CORRELATION OF (+) GOSSYPOL LEVEL IN SEEDS OF COTTON HYBRIDS WITH THEIR INSECT AND DISEASE RESISTANCE

Shadman Namazov[1], Alois A. Bell[2], Robert D. Stipanovic[2], Abbos Marupov[3], Maqsuda Ishanqulova, Rano Yuldosheva[1], Ikrom Amanturdiev[1], Tojiddin Rakhimov[1]

[1]The Uzbek Scientific Research Institute of Cotton Breeding and Seed Production, Tashkent, Uzbekistan
[2]USDA, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, TX, U.S.A.
[3]The Uzbek Scientific Research Institute of Plant Protection, Tashkent, Uzbekistan

Abstract

Cotton is the most important fiber crop and the seed from the plant is a rich source of high quality protein and oil. However, the use of cottonseed as a feed for animals is limited by the presence of gossypol, which is contained in glands in the seed. Gossypol occurs in two stereoenantiomers referred to as (+)- and (-)-gossypol (Jaroszewski et al., 1992). Most of the toxicity of gossypol resides in the (-)-enantiomer (Wu et al., 1986). Gossypol helps protect the plant from pests. However, there is very limited information on how the level (+)- and (-)-gossypol enantiomers correlates with insect and disease resistance.

To understand the influence of (+)-gossypol level in seeds on insect and disease resistance we have initiated a study to examine if a correlations exists between percent (+)-gossypol in the seed and resistance to pests. The objective of our research is to develop cotton breeding material with good agronomic qualities that are suited for growing in Uzbekistan, and with a high percentage of (+)- gossypol and determine resistance to insects and pathogens.

Results from our research shows that it should be possible to develop cotton breeding material that exhibit the high (+)-gossypol trait in seed and provide suitable insect and diseases resistance with good agronomic traits.

Introduction

Cotton is the most important fiber crop grown in Uzbekistan and its seed is a rich source of high quality protein and oil. However, the use of cottonseed as a feed for animals is limited by the presence of gossypol contained in glands in the seed.

Gossypol, was identified in 1915 (Withers and Carruth) as the toxic component in cottonseed. In 1954, McMichael described a genetic trait in cotton in which the foliage and seed are devoid of glands. The prospect of plants with glandless, edible cottonseed set off a flurry of research to incorporate and test this trait in commercial lines. However, field trials were discouraging. Glandless plants in the field were completely defoliated by insects whereas adjacent glanded plants showed little or no damage (Bottger et al., 1964). Researchers showed that gossypol was toxic to cotton aphids, lygus bugs, salt-marsh caterpillars, thurberi 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).

Gossypol occurs in two stereoenantiomers referred to as (+)- and (-)-gossypol (Jaroszewski et al., 1992). Most of the toxicity of gossypol resides in the (-)-enantiomer (Wu et al., 1986). The ratio of (+)- to (-)-gossypol in seed has been reported to vary between a high of 98:2 and a low of 31:69 (Cass et al., 1991; Stipanovic et al., 2005).

The toxicity of (+)- and (-)-gossypol to insect pests and pathogens have not been extensively investigated, especially under field conditions. 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 to pupation and the moth stage (Yang et al., 1999). The larvae raised on the (+)-gossypol diet matured more slowly, and percent survival to the adult was lower. In a recent artificial feeding study, Stipanovic et al. (2006) showed that racemic, (+)- and (-)-gossypol were equally effective at reducing days-to-pupation, pupal weights and survival of *Helicoverpa zea*.

Puckhaber et al. (2002) determined the effect of (+)- and (-)-gossypol on the growth (ED_{50}) and survival (LD_{100}) of the seedling pathogen *Rhizoctonia solani*. The turbimetric bioassay used to measure inhibitory effects showed equal inhibitory effects for (+)- and (-)-gossypol.

However, gossypol was significantly less inhibitory than desoxyhemigossypol or hemigossypol. In the survival study, again (+)- and (-)-gossypol did not contribute significantly toward killing the pathogen. Rather, hemigossypol and desoxyhemigossypol were the primary fungicides. Yildirim-Aksoy et al. (2004) found (+)-gossypol was a better bacteriostat than racemic or (-)-gossypol.

However, there is very limited information on the how level (+)- and (-)-gossypol correlates with insect and disease resistance under field conditions. We investigated resistance of some local cotton varieties, USA accessions and hybrids that exhibit different percentages of seed (+)-gossypol to spider mites (Namazov et.al., 2008). We found that the percent of (+)-gossypol in seeds does not affect spider mite resistance. We have now initiated an investigation on how the percentage of (+)-gossypol in seed could influence resistance to other insects and diseases.

Materials and Methods

Cotton lines investigated. The American cotton lines used in this study were BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 which exhibited a high percent (>93.0%) of (+)-gossypol in seeds. The hybrid progenies were developed by crossing the U.S. lines with Uzbek varieties S-6524, S-6530, S-6532, and glandless cotton lines L-10/04, and L-16/04. Individual plants were selected and seed were analyzed to determine the total and percent (+)- and (-)-gossypol. Cotton breeding materials were tested 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).

Experiments were conducted in the greenhouse during the winter and under field conditions in the summer. 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 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 completely randomized block design. Plots were single rows spaced 60 cm with single plants spaced 25 cm within rows. Plants were irrigated 3-4 times on as needed basis. Plots received 240 kg/ha N, 160 kg/ha P_2O_5, and 120 kg/ha K_2O.

Measurement of INSECT damage. We investigated resistance of F_4 progenies with different percentages of (+)-gossypol to *Heliothis armigera Hb* and the spider mite, *Tetranychus urtical*. Screening for resistance to *Heliothis armigera Hb* was conducted by artificially introducing a moth to the plant during flowering and maturing periods and counting the number of damaged bolls (Shvetsova and Em, 1991). *Tetranychus urtical* resistance was determined by counting damaged cotton leaves on several dates during the growing season.

Measurement of PATHOGEN damage. We investigated resistance of F_4 progenies with different percentages of (+)-gossypol to *Thielaviopsis basicola, Rizactonia solani,* and *Xanthomonas malvacearum D*. Experiments were conducted in the greenhouse. Infected substrat was prepared by adding 40 mg. *Rizactonia solani,* 300 mg *Thielaviopsis basicola* on 1 kg of sand, heated to 160-180^{0}C, and after mixing was added to the substrate. Fifty seed from each group of progenies with different percentages of (+)-gossypol were planted in 4 replications. Counting of susceptible plants were counted at the 4-5 leaf stage (Khitrov, 1968; Khasanov and Babanazarov, 1976).

Results and Discussion

Disease resistance. Our research indicates that susceptibility of F_4 progenies to *Thielaviopsis basicola* maybe correlated with the percent of (+)-gossypol in seeds and on genotypes of local varieties and USA accessions (Table-1). Thus, increases in the percent of (+)-gossypol correlated with an increase of resistance to *Thielaviopsis basicola*. For example, among the investigated plants of hybrid $F_4BC_3S_1$-47-8-1-17 x S-6524 with (+)-gossypol level in seeds 70.1-80.0 % and 80.1-90.0 % showed damage of 24.3 % and 24.7 %, respectively, while plants with >90.0 % (+)-gossypol were less susceptible to this disease (18.3 % damage). Other progeny from the $F_4BC_3S_1$-47-8-1-17 cross that had >90% (+)-gossypol showed a similar trend. No correlation with total gossypol was observed. Progeny with percent (+)-gossypol in seeds (from 70.1-80 % and 80.1-90.0 % up to over 90 %) among F_4 plants derived from the $F_4BC_3S_1$-47-8-1-17 x S-6530 cross also showed a similar increase in resistance to *Thielaviopsis basicola* with damage values of 49.5 %, 38.7 % and 33.3 %. A similar but less strong trend was observed with the hybrids derived by crosses between the USA accession $F_4BC_3S_1$-1-6-3-15. In addition, susceptibility of plants with (+)-

gossypol percentages of 80.1-90.0 % and over 90 % among progenies $F_4BC_3S_1$-1-6-3-15 x S-6524, showed an increase in resistance as the percentage of (+)-gossypol increased (44.8 % and 32.1 %, respectively).

An analysis (Table-1) of resistance of F_4 hybrids to *Xanthomonas malvacearum D*, showed that there was no correlation with resistance as the percent (+)-gossypol increased in brackets of 70.1-80.0%, 80.1-90.0% and >90.0%. For example, susceptibility of hybrids containing <80% (+)-gossypol ranged between 43.0-45.7% and hybrids with >90.1% (+)-gossypol from 31.7% up to 38.1%. Correlation values of (+)-gossypol content and resistance of most hybrids were positive (except progenies $F_4BC_3S_1$-47-8-1-17 x C-6532 with negative correlation r=-0.04). Correlation coefficients ranged between r=-0.04 and r=+0.002. Thus, there was no correlation between increasing the percent of (+)-gossypol in seed and susceptibility to *Xanthomonas malvacearum D*.

Table-1.*Disease* susceptibility of hybrids (%)

№	Hybrids	(+) gossypol, %	Total gossypol, %	*Thielaviopsis basicola damage*, %	*Xanthomonas malvacearum damage,%*	*Rhizactonia solani damage,%*
(+)-gossypol content (<80%)						
1	$F_4BC_3S_1$-47-8-1-17 x S-6524	75.5	0.71	24.3	43.9	18.7
2	$F_4BC_3S_1$-47-8-1-17 x S-6530	73.3	0.27	49.5	43.0	43.9
3	$F_4BC_3S_1$ -1-6-3-15 x S-6530	73.3	0.45	42.8	45.7	37.1
(+)-gossypol content (80.1 – 90.0%)						
4	$F_4BC_3S_1$-47-8-1-17 x S-6524	89.3	0.52	24.7	33.5	40.6
5	$F_4BC_3S_1$-47-8-1-17 x S-6530	89.5	0.28	38.7	34.7	34.7
6	$F_4BC_3S_1$-47-8-1-17 x S-6532	83.5	0.65	48.2	37.3	54.2
7	$F_4BC_3S_1$ -1-6-3-15 x S-6524	87.9	0.71	44.8	38.8	52.2
8	$F_4BC_3S_1$ -1-6-3-15 x S-6530	88.2	0.45	31.6	39.4	37.9
(+)-gossypol content (>90.1%)						
9	$F_4BC_3S_1$-47-8-1-17 x S-6524	91.2	0.58	18.3	31.7	36.6
10	$F_4BC_3S_1$-47-8-1-17 x S-6530	91.1	0.54	33.3	34.0	49.4
11	$F_4BC_3S_1$-47-8-1-17 x S-6532	91.6	0.59	24.6	36.5	35.0
12	$F_4BC_3S_1$ -1-6-3-15 x S-6524	90.3	0.81	32.1	38.1	51.2

The resistance to *Rhizoctonia solani*, unlike resistance to *Thielaviopsis basicola* and *Xanthomonas malvacearum D*, appears to depend more on the parental lines. For example, progenies $F_4BC_3S_1$-47-8-1-17 x S-6524 with percentages of (+)-gossypol with brackets of 70.1-80.0 %, 80.1-90.0 % and >90.0 % gave various degrees of susceptibility (18.7 %; 40.6 % and 36.6 %, respectively). Susceptibility of plants of $F_4BC_3S_1$-1-6-3-15 x S-6530, $F_4BC_3S_1$-1-6-3-15 x S-6524 and $F_4BC_3S_1$-47-8-1-17 x S-6530 with the various percentages of (+)-gossypol were similar. As a whole, the data on resistance to *Rhizoctonia solani* indicates that increasing the percent of (+)-gossypol does not influence plant susceptibility.

Correlations of resistance to the pathogens and spider mites and the percent of (+)-gossypol (Table-2) among the progeny tested were low. Correlation values for resistance to *R. solani* ranged from r=-0.19 ($F_4BC_3S_1$-47-8-1-17 x S-6524) up to r=+0.19 ($F_4BC_3S_1$-1-6-3-15 x S-6524).

Table-2.Correlation of (+)-gossypol in seeds and resistance to diseases and spider mites

№	Hybrids	*Thielaviopsis basicola*	*Xanthomonas malvacearum D*	*Rizactonia solani.*	Tetranychus urtical
		r*	r*	r*	r*
1	$F_4BC_3S_1$-47-8-1-17 x S-6524	0.02	0.07	-0.19	0.01
2	$F_4BC_3S_1$-47-8-1-17 x S-6530	-0.41	0.002	-0.02	-0.03
3	$F_4BC_3S_1$-47-8-1-17 x S-6532	-0.25	-0.04	-0.10	0.07
4	$F_4BC_3S_1$-1-6-3-15 x S-6524	0.05	0.05	0.19	0.15
5	$F_4BC_3S_1$-1-6-3-15 x S-6530	0.20	0.22	0.14	-0.10

- *r- coefficients of correlation

Analyses of susceptibility of investigated progenies to studied diseases (Table-1) in depending of their total gossypol content showed some differences. For example, decreasing the total gossypol in seeds among plants of $F_4BC_3S_1$-47-8-1-17 x C-6524 from 0.71% up to 0.58% showed an increase in resistance to *Thielaviopsis basicola*. Similar data was observed among progenies $F_4BC_3S_1$ -1-6-3-15 x C-6524 where increasing total gossypol content from 0.71% up to 0.81% was accompanied by decreasing susceptibility to *Thielaviopsis basicola* (44.8% and 32.1%, respectively). But, progenies $F_4BC_3S_1$-47-8-1-17 x C-6530 showed a different trend. Thus, increasing the total gossypol (0.27; 0.28 and 0.54%) lead to decreasing trend in susceptibility (49.5%; 38.7% and 33.3%, respectively). Similar tendencies were found in regard to susceptibility to X*anthomonas malvacearum*, and different degrees of susceptibility to *Rizactonia solani* was observed among progenies with different level of total gossypol. Only hybrid $F_4BC_3S_1$-1-6-3-15 x C-6530 showed a tendency of increasing susceptibility, among progenies containing similar levels(0.45%) and total gossypol and different level of (+)-gossypol (73.3% and 88,2%) damaged 37,1% and 37.9%, respectively. Susceptibility of plants of this hybrid with percent (+) gossypol >90.1% and total gossypol of 0.81% was higher (51.2%).

Thus, the data suggests that among the hybrids neither the total gossypol nor percent (+)-gossypol in seeds significantly affected the resistance of the investigated pathogens.

Resistance of hybrids and varieties to insects. We studied the influences of total and (+)-gossypol level in seeds to insect resistance of some local cotton varieties and the USA accessions BC_3S_1-47-8-1-17 and BC_3S_1-1-6-3-15 (Table-3). No correlation was observed between (+)-gossypol percentage and resistance to *Heliothis armigera*. For example, local varieties S-6524, S-6530 and S-6532 which have a rather high percent of (+)-gossypol in seeds (70.0 %; 67.1 % and 70.9 %, respectively), were more susceptible in comparison to other varieties. Plant damage values for these three cultivars were 19.1 %; 21.4 % and 25.9 %, respectively. However, the USA accession BC_3S_1-1-6-3-15 with 93.5% (+)-gossypol was highly susceptible to the bollworm (23.5 % damaged), but the other USA accessions (BC_3S_1-47-8-1-17) with 93.3% (+)-gossypol in the seed showed little damage (11.6 % damaged). In the case of total gossypol, there appeared to be a correlation to resistance when the percent total gossypol was above 2%.

Table-3. Susceptibility of cotton varieties, lines and USA accessions to *Heliothis armigera* (%)

№	Cotton Varieties, Lines and accessions	(+) –gossypol (%)	Total gossypol (%)	Damage (%)
1	Omad	58.8	1.78	16.9
2	S– 6524	70.0	1.05	19.1
3	S – 6530	67.1	1.08	21.4
4	S – 6532	70.9	1.78	25.9
5	S – 2610	53.8	1.64	17.7
6	S – 8288	58.1	1.85	12.1
7	L – 10/04	69.5	1.97	__
8	L – 842	59.4	1.13	15.2
9	Turon	49.3	2.26	7.5
10	Bukhara – 8	56.3	2.05	8.6
11	BC_3S_1-47-8-1-17	93.3	1.73	11.6
12	BC_3S_1 -1-6-3-15	93.5	1.70	23.5

However, the 2% level was not required to achieve resistance since BC_3S_1-47-8-1-17 with 1.73% total gossypol also showed good resistance. Thus, other factors must be involved, which will require additional study. Since no correlation was observed between the percent (+)-gossypol and resistance to *H. armigera*, this should allow plants to be breed with a high percent of (+)-gossypol in the seed and with good resistance to *Heliothis armigera* Hb.

The correlation dependence of resistance of hybrids with various (+)-gossypol level in seeds to the spider mite, *Tetranychus urtical* (Table-2) showed weak and not significant correlation.

The correlation factors ranged from r =-0.10 ($F_4BC_3S_1$-1-6-3-15 x C-6524) up to r = 0.15 $F_4BC_3S_1$-1-6-3-15 x C-6530).

Conclusions

- Plants with high (+)-gossypol do not correlate with increased resistance to *Thielaviopsis basicola* and *Rhizoctonia solani*; resistance to *Xanthomonas malvacearum D* may depend on parents of the respective progenies.
- The levels of total gossypol in seed do not correlate with disease or insect resistance.
- The percent (+)-gossypol in seed does not show significant correlation values with resistance to insects or diseases. This should allow the selection of progeny with a high percent of (+)-gossypol in seed to be combined with high resistance to insects and diseases.
- Breeding material that exhibits the high percent (+)-gossypol trait in seed can be combined with other lines such that their progeny exhibit resistance to the studied diseases and insects and combine easily with other desirable agronomic traits.

Acknowledgments

This work was supported by a grant from the USDA, ARS under project No. CRDF Uzb2-31001-TA-08.

References

Beil G.M., R.E. Atkins. 1965. Inheritance of quantitative characters in grain sorghum. Iowa State Journal of Science. 39:345–358.

Belousov M.A., Z.C. Tursunxodjaev, M.P. Mednis , I.M. Madraimov, V.P. Kondratyk, A.A. Raximbaev, and A.P. Kenzer. 1973. Methods of field and vegetative experiments with cotton in irrigation conditions. Tashkent, pp. 223.

Bell, A.A., L.S. Puckhaber, H.L. Kim, R.D. Stipanovic, E. Percival. 2000. Genetic approaches for increasing percentages of (+)-gossypol levels in cotton. p. 218–230. *In* C. Benedict and G. Jividen (ed.) Genetic Control of Cotton Fiber and Seed Quality. Cotton Inc., Cary, NC.

Bottger, G. T., Sheehan, E. T., Lukefahr, M. J. (1964). Relation of gossypol content of cotton plants to insect resistance. *J. Econ. Entomol.* 57*:*283-285.

Bottger, G.T., Patana, R. (1966). Growth, Development, and Survival of Certain Lepidoptera Fed Gossypol in the Diet, *J. Econ. Etomol.* 59:1166-1168

Cass, Q. B., E. Tiritan, S.A. Matlin, E.C. Freire. 1991. Gossypol enantiomer ratios in cotton seeds. Phytochemistry 30:2655–2657.

Dospekhov B.A. 1985. Metodika polevogo opita. Agropromizdat, Moscow, Russia pp. 351.

Jaroszewski, J.W., T. Strom-Hansen, L.L. Hanson. 1992. Optical stability of gossypol. Chirality 4:216–221.

Jenkins, J. N., Maxwell, F. G., Lafever, H. N. (1966). The Comparative Preference of Insects for Glanded and Glandless Cottons, *J. Econ. Entomol.* 59:352-356.

Jenkins, J. N. (1995). Host resistance to insects in cotton. In: *Proceedings of the World Cotton Research Conference-1,* (G. A. Constable and N. W. Forrester, Eds.) Melbourne, Australia, pp. 359-372.

Khitrova A.P. Diagnostics of cotton to black root rot. J. Cotton growing,1968, #2,pp.36-37.

Khasanov O., Babanazarov A. The estimation of Rizoctania solani resistance. J.Cotton growing,1976, #8, p.35.

McMichael, S. C. (1954). Glandless boll in Upland Cotton and its use in the study of natural crossing. *Agron. J.* 46:527-528.

Namazov, Sh.E,, Yuldosheva, R.A., Marupov, A.I., Ishanqulova, M., Bell, A., Stipanovic, R. 25th Proceedings of the International Conference "Perspectives of using of biological methods of Plant Protection from harmful organisms in agriculture, December, 2008, Tashkent, Uzbekistan.25:327-330.

Puckhaber, L.S., Dowd, M.K., Stipanovic, R.D., Howell, C.R. (2002). Toxicity of (+)- and (-)-gossypol to the plant pathogen, *Rhizoctonia solani*. *J. Agri. Food Chem.* 50:7017-7021.

Shvetsova, L.P., Em E.E. Methods of estimation resistance of cotton varieties and accessions to bollworm.Tashkent, 1991.

Stipanovic, R.D.,Puchaber, L.S., Bell, a.a., Percival, A.E., Jacobs, J. (2005) Occurrence of (+)-and (-)-gossypol in wild species of cotton and in *Gossypium gric.Food Chem.hirsutum marie galante* (Watt) Hutchinson. J. Agric.Food Chem.

Stipanovic, R.D., J. Lopez, M.K.Dowd, L.S. Puchaber, and S.E.Duke. Effect of racemic and (+) and (-) -gossypol on the survival and development of *Helicoverpazea* larvae. J. Chem. Ecol. 32:959-968.,2006.

Withers, W.A., Carruth, F.F. (1915).Gossypol, the Toxic Substance in Cottonseed. J. Agric.Res. 5:261-288.

Wu,D.F.,Y.W.Yu,Z.M..Tang, M.Z.Wang.1986. Pharmocoenetics of (±)-,(+)-, and (-)-gossypol in humans and dogs. Clin.Pharmacol. Ther.39:613-618.

Yang W.-H., Ma L.-H., Zhu H.-Q., Xiang S.-K. (1999). Effects of different gossypol enantiomers on growth and development of cotton bollworm (*Helicoverpa armigera*) and Fusarium wilt. *Acta Gossypii Sinica*, 11:31-34.

Yildirim-Aksoy M., Lim C., Dowd M. K., Wan P. J., Klesius P. H., Shoemaker C. 2004. *In vitro* inhibitory effects of gossypol from gossypol-acetic acid, and (+)- and (-)-isomers of gossypol on the growth of *Edwardsiella ictaluri*. J. Appl. Microbiol. 97:87-92.