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Field performance of second generation (BG-II) Bt cotton genotypes against bollworm complex under rainfed conditions

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Abstract

A field experiment was conducted at Main Agricultural Research Station, Dharwad, Dharwad (Karnataka, India) to investigate the performance of second generation Bt cotton genotypes under rainfed condition. All second generation Bt genotypes with *cry1Ac + cry2Ab* genes have shown high level of resistance to all the three species of bollworms. The incidence of bollworms did not cross economic threshold in BG-II hybrids. First generation Bt genotypes with *cry1Ac* genotypes bollworms crossed economic threshold level for two times. Compared with BG-I and non Bt genotypes all the BG-II genotypes were found to be better with respect to larval incidence and damage by bollworms.MRC-7351 BG-II recorded highest seed cotton yield of 20.37 q/ha being at par with KDCHH-621 BG-II (19.75), MRC-7201 BG-II (19.13), and Bunny Bt BG-II (18.60), but superior to BG-I genotypes without any protection against bollworms.

Key words: Bt cotton, Bollworms, *cry1Ac*, *cry1Ac + cry2Ab*.

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Introduction

Cotton is one of the most important commercial crops in India. Several lepidopteran pests present a major threat to economical production of cotton. Due to indiscriminate use of deadly insecticides not only cause the hazardous to environment but also contributed to the development of resistance in insect species (Srinivas et al., 2004). Insects are well known for their inherent character of developing resistance against insecticides. The use of refugia to mitigate the expected resistance development found to be inconvenient and later two genes *viz.*, *cry*1Ac + *cry*2Ab (referred as Bollgard-II) concept came in to existence. The genotypes used in India until 2006 belonged to first generation Bt cottons having *Cry 1Ac* gene only. The genotypes having more than one gene are popular in Australia and USA as a tactic of possible resistance management to *Cry* proteins (Udikeri. 2006). Hence, the present investigation was carried out to assess the performance of new Bt cotton genotypes of two genes (*Cry 1Ac + 2Ab*) which are popularly called as second generation Bt cotton.

Materials and methods

Field experiments were conducted during 2007-08 at Main Agricultural Research Station, Dharwad. The experiment consisted of six new second generation Bt transgenic hybrids with *cry*1Ac + *cry*2Ab, two first generation Bt transgenic hybrids with *cry*1Ac and two non Bt cotton hybrids (Table, 1). The experiment was laid out in RBD design with three replications having ten genotypes as treatments. The size of each experimental plot was 5.4 m x 4.5 m. The space between treatments was 0.6m and replications were placed 1m apart. All plots were non-irrigated and maintained using the standard package of practice (Anonymous, 2006). The plant protection measure for the entire experimental setup given was uniform against sucking pests. Before sowing, the seeds of each genotype were treated with Imidacloprid 70 WS @ 10.0 g /kg to check the incidence of sucking pests. Later two applications of acetamaprid 20 SP @ 10 g ai/ha were given at 60 and 110 DAS (Days after sowing) to check the buildup of thrips and also to take care of trace incidence of leaf hoppers based on ETL (Kulkarni et al., 2003). Care was also taken to avoid square and bolls loss drop due to emerging pest mirid bug incidence without affecting bollworm incidence (Udikeri et al., 2008). There was absolutely no protection rendered against bollworms for any genotype with an aim to know the season long incidence and damage due to bollworms and its influence on yield of seed cotton under no protection as suggested by Udikeri et al., (2003). Performance of second generation Bt cotton genotypes for their resistance to bollworms deserved various season long observations on different insect related parameters in each genotype under unprotected condition. The layout and observations protocols were similar to Udikeri et al., (2011). All the observations were made on randomly selected 10 plants per genotype avoiding border row plants. The larval incidence of spotted bollworm *Earias vittella* (Fab.) was recorded on 50 and 65 DAS

on whole plant basis in each genotype. Similarly incidence of *Helicoverpa armigera* (Hub) larvae was also made on whole plant basis at 65, 80, 95, 110, 125 and 140 DAS. However the observations on *E vittella* and *H. armigera* have been given as seasonal mean incidence. The damage to fruiting structure (squares/ flowers/bolls) was generated at 50, 65, 80, 95, 110, 125 and 140 DAS based on the number of total as well as damaged fruiting bodies on each plant. The fruiting structures both shed and intact were taken into account to be calculated and presented as damage percentage. Flower rosetting was observed at peak flowering (60-75 DAS) for each genotype by counting the number of rosetted flowers as well as total of number of flowers per ten plants to express in percentage. The number of pink bollworm larvae per 10 green bolls was recorded by actually plucking bolls randomly from the subplots and counting the number of larvae in each boll by dissecting. The destructive sampling for larvae has been done around 115 DAS of the crop. Similarly, immediately after harvesting the crop of 25 bolls from each genotype were collected and counted for total and damaged locules due to PBW larval infestation. The data has been presented as percentage locule damage to each genotype. Before picking of seed cotton, number of good opened bolls (GOB's) and bad opened bolls (BOB's) were recorded from 10 randomly selected plants. The data has been averaged to per plant and presented as GOB/plant and BOB/plant. The seed cotton harvested from each sub-plot (genotype) excluding border rows was extrapolated and presented as seed cotton yield (q/ha) for respective treatment. The data were subjected to statistical analysis after suitable transformation and the means were separated by DMRT (p=0.05%) as per Gomez and Gomez.1984). Based on consistency in the observations in all parameters observed only average analysis has been presented.

Table 1: Details of BG-I and BG-II Bt cotton genotypes used for comparative field performance.

Sl. No	Genotypes	Cultivar type	Transgenic generation	Insecticidal gene	Proprietary	Sector
1	MRC-7351 BG-II	HXH	II	*cry*1Ac+*cry*2Ab	MAHYCO, Jalna (MS)	Private
2	MRC-7201 BG-II	HXH	II	*cry*1Ac+*cry*2Ab	MAHYCO, Jalna (MS)	Private
3	KDCHH-621 BG-II	HXH	II	*cry*1Ac+*cry*2Ab	Krishidhan Seeds Co, Ltd., (MS)	Private
4	RCH-2 BG-II	HXH	II	*cry*1Ac+*cry*2Ab	Rasi seeds Co. Ltd., Attur (TN)	Private
5	RCH-530 BG-II	HXH	II	*cry*1Ac+*cry*2Ab	Rasi seeds Co. Ltd., Attur (TN)	Private
6	BUNNY Bt BG-II	HXH	II	*cry*1Ac+*cry*2Ab	Nuziveedu Seeds Co. Ltd.(AP)	Private
7	RCH-2 Bt	HXH	I	*cry*1Ac	Rasi seeds Co. Ltd., Attur (TN)	Private
8	BUNNY Bt	HXH	I	*cry*1Ac	Nuziveedu seeds Co. Ltd.(AP)	Private
9	RCH-2 N Bt	HXH	- -	Conventional (Non Bt)	Rasi seeds Co. Ltd., Attur (TN)	Private
10	DHH -11	HXH	- -	Conventional (Non Bt)	UAS, Dharwad Karnataka	Public

Results and Discussion

As all treatments were protected against sucking pests the incidence of major sap feeders *viz*., leafhoppers, aphids, thrips, whiteflies and key arthropod predators couldn't vary significantly across the treatments. Hence the variations in the performance have been considered as impact of bollworms complex (Onkaramurthy et al., 2011). Bt genotype with one gene (Cry1 Ac) recorded a negligible proportion of *E. vittella* larval population and those genotypes with two genes (Cry1 Ac + Cry2 Ab) did not record larval population throughout the season (Table 2). The average incidence of *E. vittella* was significantly high on RCH-2 non Bt and DHH-11. It appears that at 50-70 DAS, the expression of toxin producing gene could be high enough to take care of the pest incidence. The effectiveness of Bt cotton hybrids against *E. vittella* was endorsed earlier by Hegde et al., (2004) and Udikeri et al., (2006). *H. armigera* larval population increased slowly from square formation (65 DAS) to boll maturity stage (125 DAS) across the genotypes and later decreased reaching minimum at 140 days, however only mean data is considered for analyses. All BG-II genotypes could not allow *H. armigera* larvae to crossed the ETL but in BG-I genotypes larval population crossed ETL (> 1.0/ plant) at 110 and 125 DAS and in non-Bt crossed ETL from 95 DAS till 140 DAS. There was significant difference in the population of *H. armigera* larvae among the Bt genotypes screened during the study. The average data (Table 2) clearly shows that BG-II recorded lowest *H. armigera* larval population ranged from (0.08 to 0.22/ plant) and significantly superior to BG-I and non Bt genotypes included in the study. *H. armigera* larval population was significantly less in BG-II genotypes as compared to BG-I genotypes was reported by Chitkowski et al., (2003), Jackson et al., (2004), Strickland and Annells, (2005), Udikeri, (2006) and Bheemanna et al., (2008). In general, irrespective of the genotypes the seasonal mean damage to the fruiting bodies ranged from 3.77 to 17.30 per cent. The damage to fruiting bodies in different genotypes started at 50 DAS and increased gradually reaching peak at 110 DAS and 125 DAS which declined gradually later. Based on the average damage to the fruiting bodies, it is clear from the data that BG-II genotypes *viz*., MRC-7351 BG-II recorded minimum damage of 3.77 per cent followed by KDCHH-621 BG-II (4.35%), MRC-7201 BG-II(4.36%), Bunny Bt BG-II (4.67%) and RCH-2 BG-II (4.87%) compared to other genotypes. Further the damage to the fruiting bodies was significantly less in BG-II compared BG-I genotypes. All the Bt cotton genotypes whether they are BG-II or BG-I recorded lower damage to the fruiting bodies as compared to non Bt cotton genotypes *viz*., RCH-2 non-Bt (14.92%) and DHH-11 (17.30%) (Table 2) under unprotected condition.

Table 2: Incidence of *E. vittella, H. armigera* larvae and per cent fruiting body damage in different Bt cotton genotypes*.

Genotypes	*E. vittella* larvae/ plant*	*H. armigera* larvae/plant**	Fruiting body damage (%)***
MRC-7351BG-II	0.00 b (1.00)	0.08h (1.04)	3.77g (11.19)
MRC-7201 BG-II	0.00 b (1.00)	0.11gh (1.05)	4.36f (12.05)
KDCHH-621 BG-II	0.00 b (1.00)	0.11gh (1.05)	4.35f (12.02)
RCH-2 BG-II	0.00 b (1.00)	0.18ef (1.08)	4.87ef (12.74)
RCH-530 BG-II	0.00 b (1.00)	0.22e (1.10)	5.37e (13.39)
BUNNY Bt BG-II	0.00 b (1.00)	0.15fg (1.07)	4.67f (12.46)
RCH-2 Bt	0.25 b (1.12)	0.66c (1.29)	8.03c (16.45)
BUNNY Bt	0.18 b (1.09)	0.57d (1.25)	7.26d (15.62)
RCH-2 N Bt	1.88 a (1.69)	1.31b (1.52)	14.92b (22.71)
DHH -11	2.13 a (1.76)	1.68ª (1.64)	17.30a (24.57)
SEm±	0.05	0.01	0.24
CD at 5%	0.15	0.02	0.70

*Data in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT
**Data in parenthesis are square root x+1 transformations.
***Data in parenthesis are arc sine transformations

Table 3: Incidence of PBW larvae, flower rosetting and locule damage in different Bt cotton genotypes*.

Genotypes	PBW larvae/ 10 bolls*	Rosetting (%)**	Locule Damage (%)***
MRC-7351BG-II	0.13 d (1.21)	0.56 c (3.51)	1.88 e (7.88)
MRC-7201 BG-II	0.27 cd (1.42)	0.92 bc (5.36)	2.59 de (9.17)
KDCHH-621 BG-II	0.13 d (1.21)	0.93 bc (5.51)	2.99 cde (9.87)
RCH-2 BG-II	0.53 bc (1.72)	0.98 bc (5.64)	4.08 cd (11.56)
RCH-530 BG-II	0.40 bcd (1.63)	0.99 bc (5.71)	4.31 c (11.95)
BUNNY Bt BG-II	0.27 cd (1.42)	0.93 bc (5.51)	3.67 cd (11.02)
RCH-2 Bt	0.67 abc (1.81)	1.92 b (7.95)	8.79 b (17.18)
BUNNY Bt	0.80 abc (1.87)	1.71 b (7.25)	9.05 b (17.48)
RCH-2 N Bt	1.07 ab (2.03)	6.24 a (14.39)	22.20 a (28.12)
DHH -11	1.47 a (2.21)	7.96 a (16.31)	23.80 a (29.17)
SEm±	0.15	0.88	0.80
CD at 5%	0.44	2.62	2.36

*Data in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT
**Data in parenthesis are square root x+1 transformations.
***Data in parenthesis are arc sine transformations

Table 4: Boll opening and seed cotton yield in different Bt cotton genotypes under unprotected condition*.

Genotypes	GOB/ plant	BOB/ plant	Seed cotton yield (q/ha)
MRC-7351BG-II	28.10 a	2.30 e	20.37 a
MRC-7201BG-II	23.10 b	2.70 e	19.13 abc
KDCHH-621 BG-II	25.93 a	2.50 e	19.75 ab
RCH-2 BG-II	20.10 cd	3.00 cde	17.95 bc
RCH-530 BG-II	19.00 d	3.20 cde	17.33 c
BUNNY Bt BG-II	22.30 bc	2.90 de	18.60 abc
RCH-2 Bt	18.60 d	4.00c	17.19 c
BUNNY Bt	20.30 cd	3.77 cd	17.98 bc
RCH-2 N Bt	12.70 e	9.10 b	12.15 d
DHH -11	13.00 e	11.10 a	11.72 d
SEm ±	0.89	0.32	0.66
CD at 5%	2.63	0.96	1.97

*Data in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Thus second generation genotypes have emerged as easy to adopt solution for resistance problem to Cry 1 Ac. Two gene Bt (Cry1Ac+Cry2Ab) genotypes performance also has been convincingly acceptable in different countries tested. The present observations are in close agreement with Gore *et al*, (2001), Penn *et al*, (2001), Gore *et al*,(2002), Chitkowski *et al*, (2003), Jockson *et al*, (2003a), Jockson *et al*, (2003b), Udikeri (2006) and Bheemanna *et al*, (2008) who reported that the damage to the fruiting bodies was significantly less in BG-II genotypes compared to BG-I genotypes. The data on per cent flower rosetting in different cotton genotypes (table 3) indicated that, all the BG-II and BG-I genotypes recorded lower per cent flower rosetting being on par with each other except MRC-7351 BG-II which recorded lowest per cent flower rosetting of 0.56. However non- Bt genotypes recorded significantly higher per cent resetting (6.24 to 7.96%) compared to BG-II and BG-I genotypes. All the BG-II genotypes except RCH-2 BG-II (0.53 larvae/10 bolls) recorded lower number of PBW larvae/10 bolls. Whereas, BG-I genotypes and non-Bt cotton genotypes were similar in their performance in recording PBW larvae as they were statistically at par with each other. As regards the per cent locule damage, MRC-7351 BG-II recorded lowest of 1.88 being at par with MRC-7201 BG-II (2.59%) and KDCHH-621 BG-II (2.99%). The other three BG-II genotypes were at par with each other in recording the locule damage. Whereas, BG-I genotypes were significantly inferior to BG-II but superior to non-Bt genotypes. Non-Bt genotypes *viz*., RCH-2 non-Bt (22.20%) and DHH-11 (23.80%) recorded significantly highest locule damage compared to all BG-I and BG-II genotypes (Table 3). The present findings are in close agreement with the

reports of Marchosky *et al*, (2001) who reported that Bollgard and Bollgard-II bolls had consistently fewer PBW larvae. However, Bollgard II showed at least 10 fold better efficacy than Bollgard lines. Udikeri (2006) reported that BG-II genotypes recorded significantly lowest per cent of flower rosetting, locule damage and less incidence of PBW larvae compared to BG-I and check genotypes. Significantly higher number of GOB/plant was noticed in MRC-7351 BG-II (28.10) being at par with KDCHH-621 BG-II (25.93) (table 4). All the BG-II genotypes except RCH-2 BG-II (20.10) and RCH-530 BG-II (19.00/plant) recorded more number of GOB/plant compared to BG-I. Both the BG-I genotypes recorded significantly higher number of GOB/plant as compared to non-Bt genotypes. With regard to BOB/plant, all the BG-II genotypes recorded significantly lower number of BOB/plant compared to BG-I genotypes. BG-I genotypes recorded significantly lower number of BOB/plant compared to non Bt cotton genotypes. MRC-7351 BG-II recorded highest seed cotton yield of 20.37 q/ha being at par with KDCHH-621 BG-II (19.75), MRC-7201 BG-II (19.13), and Bunny Bt BG-II (18.60), but superior to BG-I genotypes. Further, both BG-I genotypes *viz*., RCH-2 Bt (17.19) and Bunny Bt (17.98) were significantly superior in recording higher seed cotton yield compared to two non-Bt cotton genotypes included in the study. Superiority of BG-II genotypes over BG-I and non Bt genotypes with regard to yield was reported by Strickland and Annells, (2005). In Indian rainfed condition field performance perspectives BG-II genotypes have out yielded BG-I Bt cotton suppressing all bollworms to far below ETL levels as per Udikeri *et al*., (2011). Further, upon release of some more Bt transgenic events on commercial scales in India, Hallad *et al*.,(2014) confirmed the par excellence of BG-II event genotypes over all those events expressing Cry 1 Ac, fusion gene and Cry 1c only in terms bollworm complex suppression and seed cotton yield. Thus the genotypes expressing Cry 1 Ac + 2 Ab have better advantage. However the natural phenomenon of resistance development in bollworms cannot be ignored which could quite alarming in pink bollworms due to endocarpic nature.

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