Performance of Bt-cotton genotypes under unprotected conditions

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ABSTRACT

A field experiment was conducted during kharif 2001-02 at the Agricultural Research Station, Dharwad Farm to assess the performance of three Bt transgenic hybrids under completely unprotected conditions. The experiment was laid out in a randomized block design with eight genotypes replicated three times. The genotypes were MECH-12 Bt, MECH-162 Bt, MECH-184 Bt, MECH-12 non-Bt, MECH-162 non-Bt, MECH-184 non-Bt, DHH-11 (local controlcontrol) and NHH-44 (national control). The results indicated that there was no effect of Bt-toxin on any of the sucking pests and that the presence of the cry1Ac protein in cotton hybrids created much variation in the incidence of bollworms. The population of Helicoverpa armigera *Hüb. larvae was significantly lower in the Bt genotypes compared to the non-Bt and standard control hybrids. MECH-184 Bt registered a significantly lower incidence (0.91 larvae/plant), followed by MECH-162 Bt (1.05 larvae/plant). The population of spotted bollworms (*Earias *spp.) was also lower (0.06 to 0.08/plant) in Bt genotypes as against significantly higher (0.12 to 0.16 larvae/ plant) incidence in non-Bt genotypes. Similarly the incidence of pink bollworms was also lower in Bt genotypes compared to non-Bt genotypes as indicated by the population of PBW larvae in green bolls and the difference was significant. The effect of the Bt gene was impressive in terms of damage caused to fruiting bodies in different hybrids, among which MECH-184 Bt exhibited only 4.04 percent damage followed by 162 Bt (5.02%) and 12 Bt (6.84%) which were significantly superior to respective non-Bt versions and controls (15.89 to 21.30%). The locule damage was also less in Bt genotypes. Hence the yield of seed cotton was higher in MECH-184 Bt (12.13 q/ha) followed by 162 Bt (8.44 q/ha) and 12 Bt (6.77 q/ha). The yield in non-Bt hybrids was 8.12, 4.15 and 5.34 quintals of seed cotton per hectare from MECH-184, 162 and 12 hybrids respectively in unprotected conditions. The studies clearly indicated the necessity of protection against sucking pests in Bt-cottons and possible reduction in number of insecticidal applications for management of bollworms.*

Introduction

In India an area of 169 million hectares is permanently under cropping. With the advent of the green revolution, tremendous benefits have been derived from the use of pesticides in agriculture, a sector upon which the Indian economy is largely dependent. As farr as cotton is concerned, India occupies one of the prime positions in the world and contributes 20 percent of the area under cotton cultivation (8122 thousand hectares) and 12 percent (2483 thousand tons) of the global production (Anonymous, 2002). Many cotton genotypes and hybrid cultivars are cultivated in India under rainfed, as well as irrigated conditions (Kulkarni *et al.*, 2003). In India, cotton appears to be a major consumer of insecticides (45%) although the total area under this crop is only five percent (Saiyed *et al.*, 1999). At present, resistance to insecticides in the important Heliothine species is a worldwide problem. Over-dependency on insecticides to contain an array of bollworms and sucking pests has also created the problem of insecticide resistance and resurgence of sucking pests in India . Resistance in *Helicoverpa armigera* (Hüb.) to pyrethroids and many other insecticides has been assessed in the entire country and has been documented since 1992 through network research. The resistance to pyrethroids is ubiquitous and stable at around 50-80 percent in most areas of India (Russell *et al.*, 1998). Hence under repeated control failures and inevitable conditions of searching for alternative management strategies for bollworms, the cultivation of transgenic Bt cultivars appear to be promising. Insecticidal crystal proteins found in a soil bacterium *Bacillus thuringiensis* (Bt) forms an important part of a arsenal, which offer better control than insecticides and is safe for the environment. Although plenty Bt formulations are available, the most effective way to deliver the toxin to the target pests is through the transgenic plant system (Kumar, 2002). Since 1987 when the first transgenic plants expressing the Bt toxin were developed, significant progress has been made culminating in the commercialization of Bt cotton in 1995 in USA (Krattiger, 1997). Today, Bt-transgenic cottons are widely cultivated in USA, China and Australia (Khadi *et al.*, 2001). Subsequently, the present field investigation was carried out to study the potential of three transgenic cultivars containing the Bt gene (Cry 1Ac) under unprotected conditions prior to its release in India.

Experimental procedure

The field experiment was conducted in medium deep red soil during 2001-02 kharif/rabi season at the Agricultural Research Station, Dharwad Farm (Karnataka, India) which is located at an altitude of 678 meter, latitude 15.7° N., longitude 76.0° E. The experiment was laid out in a randomized block design with three replications. The Bt genotypes studiedwere MECH-12 Bt, MECH-162 Bt and MECH-184 Bt. The same hybrids without Bt genes were used as controls along with the current local (DHH-11) and national (NHH-44) controls. The spacing allowed was 90 cm x 40 cm with fertilizer supplementation at the rate of

80:40:40 kg NPK/ha. All the genotypes were sown on 11 July 2001 and kapas was harvested on 15 February 2002. No plant protection measures were taken for any of the insect pests throughout the season. Two supplementary irrigations were given to maintain the crop. Observations for the population of both sucking pests and bollworms were made at regular intervals. The insect pest species under consideration were thrips (*Thrips tabaci*), aphids (*Aphis gossypii*), Jassids (*Ambrasca bigutulla bigutulla*) and whiteflies (*Bemisia tabaci*). Bollworms included American bollworm (*Helicoverpa armigera* (Hüb.), spotted bollworm (*Earias vitella* Fab.) and pink bollworm (*Pectinophora gossypiella* Saund.). The sucking pest population was recorded on three leaves per plant and presented as population per leaf. Similarly, the larval population was observed on a whole plant basis for American and spotted bollworm. For pink bollworm, the larval population in ten bolls was counted. The effect of insect damage on boll opening has been recorded in terms of good opened bolls (GOB/plant) and badly opened (BOB/ plant). The damage onto fruiting bodies and locules was recorded to determine the total impact of the Bt gene on bollworms. The populations of syrphids, coccinellids and chrysopa were assessed on each genotype (per plant) to determine the influence of Bt transgenic genotypse on the insect predatory population. Seed cotton yield was determined by harvesting each genotype separately and was presented in terms of quintals per hectare. All observations were statistically analyzed.

Results and Discussion

Sucking pests

The results were significant different in respect of the jassid populations, but were not significant with respect to the populations of other sucking pests such as thrips, aphids and whiteflies (Table 1). The thrips population ranged from 8.10 to 10.80 thrips/leaf. Although all the Bt genotypes had higher thrips populations, they were on a par with the non-Bt genotypes as well as the standard controls (DHH-11 and NHH-44). The aphid population was low in all the treatments including Bt genotypes (6.35 to 8.55 aphids/leaf) and there was no significant difference. There was significant variation in the jassid populations between the different genotypes. The population was lower (1.30 jassids/leaf) in MECH 184 non-Bt which was on par with MECH 184 Bt, MECH 162 Bt and MECH 162 non-Bt. In MECH 12 Bt the population was the highest (2.02 jassids/leaf), which was on a par with MECH 12 non-Bt genotype. In the rest of the genotypes, including the controls, the population was high. The whitefly population was very low during the trial (0.72 to 1.29/leaf) and was no there significant differences between the Bt, non-Bt genotypes and standard controls (NHH-44 and DHH-11). Thus, the results from the unprotected screening indicate that there was no effect of the Bt-toxin on any of the sucking pests. These genotypes need protection against sucking pests as in any other normal genotypes. It is a known fact that Bt toxins are target specific and that they have no effect on sucking pests (Xue, 2002; Kumar, 2002). The variation in the jassid populations during the present investigations was due to varietal reaction only. Kranthi (2002) observed similar differential varietal reactions of the same cultivars to jassids. However, the susceptibility of the Bt cultivars would not become a critical issue in accepting the Bt genotypes for commercial cultivation, as sucking pests can be managed in other ways, e.g. through seed treatment and spraying formulations of the more effective neonicotinyle and chloronichotinyle insecticides, such as imidacloprid, thiomethoxam and acetamiprid (David, 2002; Anomymous, 1999). However, it would better if the genotypes that have tolerance to sucking pests could be used for developing Bt transgenics (Kranthi, 2002).

Bollworms

Significantly lower incidence of *Helicoverpa* infestations were observed in all the Bt genotypes that were compared with the respective non-Bt genotypes and standard controls. Among the Bt genotypes, MECH-184 registered a significant lower incidence of *Helicoverpa* larvae (0.91 larvae/plant) compared to all the genotypes, including the other transgenics. MECH-162 Bt was the second best genotype and performed significantly better than the other genotypes. MECH-162 non-Bt has recorded significantly higher (1.97/ plant) incidence of *Helicoverpa*, indicating its susceptibility to bollworms (Table 2). A comparatively lower incidence of spotted bollworm was observed in all the Bt genotypes (0.06 to 0.08 larvae/plants) followed by the non-Bt genotypes (0.12 to 0.16 larvae/plants). Although the population was too low in all the hybrids,the difference noticed was significant, except for the non-Bt versions of MECH-162, MECH-184 and the national control, which were at a par with each other. The incidence of pink bollworm (PBW) ranged from 2 to 10.67 larvae/10 bolls. Both MECH-184 Bt (2 larvae/10 bolls) and MECH-162 Bt (2.67 larvae/10 bolls) recorded significantly lower incidence of PBW compared to the non-Bt genotypes. MECH-12 Bt was the next best treatment. MECH-12 non-Bt, MECH-162 non-Bt and NHH-44 were more susceptible and recorded a higher incidence of PBW (>10 larvae/10bolls) .

Fruiting body damage

Among the genotypes, MECH-184 Bt was the least damaged (4.04%), followed by MECH-162 (5.02%) and MECH-12 (6.84%). These genotype performed significantly betterthan the other genotypes. The control hybrids NHH-44, DHH-11 and the non-Bt genotypes were susceptible to bollworms and ahigher percentage of boll damage (15.89 to 21.30%) were recorded for them. Compared to the non-Bt genotypes and the standard controls, all the Bt genotypes recorded a significant reduction in locule damage compared. Among the Bt genotypes, MECH-184 registered (13.86%) less locule damage followed by MECH-12 and MECH-162.

Boll opening and yield

MECH-184 Bt recorded a maximum of 10.93 GOB/plant and a minimum BOB (1.53/plant), which was significantly superior to standard controls (NHH-44 and DHH-11). MECH-162 Bt and MECH-12 Bt were found to be the next lleast damaged genotypes by recording high GOB and low BOB. However, the non-Bt genotypes recorded a minimum number of GOB/plant and a maximum number of BOB/plant and were at a par with each other (Table 3). MECH-184 Bt recorded a significant higher seed cotton yield (12.13 q/ha) compared to rest of the genotypes. MECH-162 Bt and MECH-12 Bt were the next best Bt genotypes. However, these genotypes were superior to both the non-Bt genotypes as well as the standard controls (DHH-11 and NHH-44). The results of the present investigations are impressive as far as the effect of the Bt-toxin on the bollworms is concerned. The population of the three bollworms has been suppressed in this study, which is similar to the reports of Perlak *et al.* (2001). The suppression of the pink bollworm population is important, as the incidence of this insect is lately increasing in South India and it is difficult to control PBW by insecticides. The Bt transgenic cultivar NuCOTN 33B has also been found to suppress the PBW, irrespective of the growth stage of the crop (Henneberry *et al.,* 2001). The yield advantage of Bt genotypes by suppressing bollworm incidence and reduceing damage to fruiting bodies have been reported by many authors. In one the reports the yield advantage of Bt cultivars over non-Bt found were reported as 10 percent (Perlak *et al.*, 2001). Interestingly, in the present investigations the local control, DHH-11, which is a traditionally bollworm tolerant bred hybrid, were on a par with the MECH-12 Bt genotype, indicating the possibility of added advantage by transforming DHH-11 to a Bt-transgenic. Despite the presence of Bt toxins, the yield levels in the transgenic hybrids were found to dropas the production of Cry protein decreases with crop growth and the season (Olsen and Daly, 2002) and the mortality in early instars also slowly decreases.

Natural enemies

Results indicated (Table 4) that the population of syrphids was significantly higher in MECH-162 Bt compared to the non-Bt genotypes and the standard controls. With respect to the coccinellids and chrysopha populations, there was no significant difference in any of the treatments, indicating the safety of the Bt genotypes to natural enemy population. The safety of Bt transgenic plants to natural enemies, parasites and mammals has been noticed in many studies including reports of Krattiger (1997) and Xue (2002).

Conclusion

Under challenging situations of growing cotton sustainably with successful strategies for insect pest management, Bt transgenics offer a good opportunity. The fact of geographical variation in susceptibility of bollworms to the Cry protein cannot be ignored. Development of specific protection packages for Bt cotton hybrids for different localitiesand pest situations seems to be essential for better utilization of transgenic technology. For better and large-scale cultivation. of Bt transgenic genotypes, it would be a wise decision to accommodate Bt transgenic genotypes under the umbrella of IPM

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Table 1. *Population of sucking pests in Bt the non-Bt genotypes under unprotected conditions.*

Treatments	Sucking pest population/leaf[a]			
	Thrips	Aphids	Jassids	Whitefly
MECH12 Bt	8.53 a	7.05 a	2.02 a	0.72 a
MECH 12 NBt	8.10 a	7.84 a	1.89 ab	0.93 a
MECH 162 Bt	10.80 a	7.53 a	1.47 cd	0.97 a
MECH 162 NBt	9.67 a	8.55 a	1.56 cd	0.97 a
MECH 184 Bt	10.47 a	8.10 a	1.31 d	1.13 a
MECH 184 NBt	10.24 a	6.35 a	1.30 d	1.29 a
DHH-11	9.27 a	6.20 a	1.62 bc	1.23 a
NHH-44	10.03 a	7.04 a	1.65 bc	1.19 a
SEm±	0.22	0.80	0.09	0.91
CD at 5%	NS	NS	0.28	NS
CV (%)	11.94	14.31	15.12	21.44

[a]Figures in a column with the same alphabetical letter does not differ significantly by DMRT (5%).

Table 2. *Population of bollworms in Bt and non Bt genotypes under unprotected conditions and their influence on damage.*

Treatments	Bollworm population[a]			Fruiting body damage (%)[a]	Locule damage (%)[a]
	Heliothis/plant	*Earias* spp/plant	PBW/10 bolls		
MECH 12 Bt	1.18 d	0.10 cd	4.00 d	6.84 e	20.00 d
MECH12 Non-Bt	1.62 b	0.16 a	10.67 a	21.30 a	44.86 b
MECH 162 Bt	1.05 e	0.06 f	2.67 e	5.02 g	22.13 d
MECH 162 Non-Bt	1.97 a	0.11 bc	10.67 a	15.89 b	42.30 b
MECH 184 Bt	0.91 f	0.08 e	2.00 e	4.04 h	13.86 e
MECH 184 Non-Bt	1.45 c	0.12 b	7.34 b	8.06 c	51.70 a
DHH-11	1.19 d	0.09 de	6.10 c	5.94 f	42.66 b
NHH-44	1.37 c	0.12 b	10.67 a	7.07 d	37.63 c
SEm±	0.03	0.00	0.26	0.28	1.09
CD at 5%	0.09	0.01	0.79	0.85	3.28
CV (%)	3.83	5.3	6.73	5.29	13.25

[a]-Figures in the column with same alphabet does not differ significantly by DMRT (5%)

Table 3. Boll opening and seed cotton yield Bt and non Bt genotypes under unprotected conditions.

Treatments	GOB/plant[a]	BOB/plant[a]	Yield (q/ha)[a]
MECH 12 Bt	4.80 c	1.27 c	6.77 c
MECH 12 NBt	4.30 c	2.97 ab	5.34 d
MECH 162 Bt	7.97 b	1.43 c	8.44 b
MECH 162 NBt	5.37 bc	1.90 bc	4.15 d
MECH 184 Bt	10.93 a	1.53 c	12.53 a
MECH 184 NBt	4.13 c	3.00 a	8.12 b
DHH-11	5.97 bc	3.13 a	6.30 c
NHH-44	7.67 b	2.20 abc	5.16 d
SEm±	0.94	0.37	0.34
CD at 5%	2.87	1.12	1.13
CV (%)	25.68	29.56	14.64

[a]Figures in a column with the same alphabetical letter do not differ significantly by DMRT (5%).

Table 4. Predatory insect population in Bt and non Bt genotypes under unprotected conditions.

Treatments	GOB/plant[a]	BOB/plant[a]	Yield (q/ha)[a]
MECH 12 Bt	4.80 c	1.27 c	6.77 c
MECH 12 NBt	4.30 c	2.97 ab	5.34 d
MECH 162 Bt	7.97 b	1.43 c	8.44 b
MECH 162 NBt	5.37 bc	1.90 bc	4.15 d
MECH 184 Bt	10.93 a	1.53 c	12.53 a
MECH 184 NBt	4.13 c	3.00 a	8.12 b
DHH-11	5.97 bc	3.13 a	6.30 c
NHH-44	7.67 b	2.20 abc	5.16 d
SEm±	0.94	0.37	0.34
CD at 5%	2.87	1.12	1.13
CV (%)	25.68	29.56	14.64

[a]Figures in a column with the same alphabetical letter do not differ significantly by DMRT (5%).