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Comparative efficacy of interspecific cotton hybrids containing single and stacked Bt genes against pink bollworm, *Pectinophora gossypiella* (Saund.) and tobacco caterpillar, *Spodoptera litura* (Fab.)*

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Abstract : A Field experiment was conducted at Agricultural Research Station (ARS), Dharwad Farm, Dharwad, Karnataka during 2009-10 to evaluate the comparative efficacy of interspecific cotton hybrids containing single and stacked Bt genes against pink bollworm, *Pectinophora gossypiella* (Saund.) and tobacco caterpillar, *Spodoptera litura* (Fab.) under rainfed condition. Among the hybrids compared stacked Bt gene hybrids performed better against pink bollworm and tobacco caterpillar over single gene Bt hybrids. Among the stacked Bt hybrids, Steplon BG-II and Kashinath hybrids proved better compared to other hybrids. All the stacked Bt hybrids recorded significantly lower PBW and tobacco caterpillar larval population, green boll and locule damage, tobacco caterpillar infested leaves per plant and damaged area per infested leaf compared to single gene Bt hybrids. Significantly higher PBW and tobacco caterpillar population and their damaging indices were recoded on conventional hybrid, DCH-32.

Keywords: Bt cotton hybrid, single Bt gene (*Cry*1Ac), stacked Bt gene (*Cry*1Ac+ *Cry*2Ab), tobacco caterpillar

Introduction

Introduction of Bt cotton resulted in effective control of bollworms leading to significant increase in yield. The Bt cultivars have an added advantage over conventional cotton hybrids both in terms of yield as well as profit apart from the satisfactory control of pest complex. Bt cotton cultivars generally referred as Bollgard (BG) contains only one gene (*Cry*1Ac) which offers protection against lepidopteran pests and known to be effective against *Helicoverpa armigera* (Hubner), *Erias vittella* (Fab.) and *Pectinophora gossypiella* (Saund.). The area under Bt cotton in India increased from 50,000 hectares in 2002 (when Bt cotton was first commercialized) to 8.4 million ha constituting 87 per cent of the total cotton area (9.6 million ha) in 2009 representing an unprecedented 168-fold increase in eight years (James, 2009).

In recent past, PBW has assumed a major threat to first generation Bt cotton hybrids due to its progressive incidence over the seasons and has been noticed regularly on cotton in different cotton growing areas. Even in Bt cotton, it is likely to emerge as major production constraint considering its behavior (Dhaliwal *et al.,* 2010). Further, the incidence of pink bollworm, *P. gossypiella* is more pronounced in the recent past and also its loss is more pronounced in interspecific hybrid cottons which are important source of extra long staple cotton and utmost important from the point of view of textile industry and export (Patil *et al.,* 2003). Moreover, pink bollworm appears during later stages of the cropping season. This is also a period where *Cry* protein expression in Bt cotton slopping down. Although the *Cry*1Ac is toxic to the bollworm complex which is major production constraints, there are several other pests *viz., Spodoptera litura, S. littoralis* and *S. exigua* which are less sensitive to *Cry*1Ac protein and have the potential to become major pests in the emerging scenario. Hence, in an attempt to have broader spectra of activity within lepidopterans including efficacy against many pests previously controlled effectively by single gene constructs and improved efficacy against bollworms complex, stacked gene Bt cottons popularly known as Bollgard-II (BG-II) were approved in both USA and Australia since 2002 and in India since 2006 as most convenient tool for resistance management. The dual-gene cotton hybrids produce approximately the same level of the *Cry*1Ac protein as the single-gene Bollgard cultivars, but are further protected by *Cry*2Ab protein (Adamczyk *et al.,* 2003).

The information on comparative efficacy of first and second generation Bt interspecific cotton hybrids against pink bollworm and tobacco caterpillar is lacking. Keeping this in view, a field evaluation was undertaken at Agricultural Research Station, Dharwad to compare interspecific cotton hybrids containing single and stacked Bt genes against pink bollworm and tobacco caterpillar.

Material and methods

Ten interspecific cotton hybrids were evaluated in RBD with three replications against PBW and tobacco caterpillar in the field experiment laid out at Agricultural Research Station, Dharwad Farm, Dharwad during 2009-10. The hybrids comprised of six inter specific Bt cotton hybrids containing dual genes *viz.,* MRC-7918 BG-II Pratik BG-II, Minerva BG-II, Steplon BG-II, Namcot BG-II, Kashinath and three hybrids containing single gene *viz.,* Rasi XL BG-I, 6188 BG-I, MRC-6918 BG-I and one non Bt inter specific hybrid, DCH-32 as a check were used under unprotected rainfed conditions. Sowing was undertaken on 6th July 2009. Each hybrid was sown on an area of 4.5 m ´ 6 m with six rows at spacing of 90 cm between rows and 60 cm between plants by following all standard agronomical practices for hybrid cotton under rainfed conditions except plant protection measures against pink bollworm and tobacco caterpillar.

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The incidence of pink bollworm and tobacco caterpillar was recorded at fortnight interval from ten randomly selected plants from each hybrid. Observations on flower rosetting, number of PBW larvae per 20 green bolls through destructive sampling, number of damaged locules per 100 fully opened bolls were recorded from each hybrid and presented as percent rosette flowers, percent green boll damage and percent locule damage respectively. Similarly, the observations on number of *S. litura* larvae per plant, number of damaged leaves per plant and damaged leaf area due to *S. litura* were recorded and presented as number of larvae per plant, damaged leaves per plant and per cent damaged leaf area per plant respectively. The yield parameters *viz.,* good opened bolls, bad opened bolls and seed cotton yield were recorded over two pickings and data was averaged to per plant and presented as good opened bolls (GOBs) and bad opened bolls (BOBs) per plant. The seed cotton harvested from each sub-plot (hybrids) excluding border rows was extrapolated and presented as seed cotton yield (kg/ha) for the respective hybrid. The data obtained over a season was pooled and presented in respective means and subjected to analysis of variance after applying suitable transformations.

Results and Discussion

Field evaluation of interspecific cotton hybrids containing stacked genes in comparison with hybrids containing single gene revealed that there was wide variation between BG-I and BG-II hybrids regarding the incidence of pink bollworm and tobacco caterpillar. Seasonal mean of rosette flowers clearly revealed that, Kashinath hybrid recorded lowest flower rosetting (0.29%) and was on par with Steplon BG-II (0.37%) and Minerva BG-II (0.38%). Among BG-II hybrids Namcot BG-II (0.52%) recorded higher flower rosetting. All BG-II hybrids were superior to hybrids with single gene (*Cry*1Ac) *viz.,* MRC-6918 BG-I (1.18%), 6188 BG-I (1.20%) and Rasi XL BG-I (1.24%). On the contrary, the check hybrid, DCH-32 non-Bt, recorded significantly highest flower rosetting (3.76%) compared to all Bt hybrids (Table 1). Incidence of larval population of PBW during 2009-10 season revealed that, BG-II hybrid, Kashinath registered low population ranging from 0.00 to 0.66 larvae with a averaging of 0.10 larvae/20 green bolls which was on par with all BG-II hybrids. While BG-I hybrids recorded significantly higher population of PBW ranging from 1.00 to 3.00 with an

Table 1. Incidence of pink bollworm on different interspecific cotton hybrids

Hybrids	Proprietary	Incidence of pink bollworm			
		Mean flower rosetting (%)	Mean PBW larvae per 20 green bolls**	Mean boll green damage (%)*	Mean locule damage (%)*
Rasi XL BG-I	M/s. Rasi seeds	1.24 (6.38)b	1.14 (1.46)b	5.48 (13.51)b	11.33 (19.65)b
6188 BG-IM/s.	Bioseeds Research India Pvt. Ltd.	1.20 (6.28)b	1.05 (1.43)b	5.00 (12.91)b	10.67 (19.04)b
MRC-6918 BG-I	M/s Mahyco	1.18 (6.24)b	1.19 (1.48)b	5.48 (13.51)b	9.67 (18.10)b
MRC-7918 BG-II	M/s Mahyco	0.49 (3.98)cd	0.24 (1.11)c	1.19 (6.24)c	2.33 (8.74)c
Pratik BG-II	M/s Krishidhan seeds Pvt. Ltd	0.49 (4.04)cd	0.14 (1.07)c	0.71 (4.83)d	3.00 (9.97)c
Kashinath BG-II	M/s Nath Seeds Ltd.	0.29 (3.09)e	0.10 (1.05)c	0.48 (3.95)d	1.67 (6.03)c
Minerva BG-II	M/s Bayer Bioscience Pvt. Limited.	0.38 (3.55)cde	0.19 (1.09)c	0.71 (4.83)d	2.67 (9.36)c
Steplon BG-II	M/s Mansanto Holdings Pvt Ltd.	0.37 (3.49)de	0.14 (1.07)c	0.71 (4.83)d	2.00 (7.94)c
Namcot BG-II	M/s Namdhari Seeds Pvt.Ltd.	0.52 (4.13)c	0.14 (1.07)c	0.71 (4.83)d	1.33 (6.53)c
DCH-32 Non Bt	UAS, Dharwad	3.76 (11.16)a	6.24 (2.69)a	22.71 (28.44)a	47.33 (43.45)a
S.Em±		0.19	0.04	0.27	1.29
CD@ 5%		0.55	0.11	0.80	3.83
CV (%)		6.17	4.58	5.15	15.01

*Figures in parentheses are arcsine transformed values

** Figures in parentheses are square root $\sqrt{x+1}$ transformed values

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

average of 1.05 to 1.19 larvae/20 green bolls compared to BG-II hybrids. The DCH-32 non-Bt cotton hybrid was found to be highly susceptible by harboring significantly higher PBW population of 6.24 larvae/20 green bolls (Table 1). Similarly the mean green boll damage was significantly lower in BG-II hybrids compared to BG-I hybrids. The least damage was recorded in Kashinath (0.48%) and was on par with rest of BG-II hybrids except MRC-7918 BG-II (1.19%). The green boll damage in BG-I hybrids ranged from 5.00 to 5.48 per cent. Whereas the check hybrid, DCH-32 non Bt recorded 22.71 per cent and was found susceptible. The locule damage was least in Namcot BG-II (1.33%) and was on par with rest of BG-II hybrids and found significantly superior to BG-I hybrids which recorded the percent locule damage ranging from 9.67 to 11.33 per cent. The significantly higher per cent of locule damage was recorded by DCH-32 (47.33%) which confirmed its susceptibility to PBW.

The present study revealed that the interspecific hybrids containing stacked genes were found highly tolerant against pink bollworm, since the pink bollworm incidence was significantly lower compared to hybrids containing single gene with significantly lower flower rosetting, PBW larval population, per cent green boll and locule damage. The advantage of Bt genotypes with stacked genes has been reported by Adamczyk *et al.* (2001) and Jackson *et al.* (2003) where in DP 50 B-II, the genotype with *Cry*1Ac + *Cry*2Ab was found better than DP 50 B (*Cry*1Ac) with enhanced efficacy over wide range of lepidopteran pests. Further, Tabashnik *et al.* (2002) also observed that *Cry*1Ac resistant PBW had little or no survival on second generation transgenic cotton with *Cry*2Ab alone or with *Cry*1Ac plus. Thus, stacked gene Bt hybrids performance has been convincingly acceptable. Marchovsky *et al.* (2001) who reported that Bollgard and Bollgard-II bolls had consistently fewer PBW larvae. The Bollgard II showed at least 10 fold better efficacy than Bollgard lines. From the data recorded, the superiority of Bt hybrids over conventional hybrid, DCH-32 has been proved beyond doubt. The advantage of stacked gene Bt hybrids over single gene Bt hybrids against pink bollworm reported by Udikeri (2006) and Bheemanna *et al.* (2008) are in accordance with the present findings.

The mean larval population of *S. litura* on BG-I hybrids was significantly high (1.12 to 1.17/pl) compared to BG-II hybrids (0.11 to 0.35/pl). Among the BG-II hybrids Kashinath BG-II recorded lowest *S.litura* population (0.11/pl) and was statistically on par with Namcot BG-II (0.20/pl). Whereas the

Table 2. Incidence of tobacco caterpillar on different interspecific cotton hybrids

Hybrids	Proprietary	Incidence of tobacco caterpillar		
		Mean number of live larvae per plant*	Mean number of damaged leaves per infested plant*	Mean damaged leaf area per infested leaf (%)**
Rasi XL BG-I	M/s. Rasi seeds	1.12 (1.45)b	3.32 (2.08)b	18.93 (25.77)b
6188 BG-I	M/s. Bioseeds Research India Pvt. Ltd.	1.13 18.54 (1.46)b	3.33 (1.46)b	(2.08)b (25.49)b
MRC-6918 BG-I	M/s Mahyco	1.17 (1.47)b	3.34 (2.08)b	18.33 (25.34)b
MRC-7918 BG-II	M/s Mahyco	0.23 (1.11)cd	0.68 (1.30)c	4.80 (12.64)c
Pratik BG-II	M/s Krishidhan seeds Pvt. Ltd	0.35 (1.16)c	0.65 (1.28)c	4.46 (12.19)c
Kashinath BG-II	M/s Nath Seeds Ltd.	0.11 (1.05)e	0.73 (1.31)c	4.76 (12.60)c
Minerva BG-II	M/s Bayer Bioscience Pvt. Limited.	0.27 (1.29)c	(1.13)cd 5.31	0.68 (13.12)c
Steplon BG-II	M/s Mansanto Holdings . Pvt Ltd	0.30 (1.14)cd	0.71 (1.31)c	4.50 (12.24)c
Namcot BG-II	M/s Namdhari Seeds Pvt.Ltd.	0.20 (1.10)de	0.74 (1.32)c	4.97 (12.79)c
DCH-32 Non Bt	UAS, Dharwad	1.66 (1.63)a	5.16 (2.48)a	25.92 (30.58)a
SEm±			0.03	0.04 0.73
CD@ 5%			0.08	0.12 2.16
CV (%)			3.72	4.21 6.89

*Figures in parentheses are arcsine transformed values

** Figures in parentheses are square root $\sqrt{x+1}$ transformed values

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

check hybrid, DCH-32 non-Bt found to be inferior by registering significantly higher population of 1.66 larvae per plant compared to all Bt hybrids.

The wide range bioactivity of stacked genes in BG-II Bt cotton hybrids was proved by recording least number of damaged leaves of 0.65 to 0.74/plant compared to the hybrids containing single gene (3.32 to 3.34/pl) and non-Bt cotton hybrid, DCH-32 (5.16/pl) during the study. Similarly, mean data recorded over the season clearly indicated that, the BG-II hybrids suffered less foliar damage of *S. litura* (4.46 to 5.31%) compared to BG-I hybrids (18.33 to 18.93%) and non-Bt DCH-32 conventional hybrid (25.92%). The reason for the superiority of BG-II hybrids in recording lower larval incidence could be due to the presence of an additional *Cry*2Ab gene. The dual stacking of *Cry*1Ac+*Cry*2Ab genes has been reported to have 10 folds advantage over *Cry* 1Ac genotypes (Marchovsky *et al.,* 2001). The present results are in close agreement with the report of Strickland and Annells (2005) who reported that Bollgard-II block having lower number of *S. litura* larval population as compared to INGARD (BG-I) and conventional cotton blocks. Further Bheemanna *et al.* (2008) reported that *S. litura* larvae were nil in MRC-7201 BG-II compared to MRC-6322 BG-I genotype.

The higher number of good opened bolls (GOBs) of 43.60 GOBs/plant were harvested from Steplon BG-II hybrid which was on par with the rest of Bt hybrids (Fig. 3) except MRC-6918 BG-I hybrid (38.13 GOBs/pl). In general, the good opened bolls were more in BG-II hybrids compared to BG-I hybrids. On the contrary, non-Bt cotton hybrid, DCH-32 was found inferior by recording significantly lower number of good opened bolls (21.93 GOBs/plant). In contrast to GOBs/ plant, BG-II hybrids registered significantly lower number of bad opened bolls (2.32 – 3.20 BOBs/plant) compared to BG-I hybrids (4.53 – 5.13 BOBs/plant). On the contrary significantly higher number of bad opened bolls were observed in DCH-32 non-Bt cotton hybrid (10.20 BOBs/ plant) compared to Bt hybrids as the hybrid suffered more due to PBW damage owing to lack of inbuilt tolerance.

The Bt cotton hybrids produced significantly higher seed cotton yield compared to conventional hybrid, DCH-32. This was mainly because of more number of good opened bolls that contributed for higher seed cotton yield. Compared to BG-I hybrids, BG-II hybrids recorded higher seed cotton yield. The seed cotton yield of BG-II hybrids ranged between 2379.10 to 2479.92 kg/ha, where as BG-I hybrids registered seed cotton yield of 2282.77 to 2327.60 kg/ha. The Bt hybrids containing stacked genes registered higher seed cotton yield owing to the higher efficacy offered against PBW by stacked genes which inturn resulted in more number of good opened bolls. Performance of BG-II hybrids interms of less damage and more seed cotton yield compared to BG-I hybrids was reported by Gore *et al.* (2002). Superiority of BG-II genotypes over BG-I genotypes with regard to seed cotton yield was also reported by Catchot and Mullins (2003), Strickland and Annells (2005) and Udikeri (2006) which support the present findings.

Table 3. Boll opening and seed cotton yield

Hybrids	Proprietary	Boll opening		Seed cotton
		GOBs/pl	BOBs/pl	yield (kg/ha)
Rasi XL BG-I	M/s. Rasi seeds	39.40ab	4.53b	2291.53a
6188 BG-I	M/s. Bioseeds Research India Pvt. Ltd.	39.13ab	5.13b	2327.60a
MRC-6918 BG-I	M/s Mahyco	38.13b	4.60b	2282.77a
MRC-7918 BG-II	M/s Mahyco	41.53ab	3.20c	2379.10a
Pratik BG-II	M/s Krishidhan seeds Pvt. Ltd	42.73a	2.36c	2426.42a
Kashinath BG-II	M/s Nath Seeds Ltd.	43.07a	2.63c	2451.09a
Minerva BG-II	M/s Bayer Bioscience Pvt. Limited.	41.87ab	3.07c	2394.51a
Steplon BG-II	M/s Mansanto Holdings Pvt Ltd.	43.60a	2.60c	2479.92a
Namcot BG-II	M/s Namdhari Seeds Pvt.Ltd.	42.20ab	2.47c	2399.14a
DCH-32 Non Bt	UAS, Dharwad	21.93c	10.20a	1194.17b
SEm±		1.33	0.28	80.17
CD@ 5%		3.94	0.82	238.00
CV (%)		5.84	11.70	6.15

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

References

Adamczyk, J. J., Adams, L. C., Hardee, D. D. and Summerford, D. V., 2003, Correlating differences in the larval survival and development of bollworms (Lepidoptera : Noctuidae) and fall army worms (Lepidoptera : Noctuidae) to differential expression of *Cry*1Ac ä-endotoxin in various plant parts among commercial cultivars of transgenic Bacillus thruingiensis cotton. J. Econ. Entomol., 94 : 284-290.

Adamczyk, J. J. Jr., Bew, K., Adams, L. C. and Hardee, D. D., 2001, Evaluation of Bollgard® (cv. DP50BII) in the Mississippi delta: Field efficacy against various Lepidoptera while profiling

season-long expression of *Cry*1Ac and *Cry*2Ab. In: *Proc. Beltwide Cotton Conf.,* Anaheim, CA. 9-13, January, 2001, Nat. Cotton Council, A M., Memphis, TN. pp. 835-836.

Bheemanna, M., Patil, B. V., Hanchinal, S. G., Hosamani, A. C. and Bansi, A. B., 2008, Comparative performance and economics of Bollgard-II cotton under irrigated condition. J. Cotton Res. Dev., 22 (1): 118-121.

Catchot and Mullins, 2003, Bollgard II: A New Generation of Bt Genes Commercialized ICAC RECORDER.

Dhaliwal, G. S., Jindal, V. and Dhawan, A. K., 2010, Insect pest problems and crop losses: Changing trends. Indian J. Ecol., 37(1) : 1-7.

Gore, J., Leonard, B. R., Chorch, G. E. and Cook, D. R., 2002, Behavior of bollworm (Lepidoptera : Noctuidae) larvae on genetically engineered cotton. J. Econ. Entomol., 95(3): 763-769.

Jackson, R. E., Bradley, J. R. and Van, D. J. W., 2003, Field performance of transgenic cotton expressing one or two *Bacillus thuringiensis* endo toxins against bollworm, *Helicoverpa zea* (Boddie). The J. Cotton Sci., 7: 57-64.

James, C., 2009, Global status of commercialized biotech/GM crops : 2009, ISAAA Brief No. 41, Ithaca, New York, USA.

Marchovsky, R., Ellsworth, P. C., Moser, H. and Hennerberry, T. J., 2001, Bollgard and Bollgard II efficacy in near isogenic lines of DP50 Upland cotton in Arizona. Arizona Cotton Rep., pp. 236-249.

Patil, S. B., Udikeri, S. S. and Khadi, B. M. 2003, Integrated pest management with genetically modified cotton hybrids in India. In : *Proc. World Cotton Res. Conf. - III*. 9-13 March, 2003, Cape Town, South Africa, pp. 959-966.

Strickland, G. R. and Annells, A. J., 2005, The seasonal dynamics of arthropods in conventional, INGARD® and Bollgard II® cotton genotypes in a winter production system at Kununurra, Bentley Delivery Centre WA 6983 @, State of Western Australia, pp.1-13.

Tabashnik, B. E., Dennehy, T. J., Sims, M. A., Larkin, K., Head, G. P., Moar, W. J. and Carriere, Y., 2002, Control of resistant pink bollworm (*Pectinophora gossypiella*) by transgenic cotton that produces *Bacillus thuringiensis* toxin Cry2Ab. Appl. Environ. Microbiol., 68 (8) : 3790-3794.

Udikeri, S. S., 2006, Evaluation of new generation Bt cotton genotypes, Sustainability of Cry protein expression, computation of ETL, Effect on aphid predators and development of IPM module for Bt Cotton under rainfed conditions. *Ph. D. Thesis*, Univ. Agric. Sci., Dharwad, (India).