

Tolerance of Genetically Distant Cotton Hybrids to Cotton Bollworm (*Helicoverpa Armigera*) in Depending of the Level of (+)- Gossypol in Seeds

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Abstract- In this paper presents obtained data on the natural background about on tolerance of senior generations of cotton hybrids to cotton bollworm (*Helicoverpa armigera*) with different levels of (+) - gossypol in seeds. On the basis was obtained results, revealed that the level of (+) - gossypol in seeds does not significantly resistance to cotton bollworm, i.e. Confirmed the possibility of selecting new broods and lines of cotton with different levels (+) - gossypol and tolerance to *Helicoverpa armigera*. Results of researches of comparative resistance to *Helicoverpa armigera* among hybrids of different generations in petri dishes, it was established that progenies with a different levels of (+) - gossypol, a definite pattern is observed for affection. Genotypes with a low level of (+)- gossypol are affected by *Helicoverpa armigera* to a certain extent less than hybrids with high (+)-gossypol level. Although, the incidence of the initial accession BC₃S₁-1-6-3-15 of US with a high level of gossypol does not preclude the possibility of developing of resistant genotypes with a high level of (+)-gossypol, which requires additional researches in this direction.

Key words: cotton, gossypol, damage, generation, *Helicoverpa armigera*, hybridization, bolls, resistance, seeds.

Scientific area: Life Science, Plant science.

I. INTRODUCTION

Cottonseed provides a high quality protein that is currently under utilized because of the presence of a toxic compound called gossypol. Gossypol is biosynthesized by the free radical coupling of two molecules of hemigossypol. During this coupling reaction, two optically active enantiomers are formed. One of these is referred to as (+)-gossypol and the other as (-)-gossypol. In cotton (*Gossypium*) the ratio of (+)- to (-)-gossypol can vary from 98:2 to 31:69 in seed (Cass et al., 1991; Percy et al., 1996). Within the genus *Gossypium*, were found accessions from several species that have >92% (+)-gossypol in the seed. These include *G.mustelinum*, *G. anomalum*, and *G.gossypoides* (Stipanovic et al., 2005). Cass et al. (1991) first reported that *G. barbadense* had an excess of the (-)-enantiomer in the seed. It was found that some accessions of *G.darwinii*, *G.sturtianum*, *G.harknessii*, *G.longicalyx* and *G.costulatum* also produce an excess of (-)-gossypol in the seed (Stipanovic et al., 2005) [1].

Gossypol, which is found in pigment glands, was identified in 1915 (Withers and Carruth) as the toxic component in cottonseed. Bottger et al. (1964) showed that gossypol was toxic to cotton aphids, lygus bugs, salt-marsh caterpillars, thurberia weevils, and bollworms. Jenkins et al. (1966) showed that the grape colaspis and leaf beetle preferred feeding on glandless compared to glanded cotton cultivars. Gossypol inhibits the growth and development of many insect pests including the beet armyworm, bollworm, cabbage looper and the salt-marsh caterpillar (Bottger and Patana, 1966) [2].

Terpenoids that are biosynthetically related to gossypol also occur in the foliage. Besides gossypol, these terpenes include hemigossypolone and heliocides H₁, H₂, H₃ and H₄ (Gray et al., 1976; Stipanovic et al., 1977a; 1977b; 1978a; 1978b). These compounds are also involved in insect resistance. In an artificial diet study, Stipanovic and collaborators established the effective dosage that is required to reduce growth of the tobacco budworm larvae (*Heliothis virescens* (F.)) by 50% (ED₅₀) (Stipanovic et al., 1977a; Williams et al., 1987). Although gossypol is toxic, field studies show that the levels of heliocides and hemigossypolone correlate better with resistance than gossypol (Hedin et al., 1992; Jenkins, 1995) [3].

Cotton bollworm (*Helicoverpa armigera*), the main rodent pests. In particular, the elements of the cotton bollworm moth is a great loss, he is the glory and the loss of knots, dry or completely fall (Rashidov, 1982) [4]. Zhang Jhin (1985) study carried out at the United States that the resistance of cotton bollworm and sucking pests in addition to the gossypol, along with other terpenoid compounds, flavonoids, fatty acids and tannin material is provided [5]. Thus, gossypol does not appear to be the primary source of chemical resistance to *Helicoverpa* in cotton foliage.

Extensive tests on the toxicity of (+)- and (-)-gossypol to insect pests are not available. Chinese scientists report a study with *Helicoverpa armigera* in which larvae were raised on artificial diets containing either (+)- or (-)-gossypol from the 3rd instar through pupation to the moth stage (Yang et al., 1999) [6]. The larvae raised on the (+)-gossypol diet matured more slowly, and percent survival to the adult was lower. Stipanovic et al. (2006) showed that racemic, (+)- and (-)-gossypol were equally effective at reducing days-to-pupation, pupal weights and survival of *Heliothis zea*. Stipanovic et al. (2006) showed that the leaves and stems of *G. hirsutum marie galante* that exhibit high levels of

(+)-gossypol in the seed falls within the normal 3:2 range. Thus, regulation of the (+)- to (-)-gossypol ratio in foliage appears to be under separate regulation from that in seed. Therefore, we expect the plants exhibiting a high level of (+)-gossypol in the seed to behave normally with respect to susceptibility to phytophagous insects [7].

More than a decade ago, scientists at the USDA, Cotton Pathology Research Unit, Southern Plains Agricultural Research Center in College Station, Texas began a backcross breeding program to incorporate the high (+)-gossypol trait from moco cotton into agronomically acceptable cotton cultivars. This backcross breeding program shows two dominant genes are responsible for the high percentages of (+)-gossypol in moco cottonseed (Bell et al., 2000).

II. MATERIALS AND METHODS

Materials of researches were served medium fiber kind (*G. hirsutum* L.) and thin fiber kind (*G. barbadense* L.) cotton cultivars, lines and accessions of the US with high level of (+) gossypol, as well as hybrids obtained with their participation. The degree of damage by bollworm (*Helicoverpa armigera*) and its butterfly in artificial (isolated background) and field conditions was determined by the method of L.P. Shvetsova, E. Em (1991); assessment of the degree of damage to the bollworm in different hybrid generations in the laboratory according to N.Sattarov and I.Amanturdiyev (2014); determination of the level of (+) - gossypol on an HPLC instrument according to Hron et al. (1999); mathematical, statistical (MS Excel) and carried out according to B.P. Dospekhov (1985). Research work was carried out in 2016-2018, in particular, field and artificial experiments were carried out in the laboratory "Genetics and cytology of cotton" of the Research Institute of breeding and seed production, at the National University of Uzbekistan, and laboratory experiments at the Institute for Plant Protection.

By joint researches of USDA and Uzbek scientists under KX-EA-KX-2018-65 and Uzb2-31001-TA-08 projects toward developing of cultivars that exhibit a high ratio of (+)-gossypol in the seed by using of Uzbek cultivars and American lines has shown that: it is possibility to transfer the high (+)-gossypol seed trait from U.S. cotton accessions into Uzbek cultivars; these Uzbek cotton hybrids developed to date have >93% (+)-gossypol. Thus, it is possible to introduce the high (+)-gossypol seed trait into Uzbek cotton lines to provide plants with agronomic traits suitable for growing in Uzbekistan; these plants are no more susceptible to insect pests and pathogens than normal cotton cultivars.

In the current years of researches investigate the character of inheritance and variability of insect resistance and evaluation of developed progenies for resistance to *Helicoverpa armigera* in artificial conditions, the total of gossypol and (+)-gossypol in seeds and other important parameters of various hybrid generations of cotton [8-10].

III. RESULTS AND DISCUSSIONS

It is known that hybridization is a major source of genetic variation in populations. The resulting random chromosome segregation at meiosis, involving diverse

parental forms, leads to the recombination of genetic materials. In the selection of crops, including cotton, different methods of interspecific hybridization are exploited to produce a wide variability in economically valuable traits and to develop nonexistent combinations.

The goal of our researches is to develop new initial materials with a high level of (+) - gossypol in seeds and tolerant to bollworm based on the study of tolerance to bollworm hybrids obtained with the participation of cotton cultivars with different levels and forms of gossypol in seeds. Based on the above researches, we will analyse in this paper some research results regarding to *Helicoverpa armigera* tolerance of progenies developed with the participation of genetically distant hybridization.

The results of the first observation of the first set of hybrids under isolated conditions showed that the F₄BC₃S₁-47-8-1-17 x S-6532 with the level of (+) - gossypol 91.4% was not affected by *Helicoverpa armigera* (Table 1). Among the studied only in the combination F₄BC₃S₁-47-8-1-17 x S-6524 with a high level of (+) - gossypol (91.2%), a relatively high affection with *Helicoverpa armigera* was observed. In the remaining cases, there was no significant. The second observation of the damage of plants with a different level of (+)-gossypol in seeds showed resistance of high (+) - gossypol (91.4%) progenies to *Helicoverpa armigera*. It should be noted that all combinations with the level of (+)-gossypol over 91%, in contrast to the hybrids of the progenies with a low level of (+) - gossypol, were less affected (5 and lower percentages). The results of the 3-observation of the damage by *Helicoverpa armigera* showed that the pest is being moved in depending of the level of (+)-gossypol. In other words, among the hybrids of this generation with a high level of trait, only in two cases, i.e. in the combinations F₄BC₃S₁-47-8-1-17 x S-6524 and F₄BC₃S₁-1-6-3-15 x S-6524, the damage were observed at the level 15% and 10%, respectively. The remaining progenies were affected up to 5 %. However, the percentage of affection with *Helicoverpa armigera* increased (except for the combination F₄BC₃S₁-47-8-1-17 x S-6530) at hybrids with a relatively low level of (+)-gossypol.

The results of first observation of hybrids F₆ under the conditions of a greenhouse, showed that all combinations with both a high (above 90%) and low (below 70%) level of (+) - gossypol were affected by *Helicoverpa armigera* (Table 2). Among the studied, the hybrid F₆BC₃S₁-1-6-3-15 x S-6530 with a relatively high content of (+) - gossypol (90.8%), was less affected (6%). The results of monitoring of susceptibility to *Helicoverpa armigera* at the first observation showed that the pest is populated depending on the level of (+)-gossypol. In other words, among F₆ hybrids with a high level of (+) - gossypol, only in two cases, i.e. at F₆L-10/04 x BC₃S₁-47-8-1-17 and F₆BC₃S₁-1-6-3-15 x S-6524, a high degree of susceptibility were observed (23% and 21%, respectively). It can be noted that hybrids of this generation with a high level of (+) - gossypol were affected by *H. armigera* from 6% (F₆BC₃S₁-1-6-3-15 x S-6530) up to 17% (F₆BC₃S₁-47-8-1-17 x S-6532). Thus, it was found that the studied progenies F₆ with high (+)-gossypol were relatively resistant to the cotton bollworm

in comparing to progenies with low level (+)-gossypol.
These results indicate that the level of (+)-gossypol does not significantly affect on resistance to *H.armigera*.

Table 1. Dynamics of affection of cotton hybrids F₄ by *Helicoverpa armigera*

№	Hybrids-combinations	(+)-gossypol, %	Total gossypol, %	1-Observation			2-Observation			3-Observation Affected, %		
				Density of population, 10 plants, n			Affected, %	Density of population, 10 plants, n			Affected, %	
				Eggs, n	Caterpillars, n	Affected buds, n		Eggs, n	Caterpillars, n			Affected buds, n
1	F ₄ BC ₃ S ₁ -47-8-1-17 x S-6524	75.6	0.87	0.05	0	0	5.0	0.05	0	0.05	5.0	10.0
2	F ₄ BC ₃ S ₁ -47-8-1-17 x S-6530	71.9	0.46	0.05	0	0	5.0	0.05	0	0.05	5.0	5.0
3	F ₄ BC ₃ S ₁ -47-8-1-17 x S-6532	75.6	2.03	0.15	0	0.05	15.0	0.05	0	0.1	5.0	10.0
4	F ₄ BC ₃ S ₁ -1-6-3-15 x S-6524	76.7	1.36	0.05	0	0	5.0	0.05	0.05	0.05	10.0	10.0
5	F ₄ BC ₃ S ₁ -1-6-3-15 x S-6530	75.2	1.20	0.15	0	0	15.0	0.05	0.05	0.15	10.0	10.0
6	F ₄ BC ₃ S ₁ -1-6-3-15 x S-6532	78.5	0.56	0.25	0	0.1	25.0	0.05	0.05	0.15	10.0	10.0
7	F ₄ BC ₃ S ₁ -47-8-1-17 x S-6524	91.2	1.20	0.8	0	0.1	80.0	0.3	0	0.25	30.0	15.0
8	F ₄ BC ₃ S ₁ -47-8-1-17 x S-6530	92.1	1.35	0.05	0	0.1	5.0	0.05	0	0.1	5.0	5.0
9	F ₄ BC ₃ S ₁ -47-8-1-17 x S-6532	91.4	0.79	0	0	0	0	0	0	0	0	5.0
10	F ₄ BC ₃ S ₁ -1-6-3-15 x S-6524	92.4	1.45	0.15	0	0.05	15.0	0.05	0	0.1	5.0	10.0
11	F ₄ BC ₃ S ₁ -1-6-3-15 x S-6530	93.1	1.23	0.15	0	0.15	15.0	0	0.05	0.1	5.0	5.0
12	F ₄ BC ₃ S ₁ -1-6-3-15 x S-6532	92.3	1.64	0.05	0	0.05	5.0	0.05	0	0.05	5.0	5.0

Table 2. Dynamics of affection of cotton hybrids F₆ by *Helicoverpa armigera*

№	Hybrids-combinations	(+)-gossypol	Total gossypol, %	1-Observation				2-Observation				Average degree of affecting
				Density of population, 10 plants, n			Affected, %	Density of population, 10 plants, n			Affected, %	
				Eggs, n	Caterpillars, n	Affected buds, n		Eggs, n	Caterpillars, n	Affected buds, n		
1.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6524	91,1	0,65	12	1	3	13.0	1	3	9	4.0	8.5
2.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6530	91,8	1,03	14	2	8	16.0	2	4	15	6.0	11.0
3.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6532	91,8	1,32	16	1	5	17.0	3	5	11	8.0	12.5
4.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6524	90,0	1,12	18	3	6	21.0	2	6	13	8.0	14.5
5.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6530	90,8	1,45	6	0	0	6.0	0	2	6	2.0	4.0
6.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6532	91,0	0,87	10	0	2	10.0	1	0	4	1.0	5.5
7.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6524	77,6	0,89	7	0	4	7.0	0	2	0	2.0	4.5
8.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6530	74,4	1,23	6	2	8	8.0	0	5	13	5.0	6.5
9.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6532	78,4	0,92	8	0	3	8.0	1	2	9	3.0	5.5
10.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6524	75,4	0,91	17	4	8	21.0	2	8	15	10.0	15.5
11.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6530	77,3	1,16	10	1	3	11.0	1	3	9	4.0	7.5
12.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6532	78,0	0,68	12	2	4	14.0	1	5	8	6.0	10.0
13.	F ₆ L-10/04 x BC ₃ S ₁ -1-6-3-15	81,0	1,19	20	3	7	23.0	3	6	15	9.0	16.0
14.	F ₆ L-10/04 x BC ₃ S ₁ -1-6-3-15	70,0	0,98	5	1	2	6.0	0	3	5	3.0	4.5

The results of studies of another group of hybrids developed between US accessions and Uzbek cultivars with a high level of (+)-gossypol in seeds showed that their affection related to the initial form genotype involved in hybridization.

For example, such hybrids with a low level of (+)-gossypol in seeds as F₃Bukhoro-8 x BC₃S₁-1-6-3-15 (62%), F₃Turon x BC₃S₁-1-6-3-15 (67%), F₃Surkhon-14 x BC₃S₁-1-6-3-15 (79%) and F₃I-9871 x BC₃S₁-1-6-3-15 (75%), developed from a relatively resistant parent form (BC₃S₁-1-6-3-15) with high (+)-gossypol in seeds (93.8%), were affected with *H.armigera* in less degree (with respective affection 25%, 50%, 40% and 40%).

Table 3. Tolerance hybrids with different levels of (+) - gossypol in seeds to *Helicoverpa armigera* (in Petri dishes)

№	Initial forms and hybrids	(+) - gossypol, %	Affected, %
1.	S-6524	77,0	55,0
2.	S-6530	70,0	45,0
3.	S-6532	75,0	55,0
4.	L-10/04	61,0	40,0
5.	BC ₃ S ₁ -47-8-1-17	93,3	45,0
6.	BC ₃ S ₁ -1-6-3-15	93,8	35,0
7.	F ₃ Turon x BC ₃ S ₁ -47-8-1-17	62,0	75,0
8.	F ₃ Turon x BC ₃ S ₁ -1-6-3-15	67,0	50,0
9.	F ₃ Bukhoro-8 x BC ₃ S ₁ -47-8-1-17	64,0	65,0
10.	F ₃ Bukhoro -8 x BC ₃ S ₁ -1-6-3-15	62,0	25,0
11.	F ₃ Surkhon-14 x BC ₃ S ₁ -47-8-1-17	75,0	50,0
12.	F ₃ Surkhon-14 x BC ₃ S ₁ -1-6-3-15	79,0	40,0
13.	F ₃ 9871-I x BC ₃ S ₁ -47-8-1-17	60,0	65,0
14.	F ₃ 9871-I x BC ₃ S ₁ -1-6-3-15	75,0	40,0
15.	F ₃ BC ₃ S ₁ -47-8-1-17 x S-6524	90,9	55,0
16.	F ₃ BC ₃ S ₁ -47-8-1-17 x S-6530	92,8	65,0
17.	F ₃ BC ₃ S ₁ -47-8-1-17 x S-6532	92,0	60,0
18.	F ₃ BC ₃ S ₁ -1-6-3-15 x S-6524	91,3	60,0
19.	F ₃ BC ₃ S ₁ -1-6-3-15 x S-6530	91,8	50,0
20.	F ₃ BC ₃ S ₁ -1-6-3-15 x S-6532	91,5	85,0
21.	F ₃ BC ₃ S ₁ -47-8-1-17 x S-6524	78,0	35,0
22.	F ₃ BC ₃ S ₁ -47-8-1-17 x S-6530	77,1	30,0
23.	F ₃ BC ₃ S ₁ -47-8-1-17 x S-6532	80,1	15,0
24.	F ₃ BC ₃ S ₁ -1-6-3-15 x S-6524	76,3	45,0
25.	F ₃ BC ₃ S ₁ -1-6-3-15 x S-6530	71,5	35,0
26.	F ₃ BC ₃ S ₁ -1-6-3-15 x S-6532	69,0	20,0
27.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6524	71,9	30,0
28.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6530	76,7	35,0
29.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6532	78,5	40,0
30.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6524	91,2	75,0
31.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6530	91,4	55,0
32.	F ₆ BC ₃ S ₁ -47-8-1-17 x S-6532	93,1	70,0
33.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6524	92,1	55,0
34.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6530	92,4	60,0
35.	F ₆ BC ₃ S ₁ -1-6-3-15 x S-6532	92,3	65,0
36.	F ₆ L-10/04x BC ₃ S ₁ -47-8-1-17	80,0	60,0
37.	F ₆ L-10/04x BC ₃ S ₁ -47-8-1-17	65,0	40,0

Among the hybrids F₅ with a high level of (+) - gossypol in the seeds, only in one case the damage was 50% (F₅BC₃S₁-1-6-3-15 x S-6530), and in the remaining cases susceptibility were 55- 65%.

In contrast to the above, hybrids of this generation with a low level of (+) - gossypol in seeds, differed in relative tolerance to the *H.armigera*. For example, susceptibility of F₅BC₃S₁-47-8-1-17 x S-6532 and F₅BC₃S₁-1-6-3-15 x S-6532 - with low (+)-gossypol were 15% and 20%, respectively. The remaining hybrids of this generation with a low level of (+)-gossypol were affected by a *H.armigera* from 30% up to 45% (Table 3).

Among the hybrids F₆, comparative resistance with a low level of (+) - gossypol and a relatively high affection of buds with a high level of (+)-gossypol (over 90%) are also observed. For example, the damage of buds with a low (+)-gossypol was from 30% (F₆BC₃S₁-1-6-3-15 x S-6524) up to 40% (F₆BC₃S₁-1-6-3-15 x S-6532 and F₆L-10/04x BC₃S₁-47-8-1-17), and with a high level of (+) - gossypol - from 55% (F₆BC₃S₁-47-8-1-17 x S-6530 and F₆BC₃S₁-1-6-3-15 x S-6524) up to 70% (F₆BC₃S₁-47-8-1-17 x S-6532). The similar dates of damaging of buds with different levels of (+) - gossypol were observed at hybrids F₇.

IV. CONCLUSIONS

Thus, on the basis of the obtained results of researches to tolerance cotton bollworm of different progenies which developed hybrids with the participation of genetically distant forms of cotton on the natural background was found that the progenies F₆ with high (+)-gossypol were relatively tolerant to bollworm in comparing to progenies with low level (+)-gossypol. These results indicate that the level of (+) - gossypol does not significantly affect on resistance to *Helicoverpa armigera*.

Also, results of the study of comparative resistance to *Helicoverpa armigera* among hybrids of different generations petri dishes, it was established that progenies with a different levels of (+) - gossypol, a definite pattern is observed for affection. Genotypes with a low level of (+)- gossypol are affected by *Helicoverpa armigera* to a certain extent less than hybrids with high (+)-gossypol level. Although, the incidence of the initial accession BC₃S₁-1-6-3-15 of US with a high level of gossypol does not preclude the possibility of developing of resistant genotypes with a high level of (+)-gossypol, which requires additional studies in this direction.

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