C-6530 had a rather high percent of (+)-gossypol in seeds, and showed comparatively low dispersion parameters, i.e. stability of trait.

Among the populations of F<sub>2</sub>-F<sub>3</sub> hybrids, we observed recombinants with high (+)-gossypol with total gossypol that varied from 0.47% up to 2.0%.

№	Cultivars and hybrids	(+)-gossypol	hp	Total gossypol	hp
1.	BC <sub>3</sub> S <sub>1</sub> -47-8-1-17	98.8	1.7		
2.	BC <sub>3</sub> S <sub>1</sub> -1-6-3-15	98.3	1.7		
3.	C-6524	76.5	1.0		
4.	C-6530	72.2	1.1		
5.	C-6532	79.8	1.9		
6.	L-10/04	76.2	2.0		
7.	L-16/04	70.6	2.4		
8.	F <sub>1</sub> C-6524 x BC <sub>3</sub> S <sub>1</sub> -47-8-1-17	90.3	+0.2	1.53	+0.5
9.	F <sub>1</sub> C-6530 x BC <sub>3</sub> S <sub>1</sub> -47-8-1-17	89.0	+0.3	1.22	-0.6
10.	F <sub>1</sub> C-6532 x BC <sub>3</sub> S <sub>1</sub> -47-8-1-17	90.3	+0.1	1.44	-3.6
11.	F <sub>1</sub> L-10/04 x BC <sub>3</sub> S <sub>1</sub> -47-8-1-17	85.6	-0.2	1.06	-5.3
12.	F <sub>1</sub> L-16/04 x BC <sub>3</sub> S <sub>1</sub> -47-8-1-17	88.0	+0.2	0.96	-6.2
13.	F <sub>1</sub> C-6524 x BC <sub>3</sub> S <sub>1</sub> -1-6-3-15	90.7	+0.3	0.49	-2.4
14.	F <sub>1</sub> C-6530 x BC <sub>3</sub> S <sub>1</sub> -1-6-3-15	98.1	+1.0	1.14	-0.9
15.	F <sub>1</sub> C-6532 x BC <sub>3</sub> S <sub>1</sub> -1-6-3-15	91.3	+0.2	0.79	-101
16.	F <sub>1</sub> L-10/04 x BC <sub>3</sub> S <sub>1</sub> -1-6-3-15	87.7	+0.04	1.0	-5.7
17.	F <sub>1</sub> L-16/04 x BC <sub>3</sub> S <sub>1</sub> -1-6-3-15	89.0	+0.3	0.98	-3.0

Table 1. Inheritance percentage of total and (+)-gossypol in seeds of cotton F<sub>1</sub> hybrids.

## Material and Methods

**Plant Breeding.** Alois A. Bell, USDA-ARS, provided accessions BC<sub>3</sub>S<sub>1</sub>-47-8-1-17 and #19 BC<sub>3</sub>S<sub>1</sub>-1-6-3-15 for crossing with Uzbek cultivars and lines. These accessions were crossed with the following Uzbek varieties and lines: S-6524, S-6530, S-6532, L-10/04 and L-16/04.

The experiments were conducted in the greenhouse and under field conditions. The temperature in greenhouse varied as follows: seedling to bud formation day: 34-36°C, night: 18-20°C; bud formation, flowerings and fruiting day: 26-28°C, night: 18-20°C; maturing phase day: 34-36°C, night: 25-28°C. The experimental plots have typical serozem soils with low quantity of humus (up to 1.0 %) and deep ground water level (7-8 m). Average precipitation is 360 mm<sup>3</sup> per year which comes mainly in the autumn-winter-spring period. During the period of mass fruiting of cotton precipitation is at a minimum and humidity is very low. Sowing was done by hand in hill spaced 0,25 m apart and 0,60 m between rows. During the growing period plants were irrigated 3-4 times and fertilizers were applied at the following rates: N-240 kg/ha, P<sub>2</sub>O<sub>5</sub>-160 kg/ha, K<sub>2</sub>O-120 kg/ha.

**Chemical analysis.** The total and the percent (+)-gossypol in the flowers and seeds of parental forms and in progeny derived from crosses with U.S. cultivars were evaluated by HPLC or CE by the methods of Hron et al. (1999) and Golubenko et al. (2009).

## Conclusions

1. We determined an intermediate level of inheritance of (+)-gossypol with deviation aside to the parent with the high (+) gossypol and negative heterosis of total gossypol, i.e. over domination of the parent with a low level of total gossypol in seeds at ecologically remote F<sub>1</sub> hybrids.
2. We observed hybrids with high (+)-gossypol and low total gossypol among the segregating F<sub>2</sub>-F<sub>3</sub> populations, which allow developing recombinants with comparative high percentage of (+)-gossypol in seeds from ecologically distinct hybridization for further selection process.
3. Among the populations of F<sub>2</sub>-F<sub>3</sub> hybrids we observed recombinants with high (+)-gossypol and a level of total gossypol that varied from 0.47% up to 2.0%.
4. Further investigations are indicated to develop breeding materials with a combination of high percentage of seed (+)-gossypol, an optimal level of total seed gossypol, appropriate agronomic traits, and progeny that reliably transfer these traits.

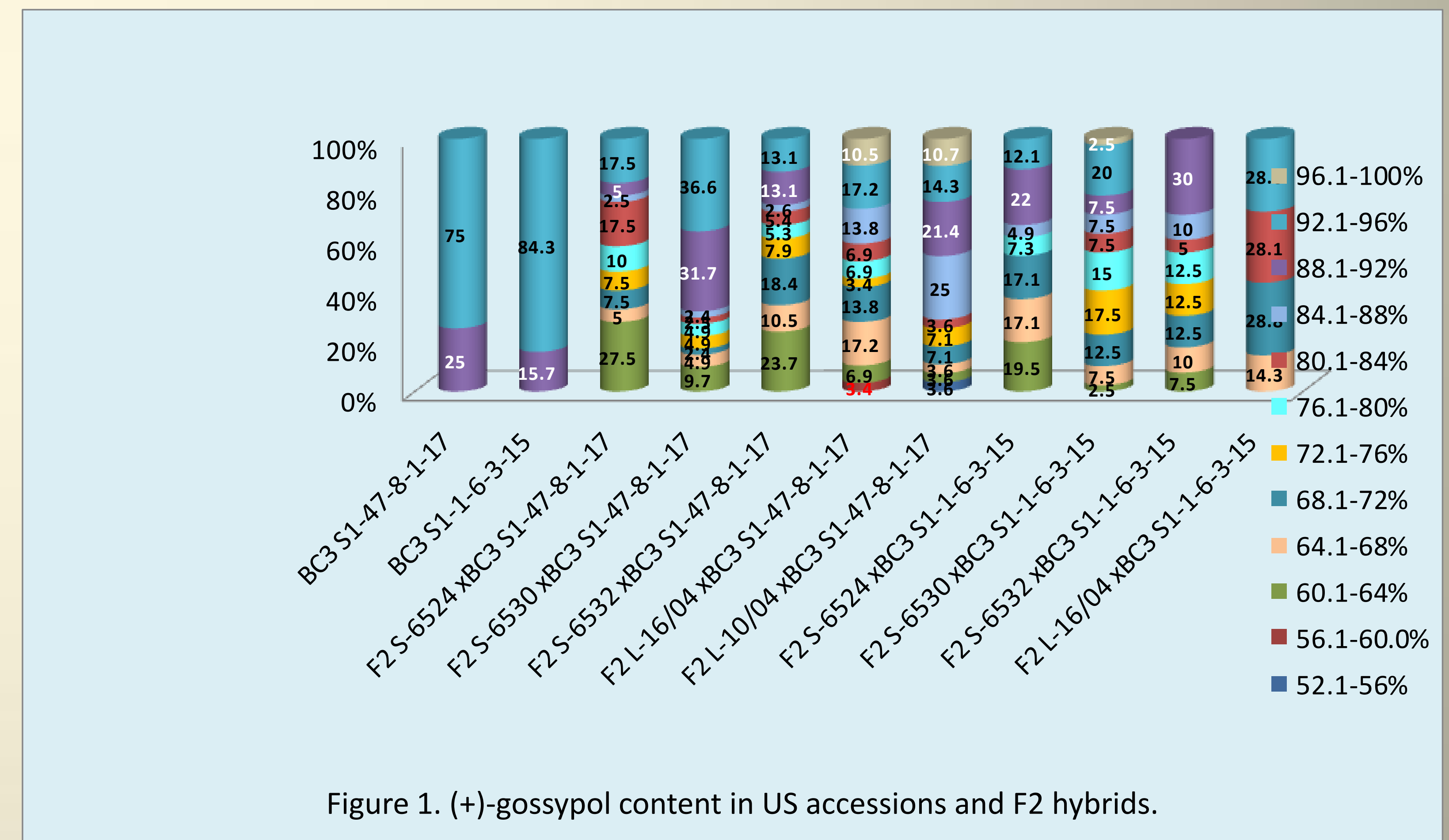


Figure 1. (+)-gossypol content in US accessions and F<sub>2</sub> hybrids.

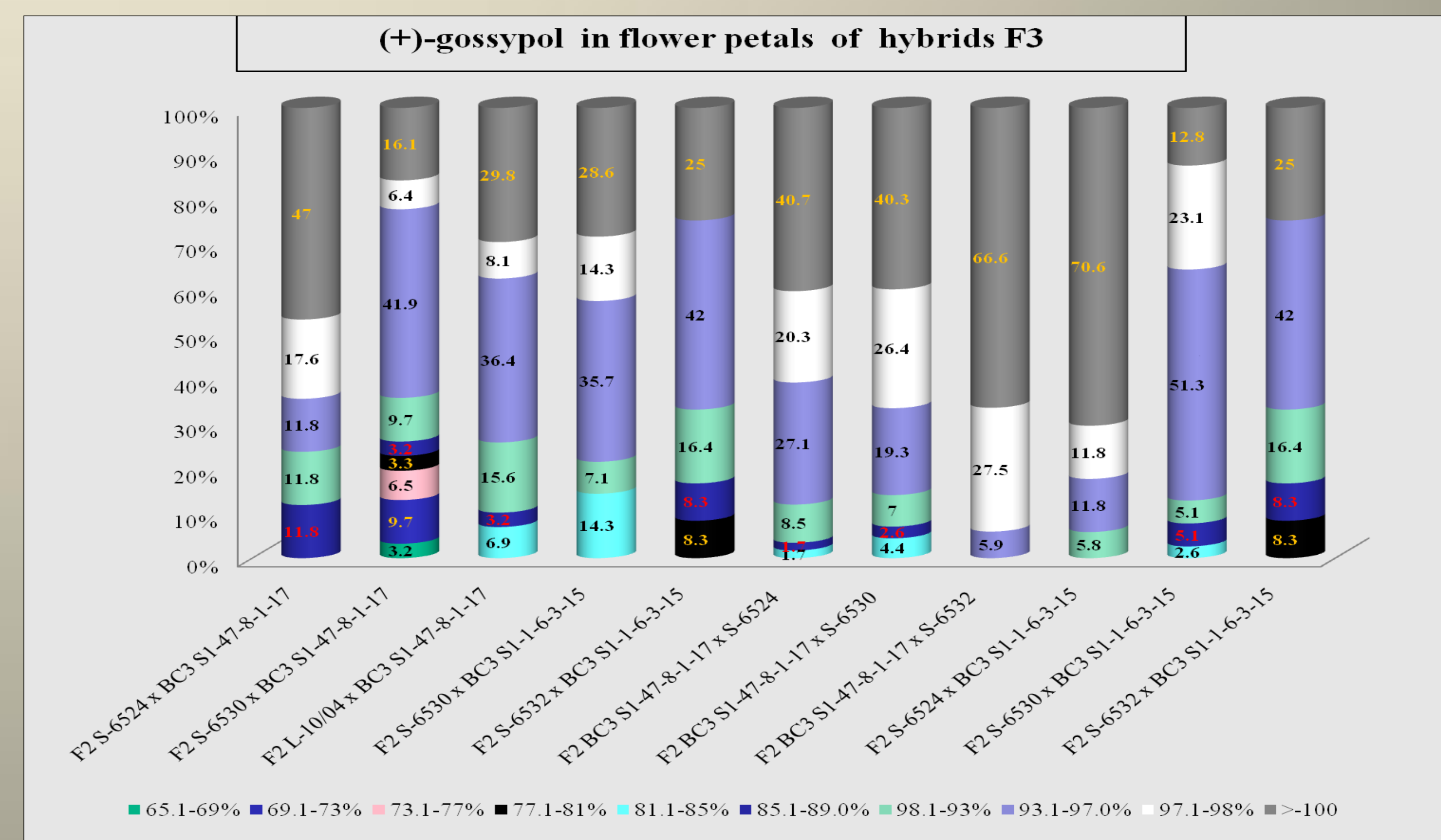


Figure 2. (+)-gossypol content of flower petals hybrids F<sub>3</sub>