Reduced use of insecticides against cotton bollworms with F_1 cotton hybrids possessing Bt-gene: A myth or reality?

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ABSTRACT

Seven F_1 hybrids with the Bt-gene (induced conventionally) along with two commercial varieties were sown in the field and monitored for lepidopteran insect activity in an unsprayed block. The results demonstrated that the hybrids with the Bt-gene were almost free from bollworm attack. In total, apparently eight bolls of six different combinations were attacked by spotted bollworm with the result that the larvae died in the holes but the combination Reshmi x Bt-cotton though visited by larvae as observed from the scars on the bolls, remained without any damage. Further, fresh, young bolls and flowers of these seven F_1 hybrids and two commercial checks were taken to laboratory and kept in separate cages. Fifteen lepidopteran larvae collected from the field were released in each cage to monitor their activity. The bolls/fruiting bodies with Bt-gene were not attacked, and if attacked, the larvae died. The bolls/fruiting bodies of two commercial checks, CRIS-134 and NIAB-78 were damaged and larvae survived except one larva which died due to unknown reasons. The studies further revealed that the combination NIAB-78 x Bt-cotton which proved to be high yielding and early maturing, could be used in commercial hybrid cotton production without spraying the crop against lepidopteran pests thus reducing the pesticide use to a greater extent and increasing the seed cotton yield to a considerable level. It is further suggested that different parents may be tested with Bt-cotton to explore the best combinations for commercial hybrid cotton production with minimum use of pesticides.

Introduction

Pakistan produced a record cotton crop in 1991, but since then the production has been facing ups and downs. The yield per acre has gone down and one main reason is the failure of insect control due to development of resistance in the bollworms and whitefly against more commonly used insecticides. The cost of production has increased due to more and more use of insecticides but the profit has reduced. Pesticides worth billions of rupees are imported every year and of these 70 to 80% are sprayed on cotton. The damage due to insect pests and importation of pesticides from 1992 are shown in Table 1. The dependence on insecticides has increased manifold and it is high time that techniques of pest control should be developed, which are safer to the environment, wild life and human beings.

Genetically engineered cotton that produces toxin derived from the bacterium *Bacillus thuringiensis* could make a substantial contribution toward such a reduction in chemical pesticides. The *B. thuringiensis* (Bt) is a soil bacterium, which produces parasporal crystals during spore formation (Koziel *et al*., 1993; Mazier, 1997). These crystals consist of one or more proteins called Delta endotoxin or insecticidal proteins (Cry).

The Delta endotoxins or Bt-toxin is insect specific and has been used since 1930 as insecticidal sprays (Aronson *et al.*, 1986) to control specific insects in bio-control systems, due to its environmental safety and safety towards non-target insects. The Delta endotoxins affect the membrane of gut epithelium of susceptible insect larvae and binds to the glycoproteins, which results in lyses of membrane as a result of which insects stop feeding, become dehydrated and ultimately die.

Bt-Delta endotoxin has been used successfully as a natural pesticide in agriculture, forestry and public health for several decades due to the adverse effects of agrochemicals including lack of selectivity towards beneficial insects, environmental hazards, health concerns as well as evolution of resistance. The host spectrum of Bt toxins is so narrow that it only kills caterpillar pests of cotton. Bt cotton is safe for human, wild life and beneficial insects (Karim & Riazuddin, 1999).

Randhawa (1999) reported that the cotton plants with Bt-gene represent a novel means to control insect pests like cotton bollworms specially *Helicoverpa* and pink bollworm. He further added that the Bt-toxin proteins are not harmful to beneficial insects due to their narrow host range and particular mode of delivery of toxin.

Keeping in view the above studies, the present work was initiated at Central Cotton Research Institute, Sakrand Sindh to induce Bt-gene conventionally from exotic Bt-cotton (the seed was received from Director Cotton Research Institute Faisalabad) into our high yielding and early maturing varieties to produce high yielding and early maturing F_1 hybrids resistant to lepidopterans and also for further development of commercial varieties with desirable traits.

Experimental procedure

During the 2000-2001 cotton season, hybridisation between exotic Bt-cotton (the seed of which was arranged through the kind courtesy of Chaudhri Waheed Sultan, Director of Cotton Research Institute, Faisalabad) and our locally adapted high yielding and early maturing varieties was initiated to develop sufficient F_0 seed of seven combinations for sowing as F_1 generation during the next cotton season (2001-2002). The F_1 generation consisting of seven combinations along with two commercial varieties NIAB-78 and CRIS-

134 was sown in May 2001 in a randomized complete block design replicated four times in a plot size of 10 x 25 feet. Thinning was done to maintain an uniform in row plant distance of 9 inches and between row distance of 2.5 feet. Normal agronomical practices were carried out as and when needed. All the combinations were monitored against the attack of lepidopterans throughout the season in the field. Fresh young bolls and flowers in sufficient quantity of these seven F_1 hybrids and two commercial checks were also brought to laboratory and kept in separate cages. Fifteen lepidopteran larvae (*Earias insulana)* collected from the field were released in each cage for monitoring their activity. The earliness studies in these combinations and two commercial checks were also carried out and the data on number of bolls per plant formed and opened at different (90, 114 and 130) days after planting (DAP) were recorded on 10 randomly selected plants from each genotype/combination per replication to single out the high yielding and early maturing combinations. The analysis of variance was performed after Steel and Torrie (1980).

Results and Discussion

The data presented in Table 2 revealed that in all the seven F_1 hybrids with Bt-gene, the larvae feeding on fruiting bodies died, while the larvae, which could not attack the fruiting bodies, turned into pupal or adult stage. Maximum mortality of seven out of 15 larvae was recorded in the FH-900 x Bt-cotton combination followed by RH-500 x Bt-cotton (six out of 15). However, the larvae feeding on two standard varieties remained healthy up to pupation. The results are in line with those of Karim and Riazuddin (1999) and Randhawa (1999) who also reported that cotton plants with Bt-gene express resistance against cotton bollworms. In field conditions, these F_1 hybrids (without spray) were also monitored throughout the season against lepidopteran larvae. All the hybrids remained safe undamaged throughout the season. The hybrids remained undamaged and if attacked the larvae died in the holes (Figures 1 and 2).

The earliness comparison studies were also conducted in these F_1 hybrids and two standards by calculating the opening percentage at 90, 114 and 130 days after planting (dap) to single out the early maturing hybrids. Further F_1 hybrids were also compared with two standards for harvesting the highest number of bolls ultimately contributing to yield.

Mean squares from analysis of variance for number of bolls formed and opened at different days (Tables 3 and 4) revealed that there existed significant differences among hybrids and standards only at 114 DAP in respect of number of bolls formed. The differences were non-significant at 90, 130 and 150 DAP. Highly significant differences were observed for number of bolls opened at all the stages of boll development (Table 4), which suggested that genotypes differed in earliness.

The data regarding opening percentage at 90 dap (Table 5) revealed that none of the hybrids were earlier than the two standard varieties, NIAB-78 with 22.33 and CRIS-134 with 41.30 percent opening, however, hybrid RH-500 x Bt-cotton with 17.74% opening was recorded earlier among other hybrids. When the percent opening data at 114 DAP were looked at (Table 6), hybrid combination VH-137 x Bt-cotton with 56.76% ranked first and was recorded as earliest hybrid in the test followed by two standards NIAB-78 (55.13%) and CRIS-134 (53.21%).

It is evident therefore that if one could make hybrid seed of the combination (VH-137 x Bt-cotton), he would harvest almost 57% of his crop only after 114 days and fetch a good price for sending his produce early to the market. However, at 130 DAP (Table 7), the two standard varieties were recorded as earliest with 89.88% (CRIS-134) and 86.86% (NIAB-78). The two hybrid combinations VH-137 x Bt-cotton (82.57%) and RH-500 x Bt-cotton (81.39%) remained a little behind the two standards in earliness. These results are in accordance with those of Soomro *et al.* (2002a), Soomro *et al*. (2002b) and Rehana *et al*. (2002).

The data regarding percent increase or decrease of hybrids over the commercial checks (Table 8) for number of bolls picked revealed that out of 7 hybrids 5 gave an increased number of bolls over NIAB-78, the range of percent increase of these hybrids in respect of bolls harvested recorded was from 31.0 to 109.5%. It means that hybrid combination NIAB-78 x Bt-cotton which produced above double (109.5%) bolls than NIAB-78 itself was the most desired combination if one has to develop high yielding varieties and to produce hybrid seed resistant to cotton bollworms. It was further observed that only this combination (NIAB-78 x BT-cotton) produced 2.3% more number of bolls against the second check variety CRIS-134; all other combinations remained behind. These results are in conformity with the results reported by Soomro *et al*. (2002a), Soomro *et al*. (2002b) and Rehana *et al*. (2002).

Conclusion

Seven F_1 hybrids with Bt-gene (induced conventionally) were observed resistant to cotton bollworms in the field as well as in laboratory conditions against two standards NIAB-78 and CRIS-134 in an un-sprayed block. All hybrid combinations except two were recorded high yielding as these produced increased number of harvested bolls against NIAB-78 with the range from 31.0 to 109.5%. As such, the combination NIAB-78 x Bt-cotton was observed high yielding as it gave 109.5% more number of bolls than NIAB-78 and 2.3% more than CRIS-134. It is therefore suggested that if any one is interested to go for hybrid seed production with high yield and bollworm resistance, to develop

NIAB-78 x Bt-cotton combination. The breeders may go for further selection in subsequent generations to develop the variety for general cultivation in Sindh. It is further suggested that until NIAB-78 x Bt-cotton combination is exploited for hybrid seed production and variety development, the next best choice is to plant CRIS-134 for getting increased yield and early crop for fetching good price of the seedcotton in earlier days when the cotton market is at the top.

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Figure 1.
F_1–hybrids with Bt-gene.

Figure 2.
Larvae of spotted bollworm attacked the bolls in field and were dead in the holes.

Table 1. *Production loss due to insect pests and import of pesticides in Pakistan.*

Year	Loss in production (000 bales)	Import of pesticides (a.i. M. Tons)
1992-93	250	5619
1993-94	1650	4919
1994-95	2500	6183
1995-96	2000	7645
1996-97	2700	7325
1997-98	2800	11209
1998-99	3050	10394

Table 2. Lepidopteran activity on fruiting bodies of hybrids with Bt gene and two standards.

Cage no.	Combination	Adults	Pupae	Died	Total
1.	BH-118 x Bt-Cotton	6	4	5	15
2.	FH-900 x Bt-Cotton	5	3	7	15
3.	MNH-552 x Bt-Cotton	8	4	3	15
4.	VH-137 x Bt-Cotton	6	5	4	15
5.	RH-500 x Bt-Cotton	5	4	6	15
6.	Reshmi x Bt-Cotton	6	5	4	15
7.	NIAB-78 x Bt-Cotton	7	3	5	15
8.	NIAB-78 (Standard)	7	8	-	15
9.	CRIS-134 (Standard)	8	6	1	15

Table 3. Mean squares from analysis of variance for number of bolls formed at different days.

		Number of bolls formed at			
Source of variance	Degrees of freedom	90 dap[1]	114 dap	130 dap	150 dap
Replications	3	157.4	177.5	139.5	79.3
Genotypes	8	188.8	339.1*	368.6	295.4
Error	24	84.3	135.3	165.7	201.4

[1]dap = days after planting

* Significant at 5% level of probability

Table 4. Mean squares from analysis of variance for number of bolls opened at different days.

		Number of bolls opened at			
Source of variance	Degrees of freedom	90 dap[1]	114 dap	130 dap	150 dap
Replications	3	9.7	12.1	60.3	47.6
Genotypes	8	31.8**	136.1**	248.6**	349.6*
Error	24	5.6	21.1	71.1	135.9

[1]dap = days after planting

*, ** Significant at 5 and 1% levels of probability respectively

Table 5. Earliness comparison of F_1 Bt-hybrids with two standards at 90 days after planting.

Hybrid combinations	Bolls formed[1]	Bolls opened[1]	Percent opening[1]
BH-118 x Bt-cotton	24.3 a	0.50 d	2.06
FH-900 x Bt-cotton	26.5 a	2.25 bc	8.49
MNH-552 x Bt-cotton	21.8 a	0.75 d cd	3.44
VH-137 x Bt-cotton	16.9 a	1.25 bc	7.40
RH-500 x Bt-cotton	15.5 a	2.75 d	17.74
Reshmi x Bt-cotton	22.8 a	0.8 d	3.51
NIAB-78 x Bt-cotton	38.0 a	4.0 b	10.53
NIAB-78 (Standard)	16.8 a	3.75 b	22.32
CRIS-134 (Standard)	23.0 a	9.50 a	41.30

[1]Means in columns followed by similar letters are not significantly different according to DMR test.

Table 6. Earliness comparison of F_1 Bt-hybrids with two standards at 114 days after planting.

Hybrid combinations	Bolls formed	Bolls opened	Percent opening
BH-118 x Bt-cotton	31.0 ab	5.25 c	16.94
FH-900 x Bt-cotton	31.3 ab	9.25 b	29.56
MNH-552 x Bt-cotton	24.8 bc	7.00 bc	28.23
VH-137 x Bt-cotton	18.5 bc	10.50 b	56.76
RH-500 x Bt-cotton	20.8 bc	9.50 b	45.68
Reshmi x Bt-cotton	32.3 ab	9.0 b	27.87
NIAB-78 x Bt-cotton	45.5 a	22.0 a	48.36
NIAB-78 (Standard)	19.5 bc	10.75 b	55.13
CRIS-134 (Standard)	39.0 ab	20.75 a	53.21

Means followed by similar letters are not significantly different according to DMR test.

Table 7. Earliness comparison of F_1 Bt-hybrids with two standards at 130 days after planting.

Hybrid combinations	Bolls formed[1]	Bolls opened[1]	Percent opening[1]
BH-118 x Bt-cotton	37.3 a	18.5 c	49.60
FH-900 x Bt-cotton	31.8 a	24.0 b	75.48
MNH-552 x Bt-cotton	26.0 a	18.3 c	70.39
VH-137 x Bt-cotton	21.8 a	18.0 c	82.57
RH-500 x Bt-cotton	18.8 a	15.3 c	81.39
Reshmi x Bt-cotton	34.8 a	22.0 b	63.22
NIAB-78 x Bt-cotton	45.8 a	35.0 a	76.42
NIAB-78 (Standard)	21.3 a	18.5 c	86.86
CRIS-134 (Standard)	41.5 a	37.3 a	89.88

[1]Means followed by similar letters are not significantly different according to DMR test.

Table 8. Percent increase or decrease of F_1 hybrids with Bt gene over two standards for number of bolls harvested.

	Percent increase (+) or decrease (-) over two standards	
F_1 Hybrids	NIAB-78	CRIS-134
BH-118 x Bt-cotton	+46.4	-28.5
FH-900 x Bt-cotton	+51.2	-26.2
MNH-552 x Bt-cotton	+31.0	-36.1
VH-137 x Bt-cotton	-4.8	-53.5
RH-500 x Bt-cotton	-9.5	-55.8
Reshmi x Bt-cotton	+65.5	-19.2
NIAB-78 x Bt-cotton	+109.5	+2.3