

Original Research Article

Field performance of first and second generation Bt cotton events for bollworm resistance in rainfed ecosystem

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ABSTRACT

Field experiment was conducted at Agricultural Research Station, Dharwad farm, Dharwad during 2010-11 to evaluate performance of ten different Bt cotton genotypes representing five different events. Second generation Bt genotypes, Tulasi 4 BG-II and Chiranjeevi-BG-II of MON-15985 event showed high degree of resistance to three species of bollworms keeping them far below ETL. The first generation Bt genotypes representing MON-531, Event-1, GFM and MLS 9124 events received two rounds of sprays for bollworms at 100 and 125 day after sowing. MLS 9124 recorded 2.7 *Helicoverpa armigera* larvae /pl at DAS which was highest among Bt cotton genotypes. *Earias vittella* incidence was > 2 /larvae/pl in all first generation genotypes at later parts of the crop growth. The incidence of pink bollworm larvae was nil green bolls and it ranged from 0.4 -0.8/ 10 green in first generation events against the maximum of 2.8 /10 green bolls in DHH-11 non-Bt. The seed cotton yield was highest in Chiranjeevi BG-II (2210 kg/ha). Among BG-I genotypes JKCH-99 (Event-1) recorded highest yield (1980 kg/ha). The least yield was recorded in DHH-11 (1581 kg/ha) with four rounds of protection against bollworm

Keywords

Bt cotton;
Bollworms;
Events;
Protected;
Unprotected.

Introduction

India is an important grower of cotton on a global scale. It ranks second in global cotton production after China with adaptation of Bt transgenic cultivars widely. Cotton is a very important cash crop for Indian farmers. However, as with many cotton growing countries the major limiting factor in the productivity is damage due to insect pests, especially the

bollworm complex (American bollworm, *Helicoverpa armigera* (Hub.) Spotted bollworm, *Earias vittella* (Fab.) Pink bollworm, *Pectinophora gossypiella* (Saund.). Sucking pests such as aphids (*Aphis gossypi*), jassids (*Amrasca biguttula biguttula*), and whiteflies (*Bemisia tabaci*) are also a problem in terms of direct damage to the plant.

Tobacco caterpillar *S. litura* has been noted as emerging pest in BG-I genotypes in India (Jeyakumar *et al.*, 2007) with advent of Bt genotypes. Thus, the transgenic cotton expressing more than one Bt toxins targeting different insect pest complex has been considered as ideal. Dual gene (stacked) genotypes known as Bollgard-II (BG-II) of MON-15985 event, where Cry2Ab is incorporated along with Cry1Ac have assumed significance which is known to provide season long control of the key bollworms. The major benefit of BG-I cotton genotypes was drastic reduction in the consumption of chemical pesticides and BG-II is expected to be even better in this regard. In US, Australia and India MON- 531 event is being phased out by MON-15985 as IRM strategy. The Bt transgenic events other than these two would also serve as better option to mitigate possible resistance in bollworms. Under Indian conditions, the transgenic cottons have shown great resistance to *H. armigera*, *P. gossypiella*, *E. vittella* under field and laboratory conditions. After five years of Cry1Ac event MON 531 cultivation in India, BG-II hybrids with Cry1Ac and Cry2Ab (MON-15985) have been commercialized to combat the possible resistance in bollworms. Deployment of BG-II genotypes in India also aimed to combat tobacco caterpillar *Spodoptera litura* Fab. a much projected threat in first generation Bt cottons. The other events which express Cry1Ac (Event-1 of JK seeds) has also been released which could efficacy similar to MON 531 BG-I hybrids. Some cultivars having fusion gene (Cry1Ab-Cry1Ac) of Chinese origin event have been approved on commercial scale that have resistance against *S. litura* also (Dong *et al.*, 2004). These genotypes representing different being cultivated simultaneously would render their own efficacy in over

bollworm and spodoptera complex. Thus there was necessity to generate the information on bio-efficacy of these events to key bollworm species and potential pest *S. litura* which has been addressed in the present investigation.

Materials and Methods

The field experiments was carried out at Agriculture Research Station (ARS), Dharwad (Hebballi) farm, Dharwad (Karnataka:India) during 2009-2010, to asses the efficacy of first and second generation intraspecific Bt cotton hybrids of *Gossypium hirsutum*. All commercially approved events of Bt transgenic technology in India were included in the experiment. Thus 10 Bt cotton genotypes representing 5 events (two / event) were selected along with a conventional (non Bt) check DHH-11 (Table 1). These genotypes were compared under two protection regimes *viz*, economic threshold level (ETL) based protection (P) and zero protection (UP) against bollworms to know the efficacy of different Bt events and to estimate the number of avoidable insecticidal sprays. The experiment was laid out in split plot design with two main (P and UP) and eleven subplots (genotypes) replicated thrice. The individual treatment plot size was 5.4 x 6.3 m². The space between subplot treatments was 0.9 m. The main plots and replications were placed 1.8 m apart. Each subplot treatment accommodated six rows with 7 plants/ row and a total of 42 plants per genotype at 90x90 cm spacing.

Sowing of crop was done on July 6th, 2009 in deep black cotton soil with all recommended agronomical practices of northern transitional zone of Karnataka. The plant protection measures for entire experimental field was uniform against

sucking pests and diseases. The seeds were pretreated (at source) with imidacloprid 70% WS / 48% FS to check the incidence of early sucking pests. One application of acetamiprid 20 SP (Pride[®]) @ 0.20 g/l at 43 DAS, Fipronil 5% SC (Regent[®]) @ 1.0 ml/l at 85 DAS and Imidacloprid 200 SL (Confidor[®]) @ 0.2 ml/l at 108 DAS was made based on ETL of *viz.*, thrips, leaf hoppers, mirid bugs and aphids respectively. No protection was rendered against bollworms for any genotype in unprotected (UP) main plots. In the protected main plot (P) the protection against bollworms was offered based on ETL (1.0 larva/plant or 10% fruiting body damage) in each genotype. Accordingly two sprays were given against bollworms i.e. Profenophos 50 EC (Curacron[®]) @ 2.0 ml/l and Indoxacarb 14.5 SC (Avaunt[®]) @ 0.5 ml/l at 102 and 127 DAS for genotypes representing other than MON 15985. For non Bt DHH-11 four sprays were given i.e. Quinalphos 25 EC (Ekalux[®]) @ 2.5 ml/l at 52 DAS (first spray), Profenophos 50 EC (Curacron[®]) @ 2.0 ml/l at 77 DAS (second spray), Indoxacarb 14.5 SC (Avaunt[®]) @ 0.5 ml/l at 102 DAS (third spray) and Deltamethrin 2.8 EC (Decis[®]) @ 0.5 ml/l at 125 DAS (fourth spray). For BG-II genotypes no spray was given as the bollworms could reach ETL any time in the season. To assess the field efficacy of Bt cotton genotypes for their resistance, against bollworms at different crop growth stages of observation were made on the incidence of *H. armigera* (Hub.), *E. vittella* (Fab.), *P. gossypiella* (Saund.) and *S. litura* (Fab.) larvae *viz.*, 50, 75, 100, 125 and 135 DAS. The observations were made on randomly selected 10 plants per genotype avoiding border row plants. The larval incidence of *H. armigera*, *E. vittella* and *S. litura* was on whole plant basis and presented as number of larvae/plant. The damage to fruiting structures

(squares/flowers/bolls) both intact and shed was taken into account to calculate the damage percentage.

Flower rosetting was observed at peak flowering for each genotype by counting the total of number of flowers in ten plants and the number of rosetted flowers amongst them. Data was presented as per cent flower rosetting. For incidence of pink bollworm, 10 green bolls were collected randomly and presented as number of PBW larvae per 10 bolls. However the data has been presented only for 135 DAS as in earlier observations the incidence was nil. Immediately after harvesting of the kapas (seed cotton) the locule damage was estimated considering all the burrs from 10 tagged plants. The data has been presented as per cent locule damage in each genotype.

Before picking of seed cotton data on, number of good opened bolls (GOB's) and bad opened bolls (BOB's) were recorded from 10 randomly selected plants denoting as GOB/plant and BOB/plant. The kapas harvested from each plot (genotype) was extrapolated and presented as seed cotton yield (kg/ha) and also the lint yield (kg/ha) after ginning 1.0 kg of kapas/treatment with the help of lillyput gins.

Results and Discussion

The larval population of American bollworm appeared at low to moderate levels in all the transgenic Bt events throughout the season (Table 2). Until 100 DAS, the bollworms could not cross the ETL in any Bt genotypes compared to the non Bt genotype DHH11 where the population was above ETL at 50 DAS observation itself. In protected plots (P) this genotype received insecticide intervention at 50, 75, 100 and 125 DAS.

Hence, the larval incidence could tend to vary significantly for DHH-11 in two main plots (UP and P) in each subsequent observation. Among different events, MON-15985 hybrids viz, Tulasi 4 BG-II and Chiranjeevi BG-II could suppress *H. armigera* almost to the zero level and thus did not receive protection. In other events also no plant protection was taken up till 100 DAS as the suppression was significant (0.1-0.8 larvae/plant).

After 100 DAS the interaction between *H. armigera* and different Bt transgenic event wise genotypes varied significantly. The incidence was least in BG-II event and highest in MH-5174 (2.8 larvae/plant). In protected main plot larval incidence was above ETL (2.2-2.7 larvae/plant) in all Bt cottons except BG-II genotypes (0.1-0.3 larvae/plant). Hence the first round of insecticide protection was offered to all first generation (BG-I) genotypes and third spray against non Bt DHH-11 to contain *H. armigera* larvae. Both interactions (protection regimes and genotypes) were non significant at this stage (df =20). Despite protection after 100 DAS the larval incidence crossed ETL at 125 DAS warranting one more round of insecticide spray to genotypes belonging to Bt events MON 531, Event-1, GFM and Cry 1C, but not in BG-II cottons. The similar trend was observed in unprotected main plot where both BG-II hybrids had least incidence (0.1-0.2 larvae/plant). The incidence in MH 5125 (4.4/plant) and MH 5174 (4.7/plant) was relatively higher in Cry 1c expressing cottons which was on par with non Bt DHH-11 (4.8/plant). In all first generation events the larval population varied between (3.3to 3.6 / plant) indicating the decrease in cry toxin protein expression by this stage. After 135 DAS the incidence was 5.5 larvae / plant in DHH-11 and 4.5 larvae / plant in 5174.

The comparison across protection levels (intraction-2,df=20) varied significantly for all Bt events except for that of MON 15985 event. After 135 DAS the larval population varied between 0 to 0.9 larvae/plant among genotypes representing all Bt events and conventional cotton DHH-11. There was no significant difference among the genotypes in protected and unprotected main plots (Interaction1).

The spotted bollworm *E. vittella* appeared late in the season. At 100 DAS (table 2) maximum incidence (3.2 larvae/plant) was noticed in DHH-11 unprotected plots. In all hybrids expressing different Bt toxins the incidence ranged from 2.0 to 2.2 larvae/plant. In Tulasi 4 and Chiranjeevi of MON-15985 event *E. vittella* was suppressed to the maximum extent (0.5-0.6 larvae/plant). At 125 DAS also there was no much increase in the incidence compared to 100 DAS, but, the variation was significant for DHH-11(4.1 larvae/plant) and rest of the genotypes. In protected main the population was 0.8 larvae/plant in DHH-11 which was highest compared to the hybrids expressing Cry1Ac, fusion gene and Cry1C as well as Cry 1Ac+2Ab. There was no significant difference between protected and unprotected means for all genotypes including conventional hybrid. At 125 DAS a spray was taken up to control the bollworms incidence below ETL in all BG-I genotypes. However in BG-II genotypes the incidence was below ETL. The larval incidence of pink bollworms observed from destructive sampling could show (table 2) the pest presence considerabley only at later part of the crop growth. At 135 DAS revealed maximum green boll damage was recorded in DHH-11 (2.8/10 bolls). On the contrary no larvae were traced in BG-II events

indicating full proof resistance against PBW. Between protected and unprotected means the data varied significantly only for DHH-11. The incidence of PBW larvae significantly more in all single / fused gene genotypes compared to dual gene hybrids.

As the key bollworm species, *H. armigera* preferred every fruiting part and the damage was almost corresponding to its incidence. At later part of the season, *E. vittella* and PBW contributed to certain extent. The damage did not cross ETL in any hybrids at 50 DAS. The BG-II genotypes (MON15985) were completely free from the damage (Table 3). In other events the damage ranged from 1.4 to 2.6 per cent irrespective of main plots and found to be significantly higher than both BG-II hybrids. The hybrids expressing Cry 1c toxins (MH -5125 and 5174) have shown relatively more (2.6%) damage among different events. The highest damage (6.1%) observed was from DHH-11 (NBt) at 50 DAS. Further at 75 DAS also DHH-11 could show higher damage in unprotected (10.7%) and protected (12.0%) damage which was significant was significant among protection regimes. Both BG-II event hybrids have shown least damage (0.3 to 0.4%). At this crop stage there was significant difference in the damage between BG-II and rest of the BG-I events. After 100 DAS the damage crossed ETL (10%) in all BG-I events and convention hybrid DHH-11 except BG-II events. A range of (10.7 – 11.4 %) damaged was noticed irrespective of main plots in Bt cottons expressing single as well as fusion gene Cry toxins. As high as 17.2% damage was recorded in the unprotected main plot of DHH-11 against 10.2% in protected plot which was significantly high over the rest of genotypes and amongst as per interaction

1 and 2. The damage could not be different significantly for MON-531, GFM, JK and MLS 9124 events in both main blocks (interaction 1). BG-II hybrids have shown the significantly least damage in the both main plots. At 125 the damage was as low as 0.9 % in BG-II hybrids. On the contrary in MH - 5125 (19.1%) and DHH-11 (22.4%) in the absence of protection the damage huge.

The damage by pink bollworm incidence at peak flowering indicated by percentage of rosetted flowers (table 3) was least in the BGII genotypes (0.2%) while in BG-I genotypes it ranged from 0.2 to 0.6 percent. DHH-11 recorded 3.8 and 1.6 percent damage in UP and P conditions respectively. The damage due to PBW was also extracted as locule damage (%) after harvest and DHH-11 has shown highest damage (23.2%) in unprotected main plot which was reduced to significantly (11.4%) in protected main plot. Among Bt events in protected condition MH -5174 (14.1%) and JK-Durga, Tulasi 4 Bt, Vishwanath, MH-5125 were on par to it. In protected condition there was no significant difference among genotypes belonging to MON-531, Event-1, Cry 1c and GFM where damage ranged between 3.9 to 5.1%. BG-II event hybrids had least (1.5 to 3.1%) locule damage. The variation in locule damage was significant between two main plots for each genotype (interaction 2) as well for any cross comparisons.

As a parameter of better bollworm suppression the maximum good open bolls GOB's (31.7/plant) were recorded from DHH-11 (NBt) and least in JK-Durga (23.3/pl) where ETL based protection was rendered, however, the data (table 3) did not very significantly among genotypes in protected main (P) blocks. On the contrary

the range of GOB/pl varied from 19.1 (Chiranjeevi) to 27.2 (JKCH-99). In unprotected plot GOB count varied significantly between different genotypes and also with protected plots. The genotypes with dual gene efficacy had similar load of GOBs in both main plots. The number of bad open bolls (BOBs) was least (2.9/pl) in unprotected Chiranjeevi BG-II followed Tulasi-4 BG-II. The maximum BOB's were recorded in DHH-11 (10.2/pl). In other genotypes BOB count was upto 9.5 /pl (MH-5174) however the data did not very significantly. The comparison between two main plots (interaction-2) indicated significantly difference for all genotypes / events except BG-II and DHH-11.

Further, there was a significant difference between protected and unprotected main plots for each genotype (Table 3) for yield. The highest seed cotton yield was harvested from Chiranjeevi BG-II ie 2210 and 2187 kg/ha in protected and unprotected main plots respectively. Since no protection was offered in BG-II events the variation existing could be natural. Similarly Tulasi 4 BG-II recorded 1846 kg/ha (protected) and 1829 kg/ha (Un protected) seed cotton. Among other Bt genotypes JKCH-99 recorded 1980 kg/ha in protected main plot which was maximum after Chiranjeevi BG-II. It was followed by Chiranjeevi BG-I 1840 kg/ha (protected). In unprotected condition the maximum yield (1681 kg/ha) after BG-II event was harvested from JKCH-99 which was significant superior over the rest and including DHH-11 (1150 kg/ha). The lint yields were corresponding to seed cotton yield. Under protected conditions the least yield was recorded from DHH-11 (1581 kg/ha). In unprotected condition the maximum yield (except BG-II events) was recorded from JKCH 99 (1681 kg/ha)

which was significantly superior over rest including DHH-11 with 1150 kg/ha the least mean in yields. The lint yields were corresponding to seed cotton yield. Since the cultivars selected for experiment were not isohybrids yield variation, bollworms incidence and damage amongst would not be ideal for comparisons to draw conclusion on the event efficacy. However the difference between two main ploys (P and UP) for respective genotypes indicate the acceptable results. The yield largely dependent on no of bolls/plant, boll size and indicates boll weight. The resistance offered by Bt toxin against bollworms have significant impact on yield.

The advantages of Bt cotton genotypes have been well proved throughout the world by this time. Since its inception from 1996 in US and Australia with 50 million ha, the area occupied by Bt cotton has been increasing globally. Similarly in India with approval of three genotypes in 2002, area of Bt cotton has covered from an initial 24,000 ha to >10.0 m.ha now.

Since the insecticide resistance in bollworms particularly Heliothis was very much alarming, development and deployment of transgenic Bt technology was aimed to contain *Helicoverpa zea*, *Heliothis virescens* and *Helicoverpa armigera* worldwide through the most toxic delta endotoxin expressed by Cry1Ac gene. Thus MON-531 event has reached every nuke and corner of the world suppressing dreaded bollworms. These genotypes producing Cry1Ac protein also found effective to *Earias vittella*, *Earias insulana* and *Pectinophora gossypiella* which are major insect pests in India also. However the first generation Bt cotton genotypes with Cry1Ac toxin were not able to provide full protection for

entire season, which showed buildup of bollworm population in later part of the season. Since the Cry toxin families produced by *Bacillus thuringiensis* (Berlinier) are target specific, it was accepted that the tobacco caterpillar *Spodoptera litura*, fall armyworm *Spodoptera frugiperda* (Smith) would emerge as key pests in long term cultivation and wide scale adoption of Bt cottons (Singh *et al.*, 2008, Stewart *et al.*, 2001). In India the bollworm complex though dominated by *H. armigera*, incidence of other species *E. vittella*, *E. insulana* and *P. gossypiella* has been predicted as considerable since long (Qiam and Zilberman, 2002). The incidence of *Spodoptera litura* has also historical (but not regular) association with cotton cultivation in India (Jeyakumar *et al.*, 2007). Due to large scale adoption of first generation Bt cottons in India the variable efficacy against different Lepidopterans was expected to create problem. The development of resistance to Cry1Ac (Kranthi *et al.* 2001, Tabashnik *et al.*, 2002) has been considered as quite natural phenomenon for *H. armigera* and other bollworms worldwide considering the biotic potential of this insect and the pattern of selection pressure. However, so far no control failures have been experienced in Bt cotton anywhere in the world. This could be mainly due to high dose strategy and refugia compulsions adopted. But, the variable response of bollworms, decline in expression over season, survival of pest in first generation Bt cotton emerged as bottleneck in sustainability of Bt technology dominated by Cry 1 Ac toxin. This led the way for emergence of second generation Bt genotypes expressing ry1Ac+Cry2Ab. The Cry2Ab gene in Bollgard II enhance *H. armigera* mortality and provides equally good control of tobacco caterpillar *S*

litura, fall armyworm *S. frugiperda*, beet armyworm *S. exigua*, cabbage looper *Trichoplusia ni*, and soybean looper *Pseudoplusia includens*, which are likely to survive from Cry1Ac (Stewart *et al.*, 2001). Besides dual gene genotypes with the alternate Bt toxins have been considered as better options to contain the resistance. The major candidates in the list are Cry1A (Fusion gene), Cry1F, Cry1a5, Vip Cry1C and Cry1E etc. Hence the genotypes belonging to MON 15983 expressing Cry1Ac+Cry2Ab have been approved in USA, Australia (2002) and India (2006). Though Bt transgenics entered India with MON-531 during 2002, the diversity of events deployed in the country is more significant over rest of the world. Despite larger adoption and significant benefits through MON-531 the Bollgard-II genotypes entered Indian cotton scenario. Almost during same period the best bet indigenous Bt technology of China *i.e.* GFM event expressing Cry1Ac+Cry1Ab (Cry1A) also entered India. The Indian origin Cry1Ac event developed at IIT Kharkpur (event-1) was also approved on commercial scale in 2006. Later Metahelix events (MH) expressing Cry1C have also been approved during 2009 respectively. This creates a mosaic option for activity of bollworms in the field for which variable level of toxicity would be made available. Though provision of alternate events in a geographical area would hinder the development of resistance, the survival of insects on weaker events as well as survival of insect species for which particularly event does not specify efficacy would lead to a complex situation. In India the *H. armigera*, *S. litura* and *Earias spp.* have many alternate hosts for their survival which could provide platform for accumulation of resistant alleles in these species. Thus, in order to generate the

information on efficacy of different events used in India against bollworm complex and tobacco caterpillar a field investigation was carried out at Dharwad. In the present study *H. armigera* larval population increased slowly from square formation (50 DAS) to boll maturity (125 DAS) across the Bt cotton genotypes later decreased reaching minimum at 135 DAS. Throughout cropping period both BG-II genotypes could not allow *H. armigera* to cross ETL and in other Bt genotypes the incidence crossed ETL by 100 DAS. In conventional hybrid DHH-11 larval incidence was above threshold from 50 DAS itself warranting four rounds of spray in the season. Two rounds of insecticides were applied for BG-I genotypes at 100 and 125 DAS in the protected plots to check the incidence. The incidence of *E. vittella* also crossed ETL in all the genotypes of BG-I at 100 DAS in unprotected conditions except BG-II hybrids. In DHH-11 the incidence was highest. Due to plant protection the incidence was checked significantly in all genotypes. BG-II hybrids could contain the incidence on their own through inbuilt toxicity.

The present findings are in close agreement with Burd *et al.* (1999), Rao *et al.* (2002), Gore *et al.* (2002), Jackson *et al.* (2003), Vennila *et al.* (2004) and Radhika *et al.* (2004) and justify better results obtained in the Bollgard-I genotypes with one or two sprays. The average larval load of *H. armigera* was negligible in BG-II cottons compared BG-I and Non Bt hybrids in a earlier study at Dharwad (Udikeri *et al.*, 2011) The BG-II events viz., Tulasi 4BG-II and Chiranjeevi BG-II (MON15985) recorded less fruiting body damage throughout cropping period (0.0 to 1.5%) and remained superior all other genotypes (Fig. 1, Table 2). There

was significant difference in damage between protected and unprotected plots of each Bt cotton genotype except BG-II hybrids. This clearly indicated the superiority of BG-II (Cry1AC + Cry2Ab MON-15985) toxins over Cry1Ac, Cry1A and Cry1C. Further all the Bt genotypes (BG-II and BG-I) recorded minimum damage as compared to non Bt genotype DHH-11. The present observations are in close agreement with Gore *et al.* (2002), Chitkowski *et al.* (2003), Jackson *et al.* (2003a), Jackson *et al.* (2003b), Udikeri *et al.* (2011), and Somashekara (2009) who reported significantly less damage in BG-II genotypes compared to BG-I genotypes.

The present study was quite convincing about Bt toxin efficacy against pink bollworm also as greenboll incidence, flower resetting and locule damage was negligible in BG-II, least in first generation Bt cottons of different events and significantly high in non-Bt cotton DHH-11. Thus all events presently under cultivation in India proved quite effective in suppressing PBW. It was also evident from the study that pink would be detrimental for cotton production if not checked through either Bt toxins or insecticides. The present findings are in close agreement with Gujar (2001) who reported the pink bollworm high level susceptibility to the Cry 1Ac protein. The effectiveness of Bt genotypes (Cry1Ac) against PBW has been reported by Bhosle *et al.* (2004) (less locule damage), Henneberry and Jech (2000) (less green boll infestation) and Wilson *et al.* (1992) (least rosetting) in Bt cotton plants. Udikeri *et al.*, (2011) also observed significantly low rosette flowering, locule damage and less incidence of PBW larvae in BG-II genotypes compared to BG-I and conventional genotypes. In a seasonlong

Table.1 The Bt and conventional cotton genotypes used in the study

Genotype/Cultivar	Bt toxin/Toxin	Transgenic events	Source
Tulasi 4 BG-I	Cry 1Ac	MON-531	Tulasi Seeds Pvt Ltd, Guntur
Chiranjeevi BG-I	Cry 1Ac		Sri Ram Bio Seed Genetics, Hyderabad
Tulasi 4 BG-II	Cry 1Ac + Cry 2Ab	MON-15985	Tulasi Seeds Pvt Ltd, Guntur
Chiranjeevi BG-II	Cry 1Ac + Cry 2Ab		Sri Ram Bio Seed Genetics, Hyderabad
JK Durga	Cry 1Ac	Event-1	JK- Agri Genetics Ltd, Hyderabad
JKCH-99	Cry 1Ac		JK- Agri Genetics Ltd, Hyderabad
Nathbaba	Cry1Ab - Cry1Ac	Chinese event (GFM)	Nath Biogene Ltd, Aurangabad
Vishwanath	Cry1Ab - Cry1Ac		Nath Biogene Ltd, Aurangabad
MH 5125	Cry 1C	MLS 9124	Metahelix Life Sciences, Bangalore
MH 5174	Cry 1C		Metahelix Life Sciences, Bangalore
DHH-11	Non Bt	Non Bt (Conventional)	UAS,Dharwad hybrid seed produced by Karnataka hi-tech Agro Enterprises, Gadag

dynamics study with respect to PBW (Nadaf and Goud ,,2007) the Bt-cottons had less incidence compared to non-Bt hybrids. Marchovsky *et al.* (2001) reported that Bollgard and Bollgard-II bolls had consistently fewer PBW larvae. All the Bt genotypes recorded less green boll infestation as compared to conventional non Bt hybrids. Since the cultivars selected for present experiment were not

iso-hybrids, relating the yield variation with incidence of bollworms and damage amongst would not be ideal. The yield largely depends on the fruit bearing ability, boll size and weight of individual bolls at harvest and loss in the fruiting bodies in each event would also matter much. Thus resistance offered by different Bt events against bollworms has been considered to have significant impact on

Table.2 Larval incidence of bollworms in different cotton genotypes under protected (P) and unprotected (UP) conditions

Genotypes	<i>H.armigera</i> larvae/plant at										<i>E.vittella</i> larvae /plant at				Pinkbollworm larvae/10green bolls	
	50 DAS		75 DAS		100 DAS		125 DAS		135 DAS		100 DAS		125 DAS			
	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
JK Durga	0.2 (1.1)	0.1 (1.0)	0.3 (1.1)	0.5 (1.2)	2.2 (1.8)	2.3 (1.8)	3.3 (2.1)	1.4 (1.5)	4.2 (2.3)	0.6 (1.3)	2.1 (1.8)	1.1 (1.4)	2.2 (1.8)	0.3 (1.1)	0.4 (1.2)	0.3 (1.1)
JKCH 99	0.2 (1.1)	0.3 (1.1)	0.4 (1.2)	0.5 (1.2)	2.3 (1.8)	2.5 (1.9)	3.4 (2.1)	1.3 (1.5)	4.2 (2.3)	0.8 (1.3)	2.2 (1.8)	1.0 (1.4)	1.8 (1.7)	0.4 (1.2)	0.6 (1.3)	0.4 (1.2)
Tulasi 4 Bt	0.0 (1.0)	0.1 (1.0)	0.3 (1.1)	0.6 (1.3)	2.6 (1.9)	2.3 (1.8)	3.3 (2.1)	1.3 (1.5)	4.3 (2.3)	0.7 (1.3)	2.2 (1.8)	1.1 (1.4)	2.1 (1.8)	0.6 (1.3)	0.5 (1.2)	0.4 (1.2)
Tulasi 4 BG-II	0.0 (1.0)	0.0 (1.0)	0.1 (1.0)	0.0 (1.0)	0.1 (1.0)	0.3 (1.1)	0.1 (1.0)	0.0 (1.0)	0.2 (1.1)	0.0 (1.0)	1.0 (1.4)	0.5 (1.2)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
Chiranjeevi Bt	0.3 (1.1)	0.2 (1.1)	0.3 (1.1)	0.5 (1.2)	2.3 (1.8)	2.2 (1.8)	3.5 (2.1)	1.3 (1.5)	4.3 (2.3)	0.6 (1.3)	2.2 (1.8)	1.1 (1.4)	1.8 (1.7)	0.3 (1.1)	0.8 (1.3)	0.4 (1.2)
Chiranjeevi BG-II	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.2 (1.1)	0.1 (1.1)	0.2 (1.1)	0.0 (1.0)	0.3 (1.1)	0.0 (1.0)	1.0 (1.4)	0.6 (1.2)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
DHH 11	1.8 (1.7)	2.0 (1.7)	2.8 (1.9)	1.9 (1.7)	2.5 (1.9)	2.2 (1.8)	4.8 (2.4)	1.8 (1.7)	5.5 (2.5)	0.8 (1.3)	3.2 (2.0)	1.6 (1.6)	4.1 (2.3)	0.8 (1.3)	2.8 (1.9)	1.2 (1.5)
Nathbaba	0.1 (1.0)	0.1 (1.0)	0.8 (1.3)	0.9 (1.4)	2.7 (1.9)	2.5 (1.9)	3.5 (2.1)	1.3 (1.5)	4.3 (2.3)	0.5 (1.2)	2.2 (1.8)	1.1 (1.4)	2.2 (1.8)	0.4 (1.2)	0.4 (1.2)	0.3 (1.2)
Vishwanath	0.3 (1.1)	0.2 (1.1)	0.5 (1.2)	0.7 (1.3)	2.4 (1.8)	2.6 (1.9)	3.6 (2.1)	1.5 (1.6)	4.3 (2.3)	0.7 (1.3)	2.1 (1.8)	1.1 (1.4)	2.1 (1.8)	0.6 (1.3)	0.4 (1.2)	0.3 (1.1)
MH-5125	0.3 (1.1)	0.2 (1.1)	0.9 (1.4)	0.8 (1.3)	2.5 (1.9)	2.7 (1.9)	4.4 (2.3)	1.4 (1.5)	4.4 (2.3)	0.9 (1.4)	2.2 (1.8)	1.1 (1.4)	2.2 (1.7)	0.7 (1.3)	0.5 (1.2)	0.4 (1.2)
MH-5174	0.2 (1.1)	0.4 (1.2)	0.7 (1.3)	0.9 (1.4)	2.8 (1.9)	2.5 (1.9)	4.7 (2.4)	1.5 (1.6)	4.5 (2.3)	0.8 (1.3)	2.1 (1.8)	1.1 (1.4)	2.2 (1.8)	0.5 (1.2)	0.4 (1.2)	0.3 (1.1)
Mean	0.3 (1.1)	0.3 (1.1)	0.6 (1.3)	0.7 (1.3)	2.1 (1.7)	2.0 (1.7)	3.2 (2.0)	1.2 (1.5)	3.7 (2.1)	0.6 (1.3)	2.0 (1.7)	1.0 (1.4)	1.9 (1.7)	0.4 (1.2)	0.6 (1.2)	0.4 (1.2)
	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)
Protection	0.01	0.03	0.03	0.09	0.01	0.03	0.04	0.11	0.03	0.09	0.02	0.06	0.02	0.06	0.01	0.03
Genotypes	0.04	0.11	0.06	0.17	0.08	0.23	0.09	0.25	0.09	0.25	0.08	0.23	0.08	0.23	0.04	0.11
Interaction 1	0.06	0.17	0.09	0.24	0.12	0.33	0.12	0.34	0.15	0.41	0.12	0.34	0.11	0.31	0.06	0.17
Interaction 2	0.06	0.17	0.07	0.20	0.08	0.23	0.16	0.42	0.19	0.57	0.21	0.58	0.21	0.58	0.11	0.31

Figures in the parentheses are square root ($x + 1$) transformation

Interaction 1: CD for comparison between two genotype means at the same protection

Interaction 2: CD for comparison between two protection means at the same or different genotypes

Table.3 Fruiting body damage (%) by bollworms in different cotton genotypes under protected (P) and unprotected (UP) conditions

Genotypes	Damage due to <i>Helicoverpa armigera</i> incidence*										Damage by <i>Pectinophora gossypiella</i>			
	50 DAS		75 DAS		100 DAS		125 DAS		135 DAS		Flower rosetting		Locule damage	
	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
JK Durga	1.7 (7.5)	1.8 (7.7)	3.8 (11.2)	4.0 (11.5)	11.0 (19.4)	11.4 (19.7)	14.5 (22.4)	11.2 (19.5)	9.0 (17.5)	3.4 (10.6)	0.2 (2.6)	0.3 (3.1)	12.7 (20.9)	4.3 (12.0)
JKCH 99	1.4 (6.8)	1.8 (7.7)	4.1 (11.7)	3.8 (11.2)	10.5 (18.9)	10.8 (19.2)	15.8 (23.4)	11.6 (19.9)	8.8 (17.2)	3.0 (10.0)	0.5 (4.1)	0.4 (3.6)	11.3 (19.6)	4.2 (11.8)
Tulasi 4 Bt	1.4 (6.8)	1.6 (7.3)	4.4 (12.1)	4.6 (12.4)	10.8 (19.2)	10.7 (19.1)	15.2 (22.9)	11.5 (19.8)	8.8 (17.2)	3.4 (10.6)	0.4 (3.6)	0.3 (3.1)	13.5 (21.5)	4.1 (11.4)
Tulasi 4 BG-II	0.0 (0.0)	0.0 (0.0)	0.5 (4.0)	0.3 (3.1)	0.6 (4.4)	0.8 (5.1)	1.0 (5.7)	1.2 (6.3)	1.5 (7.0)	1.0 (5.7)	0.0 (0.0)	0.1 (1.8)	2.9 (9.8)	3.1 (10.1)
Chiranjeevi Bt	1.4 (7.2)	1.6 (7.4)	4.2 (11.8)	4.0 (11.5)	11.2 (19.5)	10.7 (19.1)	14.4 (22.3)	11.8 (20.1)	9.1 (17.6)	3.2 (10.3)	0.5 (4.1)	0.4 (3.6)	10.4 (18.8)	5.1 (13.0)
Chiranjeevi BG-II	0.0 (0.0)	0.0 (0.0)	0.3 (3.1)	0.4 (3.6)	0.5 (4.1)	0.7 (4.8)	0.9 (5.4)	1.0 (5.7)	1.2 (6.3)	1.4 (6.8)	0.1 (1.8)	0.2 (2.6)	2.3 (8.7)	1.5 (7.0)
DHH 11	5.0 (12.9)	6.1 (14.3)	12.0 (20.3)	10.7 (19.1)	17.2 (24.5)	10.2 (18.6)	22.4 (28.2)	10.9 (19.3)	16.7 (24.1)	4.7 (12.5)	3.8 (11.2)	1.6 (7.3)	23.2 (28.8)	11.4 (19.7)
Nathbaba	1.5 (7.0)	1.6 (7.3)	3.8 (11.2)	4.2 (11.8)	10.6 (19.0)	10.8 (19.2)	13.5 (21.5)	11.7 (20.0)	8.7 (17.1)	3.1 (10.1)	0.6 (4.4)	0.4 (3.6)	10.7 (19.1)	4.7 (12.5)
Vishwanath	1.7 (7.5)	1.9 (7.9)	4.0 (11.5)	4.4 (12.1)	10.5 (18.9)	10.8 (19.2)	14.2 (22.1)	11.5 (19.8)	8.8 (17.2)	3.8 (11.2)	0.3 (3.1)	0.4 (3.6)	11.9 (20.2)	4.3 (12.0)
MH-5125	2.1 (8.3)	2.2 (8.5)	6.5 (14.8)	7.0 (15.3)	11.2 (19.5)	11.1 (19.5)	19.1 (25.9)	11.7 (20.0)	9.1 (17.6)	4.1 (11.7)	0.4 (3.6)	0.5 (4.1)	13.2 (21.3)	3.9 (11.4)
MH-5174	2.2 (8.5)	2.6 (9.3)	6.9 (15.2)	6.7 (15.0)	10.8 (19.1)	10.7 (19.1)	17.0 (24.3)	12.2 (20.4)	8.7 (17.1)	4.2 (11.8)	0.6 (4.4)	0.3 (3.1)	14.1 (22.0)	4.4 (12.1)
Mean	1.7 (6.6)	1.9 (7.0)	4.6 (11.5)	4.6 (11.5)	9.5 (17.0)	9.0 (16.6)	13.5 (20.4)	9.7 (17.4)	8.2 (16.0)	3.2 (10.1)	0.7 (3.9)	0.3 (3.6)	11.5 (19.2)	4.6 (12.1)
	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)	SEm±	CD (5%)
Protection	0.03	0.09	0.34	0.94	0.51	1.41	0.50	1.39	0.40	1.11	0.01	0.06	1.39	3.85
Genotypes	0.34	0.94	1.04	2.88	1.03	2.85	0.99	2.74	1.02	2.82	0.11	0.32	0.61	1.69
Interaction 1	0.48	1.33	1.48	4.09	1.45	4.01	1.41	3.90	1.44	3.98	0.16	0.46	0.87	2.41
Interaction 2	0.25	0.69	0.29	0.81	0.29	0.81	0.78	2.16	0.72	1.99	0.17	0.13	0.54	1.50

* The data of 100 and 125 DAS may have contribution from *Earias vittella* also to little extent DAS: Days after sowing UP: Unprotected condition P: Protected condition
Figures in the parentheses are arc sine transformation

Interaction 1: CD for comparison between two genotype means at the same protection

Interaction 2: CD for comparison between two protection means at the same or different genotypes

Table 4 Boll opening, seed cotton and lint yield in different Bt cotton genotypes under protected and unprotected conditions

Genotypes	GOB/Plant		BOB/Plant		Seed cotton yield (kg/ha)		Lint yield (kg/ha)	
	UP	P	UP	P	UP	P	UP	P
JKDurga	19.2 (4.5)	23.3 (4.9)	7.2 (2.9)	3.9 (2.2)	1397	1675	562	671
JKCH 99	27.2 (5.3)	32.2 (5.8)	6.8 (2.8)	3.6 (2.1)	1681	1980	642	757
Tulasi 4 Bt	20.5 (4.6)	27.4 (5.3)	7.6 (2.9)	4.1 (2.3)	1159	1558	473	636
Tulasi 4 BG-II	28.2 (5.4)	31.3 (5.7)	3.5 (2.1)	2.9 (2.0)	1829	1846	726	733
Chiranjeevi Bt	19.1 (4.5)	22.9 (4.9)	7.4 (2.9)	3.7 (2.2)	1431	1840	524	674
Chiranjeevi BG-II	25.9 (5.2)	27.5 (5.3)	2.9 (2.0)	2.5 (1.9)	2187	2210	792	801
DHH 11	21.3 (4.7)	30.8 (5.6)	10.2 (3.3)	8.1 (3.0)	1150	1581	465	639
Nathbaba	25.8 (5.2)	31.7 (5.7)	8.1 (3.0)	3.5 (2.1)	1445	1765	526	642
Vishwanath	24.2 (5.0)	29.3 (5.5)	7.8 (3.0)	3.8 (2.2)	1410	1742	547	676
MH-5125	20.9 (4.7)	26.1 (5.2)	9.3 (3.2)	6.6 (2.8)	1389	1640	517	610
MH-5174	22.5 (4.8)	30.5 (5.6)	9.5 (3.2)	5.8 (2.6)	1367	1745	524	641
Mean	23.2 (4.9)	28.5 (5.4)	7.3 (2.9)	4.4 (2.3)	1495	1780	573	680
	S.Em±	CD (5%)	S.Em±	CD (5%)	S.Em±	CD (5%)	S.Em±	CD (5%)
Protection	0.06	0.17	0.12	0.34	73.08	443.35	3.59	9.9
Genotypes	0.23	0.64	0.15	0.42	37.11	105.75	8.90	24.6
Interaction 1	0.32	0.89	0.21	0.58	52.48	149.56	12.58	34.8
Interaction 2	0.14	0.39	0.24	0.62	40.0	110.5	3.72	10.3

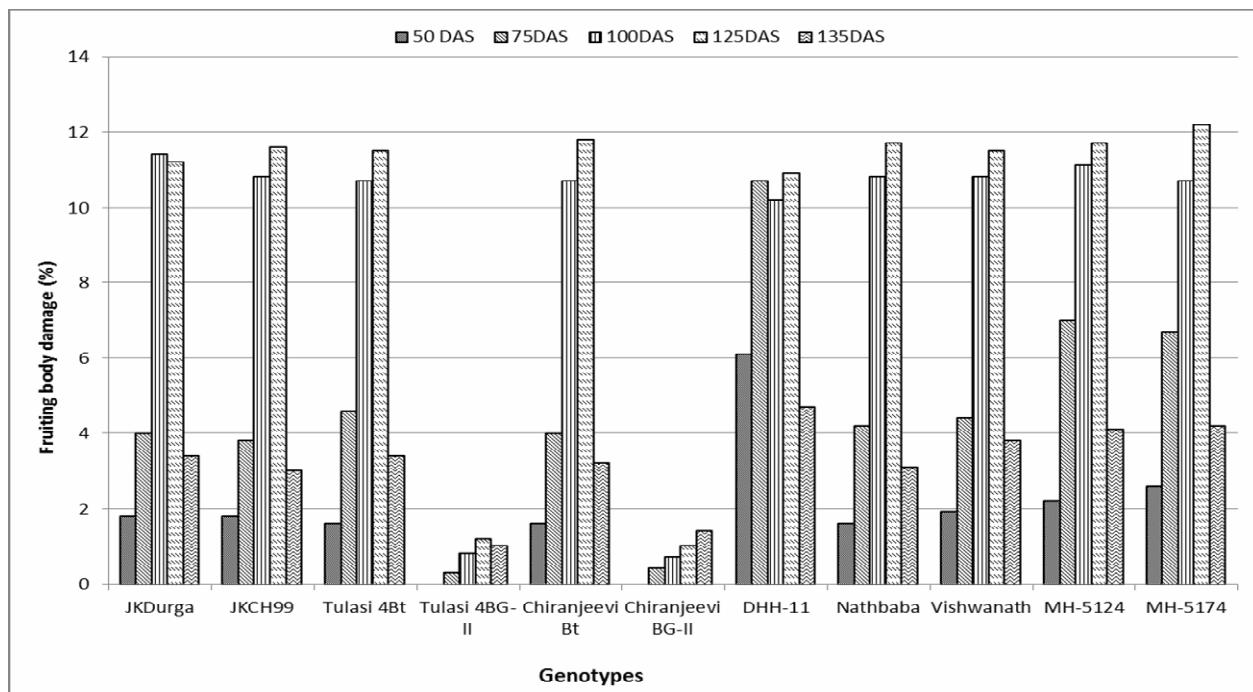
DAS: Days after sowing UP: Unprotected condition P: Protected condition

Figures in the parentheses are $\sqrt{x} + 1$ transformation

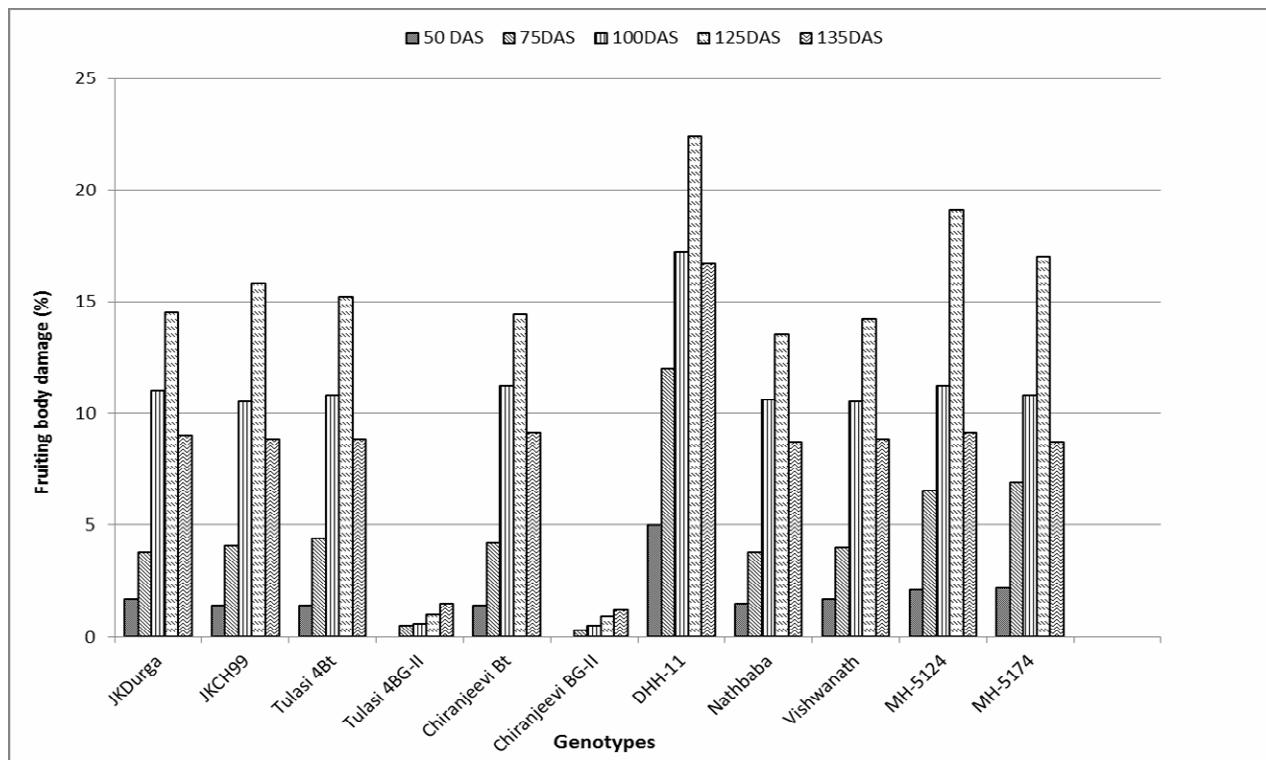
Interaction 1: CD for comparison between two genotype means at the same protection

Interaction 2: CD for comparison between two protection means at the same or different genotypes

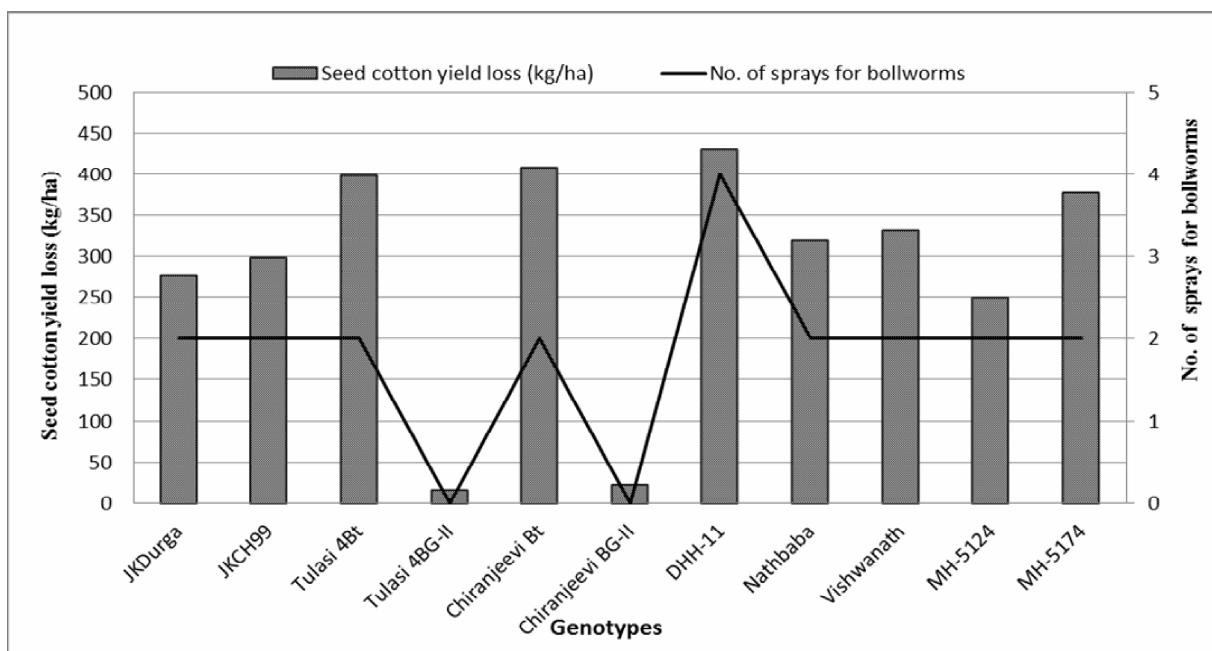
Fig.1 Damage due to bollworms in different Bt cotton genotypes



a) Damage in unprotected conditions



b) Damage in protected conditions

Fig.2 Number of sprays and avoidable yield loss in different Bt cotton genotypes

yield. The highest yield was recorded in JKCH-99 (1681 kg/ha) in unprotected main plot and 1980 kg/ha in protected main plot. The lowest yield was from Tulasi 4BG-I (1159 kg/ha) in unprotected main plot and 1558 kg/ha in protected main plot among Bt cottons. However, DHH-11 recorded 1150 kg/ha unprotected main plot and 1581 kg/ha in protected main plot. BG-II (MON-15985 Tulasi 4BG-II and Chiranjeevi BG-II) recorded 1829 kg/ha unprotected main plot 1846 kg/ha protected main plot and 2187 kg/ha unprotected main plot and 2210 kg/ha protected main plot respectively. The exact influence of Bt events on seed cotton yield has been extrapolated through avoidable loss (Fig.2) in respective genotypes by comparing the yield in protected and un protected situations. The yield advantage of BG-II genotype is negligible as no insecticide intervention was offered. But in others, which had varied levels of protection, avoidable yield loss was significant. Thus maximum

avoidable yield loss recorded (431 kg/ha) was in DHH-11. It was followed Chiranjeevi Bt (409 kg/ha), Tulasi 4 Bt (399 kg/ha) and the rest. The Bt genotypes belonging to second generation Tulasi 4BG-II and Chiranjeevi BGII recorded at par yield under protected and unprotected condition indicating the inclusion of one more toxin producing gene strengthened the protection. Further it could not require any additional protection against bollworms. In the present study BG-I and BG-II genotypes could save two and four insecticidal sprays respectively. This has been justified in the earlier reports of Udikeri *et al.*, (2011) and Somashekara (2009).

The present field investigation justified the better performance of MON 15985 event (Cry1Ac+2Ab) over other events including MON 531(Cry 1Ac). The high degree of resistance by dual toxins to a range of insect pests viz. *H. zea*, *S. frugiperda*, *S. exigua* has been reported by

Stewart *et al.*, (2001) long back who suggest this as future technology in place of Cry 1Ac. Jackson *et al.* (2003b) also strongly justify overhauling performance of stacked Bt genes. Due to lack of literature the results of present investigation with respect to GFM/Event-1/MH events have not been discussed much. However Cry 1 Ac expressing events (MON 531 and Event-1) as well as GFM events were on par in majority parameters of pest incidence and yield. Presently in China GFM is the ruling event where in bollworm and *S. litura* incidence status is almost similar to our country. The number of genotypes belonging to GFM and Event -1 are increasing year by year in India. However going for stacked gene option with Cry 2Ab in case of Event-1 (Cry 1 Ac) has been suggested for sustainability of Bt technology. It could be ideal for MH event to have Cry Ac combination for better control over Heliothinae.

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