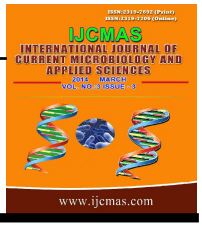
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 594-600**
http://www.ijcmas.com



Original Research Article

Characterization of resistance to all bollworms and *Spodoptera litura* (Fab.) in different *Bt* transgenic events of cotton

Anand V. Hallad[1], S. S. Udikeri[2], S. B. Patil[3] D. P. Biradar[2], and B. M. Khadi[1]

[1]College of Agriculture, Bijapur, University of Agricultural Sciences, Dharwad, India
[1]Director of Research, University of Agricultural Sciences, Dharwad, India
[2]Vice-chancellor, University of Agricultural Sciences, Dharwad, India
[3]Agricultural Research Station, University of Agricultural Sciences, Dharwad, India
Corresponding author

K e y w o r d s

Bioassay; Bollworms; Tobacco caterpillar and Bt events.

A B S T R A C T

In the present investigation five different transgenic events of Bt cotton and Non Bt were studied for their efficacy against different cry proteins expressed at different days of interval (*viz.*,50, 75, 100, 125 and 135 days). The highest mortality of *Helicoverpa armigera* (Hub.) was recoded in Tulasi 4 BG-II (MON - 15985) 94.8% followed by Tulasi 4 BG-I (MON-531) 98.8 per cent and MH 5174 (MLS 9124) shown least efficacy 70.4% at 50 day after sowing (DAS). At 135 DAS 75.9 % mortality was observed in Tulasi 4 BG-II and 21.9 % in JKCH 99 (Event-1) and 10.3% in MH 5174. The mortality of *Earias vittella* (Fab.) was same as that of *H. armigera* in Tulasi 4 BG-II shown 100.0 per cent mortality followed by Tulasi 4 BG-I (99.4%) and Nathbaba (99.3%), the morality was decreased to 87.7 % and 65.7 per cent in Tulasi 4 BG-II and Nathbaba at 135 DAS respectively. The efficacy of *Pectinophora gossypiella* (Saund.) was recoded highest in Tulasi 4 BG-II (92.9%) and least mortality was observed in MH 5174(78.3%). Mortality of *Spodoptera litura* (Fab.) was highest in Tulasi 4 BG-II (83.7%) followed by Nathbaba (81.6%) and least mortality was in Tulasi 4 BG-I (12.1%) at 50 DAS. The mortality was decreased to 57.3, 52.1, 47.9 per cent (Tulasi 4 BG-II, Nathbaba & MH 5174) respectively and Tulasi 4 BG-I recorded least mortality (3.2%) at 135 DAS respectively.

Introduction

Cotton, *Gossypium hirsutum* (L.) expressing Cry 1Ac endotoxin of *Bacillus thuringiensis* (Ber.) Bt has been reported to be highly effective against bollworm complex of cotton.Though BG-IBt cotton expressing Cry 1Ac are effective against bollworms slowly it is being replaced by BG-II expressing dual genes (Cry 1Ac+Cry2Ab) as IRM strategy. Bollgard-II cotton expressing

both Cry1Ac and Cry2Ab proteins have provided increased efficacy against the budworm-bollworm complex and enhanced spectrum of activity against beet armyworm, *S. exigua* (Hub.) fall armyworm, *S. frugiperda* (Smith) and common cutworm or tobacco caterpillar *Spodoptera litura* (Fab.) which have been predicted to be major pests in emerging scenario.(Jackson *et al.,* 2004, Adamczyk *et al.,* 2001 and Gore *et al.,* 2001).

The toxicity of Bt crystal proteins has been highly appreciated and hence the gene has been transferred to express in the plant system. Though transgenic cotton produces Bt toxin in all parts in larger quantities especially in leaves the performance of these genotypes could not remain similar throughout the season. Although the studies where Cry1Ac (other toxins also) using pure proteins have given high level of mortality, investigations on bioefficacy of Cry1Ac and other genes as plant incorporated protection system (PIP) have shown variable response. Hence spatio temporal variation in expression has been considered an issue related to the performance of Bt genotypes (Kranthi *et. al.,* 2005).

Materials and Methods

Test insects *viz., H. armigera* (Hub.), *E.vittella* (Fab.), *P. gossypiella* (Saund.) and *S. litura* (Fab.) have been obtained from the culture maintained at ARS, Dharwad. The insect cultures were maintained in this laboratory on semi-synthetic diet for test insects with the better standards of rearing. The procedure and dietary details were adopted as per Udikeri (2006).

The quantitative bioassays were carried out in laboratory at 50, 75, 100, 125 and 135 days after sowing (DAS) by leaf feeding method for *H. armigera* and *S. litura.* The squares and tiny bolls were used for *E. vittella* and *P. gossypiella* bioassays, respectively. In each treatment, there were 10 larvae replicated 4 times and in all cases, two days old neonates were used for bio-assay. The larvae were released at one/well of multicell trays on leaf disc of 2.0 cm diameter and closed tightly with serene wrap and lid. The discs and squares were changed every day. The leaf discs were placed on semi-wet filter paper disc of similar size to avoid drying. Rearing trays of 25 wells were used for bioassay. Small plastic cups and pet jars were used for squares and boll based assays. The number of larvae used was at the rate of one per square or boll. The lid of the jar/cup with provision for aeration was closed tightly to prevent escape of larvae. The mortality of the larvae at 24 hour interval till three days was recorded and converted as per cent mortality. Only corrected mortality (as per Abbots formula) in each treatment with respect to non Bt control treatment (DHH-11) was considered for analysis. Different genotypes used for the bioassay has given below (Table 1).

Results and Discussion

H. armigera mortality at 50 DAS was 98.4 per cent in the larval population fed with Tulasi 4 BG-II leaves and lowest mortality (70.4%) was recorded in MH-5174. Other 3 events could revealed the mortality range between 93.8 to 94.8 per cent being statistically on par. Further, at 75 DAS the mortality was 95.9 per cent in Tulasi 4 BG-II. Its counterpart (Tulasi 4BG-I) showed 91.1 per cent kill. JKCH 99 and Nathbaba remained on par to Tulasi 4BG-I (Table 2). The effectiveness of MON-15985 represented by Tulasi 4 BG-II remained significantly high over the rest during season long observations; however

it was reduced to 75.9 per cent at 135 DAS. The similar trend was observed in all the genotypes/events, but, the mortality was greatly reduced after 100 DAS. The lowest resistance at 100 DAS was recorded from MH 5174 (61.5%). At 125 and 135 DAS the mortality in GFM/ Event-1/ MON-531/MH events was less than 50 and 25 per cent respectively by Onkarmurthy (2008) and Adamczyk and Douglas.(2001) reported that there was a 95 per cent mortality at 55 DAS and reached 20.0 per cent by 95 DAS and by 120 DAS there was no effect of Cry toxin.

Udikeri (2006) studied the decline in *Cry* protein expression through bioassay. In RCH-2 Bt with Cry1Ac, maximum mortality (>90%) of *H. armigera* from 60 to 80 DAS was noticed. Somashekara (2009) recorded mortality of *H. armigera* was (98.53 & 98.08 %) in RCH 2 and Bunny BG-II. Whereas, in RCH 2 Bt and Bunny Bt showed 95.13 and 95.40 per cent mortality at 60 and 120 DAS respectively.

The mortality of *E. vittella* was significant across genotypes/events compared to *H. armigera.* At 50 DAS the mortality was 99.2 to 100 per cent indicating the cent per cent effect of each genotype (Table 3). Among five events MON-15985 represented by Tulasi 4 BG-II had shown 100 per cent mortality at 50 and 75 DAS, 99.2 per cent at 100 DAS and the least of 87.67% at 135 DAS. The rest of the genotypes have shown > 97 per cent mortality at 75DAS, > 95 per cent at 100 DAS, being statistically on par each other. By 125 DAS the mortality ranged between 73 -76 per cent in the events other than BG-II which was still reduced to 62-64 per cent at 135 DAS. Though the events other than MON-15985 were on par in all observations relatively lower resistance was offered by MH-5174. The results is close agreement with the field and laboratory studies involving five Bt cottons expressing Cry1Ac and two conventional varieties in Pakistan have shown 100 percent larval mortality of *E. vittella* (Abro *et al*., 2004). Udikeri (2006) studied the decline in *Cry* protein expression through bioassay. In RCH-2 Bt with Cry1Ac, maximum mortality (>90%) of *E. vittella* (Fab.) from 60 to 80 DAS was noticed. The mortality of *E. vittella* was (100 %) in RCH 2 and Bunny BG-II. Whereas, in RCH 2 Bt and Bunny Bt showed 98.50 and 99.05 per cent mortality at 60 and 120 DAS respectively (Somashekara 2009).

The PBW larval mortality was 92.9 to 69.1 per cent considering different days of observation indicating better efficacy of all events (Table 4). As in case of spotted bollworm, Tulasi 4 BG-II remained significantly superior over the rest with 92.9 (75 DAS), 91.6 (100 DAS), 88.1 (125 DAS) and 80.3 (135 DAS) per cent resistance. Similar trend was observed in all events. Thus 84.7 per cent mortality at 75 DAS was brought down to 76.0 per cent by 135 DAS in Nathbaba expressing fusion gene. The impact of Cry1Ac irrespective of events was similar and statistically on par with GFM event. MH event had comparatively lower resistance at 125 and 135 DAS. Udikeri (2006) reported that Mortality of *P. gossypiella* larvae was 85.35 per cent at 80 DAS. Mortality of all the three boll worm neonates was maximum (>99%) between 40 and 80 DAS in RCH-2 BG-II with Cry1Ac + Cry2Ab genes. Somashekara (2009) reported the PBW mortality was (91.47 & 91.2%) in RCH 2 and Bunny BG-II. Whereas, in RCH 2 Bt and Bunny Bt recorded 82.15 and 81.7 per cent

Table.1 Genotypes used for characterization of resistance

Genotypes	Event	Bt toxin/Toxin
Tulasi 4BG-I	MON-531	Cry1Ac
Tulasi 4BG-II	MON-15985	Cry 1Ac + Cry 2Ab
JKCH-99	Event -1	Cry 1Ac
Nathbaba	GFM	Cry 1Ab – Cry 1Ac
MH 5174	MLS9124	Cry 1C
DHH-11	Non Bt	Non Bt

Table.2 Bio-efficacy of different transgenic events against American bollworm *Helicoverpa armigera* Hub.

Genotypes	Neonate mortality (%)				
	50 DAS	75 DAS	100 DAS	125 DAS	135 DAS
Tulasi 4 BG-II	98.4a (82.8)	95.9a (78.6)	90.8a (72.4)	83.1a (65.7)	75.9a (60.6)
JKCH-99	93.8b (75.6)	90.3b (71.8)	73.2b (58.8)	37.8d (37.9)	21.9b (27.8)
Tulasi 4 BG-I	94.8b (76.9)	91.1b (72.6)	72.2b (58.1)	39.2b (38.7)	20.1b (26.5)
Nathbaba	94.4b (76.4)	90.7b (72.3)	73.1b (58.7)	41.9bc (40.3)	20.3b (26.6)
MH5174	70.4c (57.1)	66.1c (54.4)	61.5c (51.6)	20.0c (26.6)	10.3c (18.7)
SEm±	0.9	0.8	0.7	0.9	0.3
CD @ 5%	2.2	2.4	2.1	2.3	1.4
CV (%)	2.4	2.3	2.3	3.7	1.3

Note: DAS: Days after sowing
Figures in the parentheses are arc sine transformations
Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

Table.3 Bio-efficacy of different transgenic events against spotted bollworm *Earias vittella* Fab.

Genotypes	Neonate mortality (%)				
	50 DAS	**75 DAS**	**100 DAS**	**125 DAS**	**135 DAS**
Tulasi 4 BG-II	100.0a (90.0)	100.0a (90.0)	99.2a (85.6)	95.1a (77.3)	87.7a (69.5)
JKCH-99	99.2a (85.5)	98.1b (82.3)	95.1b (77.2)	76.1b (60.8)	64.2b (53.2)
Tulasi 4 BG-I	99.4a (87.8)	97.8b (82.6)	96.9b (79.9)	75.7b (60.4)	62.6b (52.3)
Nathbaba	99.3a (86.5)	98.0b (82.0)	95.3b (77.4)	74.9b (59.9)	65.7b (54.1)
MH5174	99.2b (85.5)	98.0b (82.9)	95.2b (77.4)	73.6b (59.1)	62.5b (52.2)
SEm±	1.6	1.6	1.0	0.9	1.0
CD @ 5%	4.9	5.0	2.9	2.6	3.1
CV (%)	3.7	3.9	2.4	2.7	3.6

Note: DAS: Days after sowing

Figures in the parentheses are arc sine transformations

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

Table.4 Bio-efficacy of different transgenic events against pink bollworm *Pectinophora gossypiella* Saund

Genotypes	Neonate mortality (%)			
	75 DAS	**100 DAS**	**125 DAS**	**135 DAS**
Tulasi 4 BG-II	92.9a (74.6)	91.6a (73.2)	88.1a (69.8)	80.3a (63.6)
JKCH-99	84.3b (66.7)	80.3bc (63.6)	77.8b (61.9)	75.4b (60.3)
Tulasi 4 BG-I	83.8b (66.3)	81.3bc (64.4)	78.3b (62.2)	74.6b (59.7)
Nathbaba	84.7b (67.0)	82.1b (64.9)	79.1bb (62.8)	76.0b (60.7)
MH5174	78.3b (62.2)	74.9c (59.9)	71.2c (57.5)	69.1c (56.2)
SEm±	0.7	0.7	0.9	0.7
CD @ 5%	2.2	2.1	2.6	2.1
CV (%)	2.2	2.1	2.7	2.3

Note: DAS: Days after sowing

Figures in the parentheses are arc sine transformations

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

Table.5 Bio-efficacy of different transgenic events against tobacco caterpiller *Spodoptera litura* Fab.

Genotypes/Events	Neonate mortality (%)				
	50 DAS	75 DAS	100 DAS	125 DAS	135 DAS
Tulasi 4 BG-II	83.7a (66.2)	82.6a (65.4)	73.8a (59.2)	71.6a (57.8)	57.3a (49.2)
JKCH-99	14.8c (22.6)	13.2c (21.3)	9.5c (17.9)	7.8c (16.2)	4.9c (12.5)
Tulasi 4 BG-I	12.1c (20.3)	10.7c (19.2)	7.2c (15.6)	5.8c (13.9)	3.2c (10.3)
Nathbaba	81.6a (64.6)	79.9a (63.3)	70.5a (57.1)	68.3a (55.7)	52.1a (46.2)
MH 5174	76.1b (60.7)	74.3b (59.5)	66.7b (54.7)	61.2b (51.5)	47.9b (43.8)
SEm±	0.5	0.4	0.6	0.5	0.4
CD @ 5%	3.1	2.6	2.2	1.8	1.5
CV (%)	2.3	2.2	2.4	2.6	2.3

Note: DAS: Days after sowing

Figures in the parentheses are arc sine transformations, Figures in the same column with similar alphabets do not differ significantly at P=0.05 by DMRT

mortality at 60 and 120 DAS respectively. The resistance in different events of Cry toxins for *S. litura* was not similar to the bollworms. Among five events only MON-15985, GFM and MH could exercise better suppression of this pest. The range of lethality exerted by Cry 1Ac + Cry 2Ab (BG-II) on *S. litura* was 83.7 to 57.3 per cent which differed significantly from the mean mortality recorded from Nathbaba (81.6 to 52.1%) and MH 5174 (76.1 to 47.9%) (Table 5). In Cry1Ac expressing events the maximum mortality was limited to 12.1 per cent at 50 DAS, as recorded in Tulasi 4 Bt. Somashekara (2009) reported mortality of *S. litura* was 82.95 and 82.14 per cent (RCH 2 & Bunny BG-II). Whereas, 12.61 and 13.05 per cent mortality recorded (RCH 2 Bt & Bunny Bt) at 60 and 120 DAS respectively. The genotype belonging to MON-15985 (Cry1Ac+Cry2Ab) shown maximum efficacy to all bollworms and *S. litura*. The other two events expressing Cry1Ac (BG-I and Event-1) found to be equal in resistance to all bollworms and *S. litura.* GFM event expressing (Cry1Ac-Cry1Ab) in fusion manner also had better efficacy against bollworms and *S .litura.*

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