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RESEARCH ARTICLE

Cry 1 Ac delta-endotoxin expression in transgenic Bt cotton hybrids as influenced by fertilizer levels for targeted yields

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ABSTRACT

A field experiment was carried out at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad to estimate the delta endotoxin concentration in transgenic cotton hybrids as influenced by fertilizer levels. The field trial was laid out in Complete Randomized Block Design with four Bt cotton hybrids and three target yield levels. Delta-endotoxin concentration in different cotton hybrids differed significantly at 75 DAS. Significantly higher endotoxin content (1.97 µg g^{-1} fw) was recorded with MRC-7201, inturn it resulted in less number of bad opened bolls per plant and lowest was observed in MRC-6918, it resulted in significantly higher number of (8.89) bad opened bolls per plant. Increase in fertilizer levels increased the delta-endotoxin concentration and significantly higher delta-endotoxin concentration was recorded with F_3 level (2.04 µg g^{-1} fw) followed F_2 (1.80 µg g^{-1} fw) and F_1 (1.62 µg g^{-1} fw) level and the latter being the lowest. Higher endotoxin concentration was recorded particularly in Bollgard-II (2.19 µg g^{-1} fw) with F_3 level.

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INTRODUCTION

The *Bacillus thurerngiensis* (Bt) transgenic cotton expressing Cry 1 Ac delta-endotoxin is rapidly dominating world agriculture, which is called as first generation Bt cottons. They have been regarded as novel alternative for management of certain lepidopteron pests. Second generation transgenic Bt cotton produced two endotoxins, Cry 1 Ac and Cry 2 Ab. The duel-gene cottons produced approximately the same level of the Cry 1 Ac protein as the single-gene Bollgard cultivars, but were further protected by the Cry 2 Ab protein (Greenplate *et al*., 2000 and Adamczyk *et al*., 2001). There is potential increase efficacy against bollworm due to the additional cry toxin compared with Bollgard cultivars. All the cultivars of transgenic cotton however, did not provide the same level of bollworm control as delta-endotoxin expression level varied. The introduction of commercial cotton varieties producing the insecticidal protein was expected to improve grower profitability, reduce environmental pollution from synthetic insecticides. The synthesis of Bt protein and its cycle in plant are also the physiological process of nitrogen metabolism, which were controlled by several key enzymes such as NR, GPT, GOT protease and peptidase. Chen *et al.* (2003) showed that the leaf insecticidal proteins content of Bt cotton had close correlation with key enzymes and nitrogen metabolism key enzymes and nitrogen metabolism of Bt cotton affected the Bt protein content. Increase in the nitrogen level increased the endotoxin concentration in the transgenic cotton hybrids to certain extent. Next to bollworm, the important issue that needs to be addressed in crop production is nutrient usage. Cotton, particularly hybrids being exhaustive draw plenty of soil nutrients and thus under continuous cropping systems/

pattern nutrient management assumes importance. Nutrient recommendation varies with crop response, soil condition and targeted yield levels. A new approach called Site Specific Nutrient Management (SSNM) which advocates the need of based supply of nutrients ensures application of nutrient at right time in desired quantity by the crop for obtaining targeted yields. Therefore, the present investigation was carried out to know the effect of nitrogen fertilizer on expression of endotoxin concentration in Bollgard hybrids.

MATERIALS AND METHODS

The experiment was conducted at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad on medium black soil. The treatments comprised of four Bt hybrids and three fertilizer levels for targeted yield of 2, 2.5 and 3 t ha^{-1} were laid in completely Randomized Block Design in plot size of 5.6 m x 6.0 m design with 12 treatments replicated thrice. The genotypes considered for experiment were two first generation Bt hybrids MRC-6918, MRC 6322 and two second generation hybrids MRC-7351, MRC-7201. Fertilizer levels calculation based on soil nutrient availability, nutrient uptake by cotton plants for one q/kg and target yield levels. The fertilizer doses were, F_1 - 145:39:99 NPK kg ha^{-1} for 2 t ha^{-1}, F_2 - 181:49:124 NPK kg ha^{-1}for 2.5 t ha^{-1} and F_3 - 217:59:148 NPK kg ha^{-1} for 3 t ha^{-1}. The endotoxin concentration in plant parts was quantified at 75, 105 and 120 DAS using the commercial available design Cry 1 Ac quantufication *i.e.* 'ELISA –Kit (Enzyme-Linked Immunosorbent Assay)', endotoxin concentration was estimated from three replicate samples of leaves. ELISA was performed according to the described design. Quantification of Cry 1 Ac was done by plotting absorbance value of the test samples on the standard curve generated with purified Cry 1 Ac standards on each of the ELISA plates and expressed as µg g^{-1} weight of tissue.

RESULTS AND DISCUSSION

Results indicate that, protein content was higher in early stages and later declined steadily with the advancement of season and plant age which was in line with observations made by Adamzyck *et al.* (2001), Jing Sun *et al.* (2002) and Kranthi *et al.*(2005b). Delta-endotoxin concentration in different cotton hybrids differed significantly at 75 DAS and there after the differences were not-significant (Table 1). Significantly higher endotoxin content (1.97 µg g^{-1} fw) was recorded with MRC-7201, inturn it resulted in less number of bad opened bolls per (3.38, 3.16 and 5.20) plant as (Table 2) compared to other hybrids at all the pickings (1st, 2nd 3rd and 4th, respectively) and it also recorded seed cotton yield of 2881 kg ha^{-1}. Lowest endotoxin content was observed in MRC-6918, it resulted in significantly higher number of (8.89) bad opened bolls per plant as compared to all the other hybrids and it also recorded significantly lower kapas yield (2578 kg ha^{-1}) compared to other hybrids (Table 2). The results are confirmed with the findings of Kranthi *et al.* (2005a) with regard to genotypic variation Bollgard-II hybrids that had higher endotoxin concentration.

The delta-endotoxin concentration also differed significantly due to different levels of fertilizer application for target yield at 75 DAS. Increase in fertilizer level increased the delta-endotoxin concentration, significantly (Table 1) higher delta-endotoxin concentration was recorded with F_3 level (2.04 µg g^{-1} fw) followed F_2 (1.80 µg g^{-1} fw) level and F_1 (1.62 µg g^{-1} fw) the latter being the lowest. With the increase in the fertilizer

Table 1 : Delta-endotoxin concentration (µg g^{-1} fresh weight of leaves) in Bt-cotton hybrids as influenced by fertilizer levels

Treatments	75 DAS				105 DAS				120 DAS			
Bt Hybrid	Fertilizer levels				Fertilizer levels				Fertilizer levels			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
MRC-6322	1.63^{e}	1.89^{cd}	1.99^{abc}	1.84^{b}	0.78^{a}	081^{a}	1.04^{a}	0.88^{a}	0.20^{a}	0.49^{a}	0.46^{a}	0.39^{a}
MRC-6918	1.42^{f}	1.73^{de}	1.83^{cde}	1.66^{c}	0.72^{a}	0.87^{a}	0.92^{a}	0.83^{a}	0.20^{a}	0.31^{a}	0.45^{a}	0.32^{a}
MRC-7351	1.73^{de}	1.93^{bcd}	2.13^{ab}	1.93^{ab}	0.82^{a}	0.89^{a}	1.01^{a}	0.91^{a}	0.18^{a}	0.37^{a}	0.57^{a}	0.37^{a}
MRC-7201	1.71^{de}	1.94^{bcd}	2.19a	1.97^{a}	0.85^{a}	0.97^{a}	1.07^{a}	0.96^{a}	0.36^{a}	0.41^{a}	0.44^{a}	0.40^{a}
Mean	1.62^{c}	1.80^{b}	2.04^{a}	-	0.79^{a}	0.89^{a}	1.01^{a}	-	0.24^{a}	0.40^{a}	0.48^{a}	-
	S.E.±		LSD (P=0.05)		S.E.±		LSD (P=0.05)		S.E.±		LSD (P=0.05)	
Hybrid (G)	0.02		0.12		0.06		NS		0.06		NS	
Fertilizer (F)	0.02		0.10		0.05		NS		0.05		NS	
Interaction (G x F)	0.04		0.20		0.09		NS		0.11		NS	

Note: F_1: 145:39:99 kg ha^{-1} NPK (2 t ha^{-1}), F_2: 181:49:124 kg ha^{-1} NPK (2.5 t ha^{-1}), F_3: 217:59:148 kg ha^{-1} NPK (3 t ha^{-1})
DAS: Days after sowing, NS: Not significant

levels, there was non-significant difference between numbers of bad opened bolls per plant at different harvesting times, but kapas yield increased with increase in the fertilizer levels. Significantly higher seed cotton yield was recorded at F_3 level (3286 kg ha^{-1}). Plant delta-endotoxin content increased with fertilizer levels which could be due to increased nitrogen fertilizer with each successive dose as reported by Pettigrew and Adamczyk (2006) and Bruns and Abel, (2003).

Increase in fertilizer level targeted for higher yield recorded higher endotoxin concentration particularly in Bollgard-II and MRC-7201 recorded highest endotoxin concentration (2.19 µg g^{-1} fw) with F_3 level and MRC-7351 (2.13 µg g^{-1} fw) was at par with it. Increase in fertilizer levels significantly decuased higher number of bad opened bolls per plant and was recorded with MRC-6918 at all the levels of fertilizer application as compared to other hybrids. Among different Bollgard hybrids, MRC-6322, recorded the maximum kapas yield at any given level of fertilizer.

In general, cost of cultivation (Table 3) followed the level of fertilizer applied, MRC-6322 cotton hybrid recorded significantly higher net returns (Rs.65620 ha^{-1}) at high (F_3) yield level. The lowest net return was obtained with MRC-7201 (Rs.38009 ha^{-1}) at normal (F_1) yield level. However, in case of Bollgard-II cultivars cost was also higher due to high cost of seeds. Among Bt cotton genotypes, MRC-6322 Bt hybrid recorded the higher gross returns, net returns and also

Table 2 : Number of bad opened bolls per plant in cotton hybrids at different harvesting time and yield (kg ha^{-1}) as influenced by fertilizer levels

Treatments	1st picking				2nd picking				3rd picking				Yield (kg ha^{-1})			
Bt Hybrid	Fertilizer levels				Fertilizer levels				Fertilizer levels				Fertilizer levels			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
MRC-6322	3.27^{b}	2.60^{b}	2.60^{b}	2.82^{b}	2.47^{c}	2.47^{c}	2.40^{c}	2.44^{b}	4.47^{bc}	5.40^{bc}	4.27^{c}	4.71^{b}	3062^{ab}	3067^{ab}	3730^{a}	3286^{a}
MRC-6918	8.87^{a}	8.33^{a}	9.47^{a}	8.89^{a}	6.07^{ab}	6.13^{ab}	7.87^{a}	6.69^{a}	6.93^{abc}	8.00^{ab}	9.34^{a}	8.09^{a}	2472^{b}	2494^{b}	2769^{b}	2578^{b}
MRC-7351	3.27^{b}	3.93^{b}	3.33^{b}	3.51^{b}	3.20^{c}	3.00^{c}	3.13^{c}	3.11^{b}	5.00^{bc}	5.67^{bc}	6.00^{abc}	5.59^{b}	2857^{b}	3006^{ab}	3290^{ab}	3051^{a}
MRC-7201	3.93^{b}	2.80^{b}	3.40^{b}	3.38^{b}	2.27^{c}	3.00^{c}	4.20^{bc}	3.16^{b}	4.47^{bc}	6.00^{abc}	5.13^{bc}	5.20^{b}	2561^{b}	2997^{ab}	3086^{ab}	2881^{ab}
Mean	4.83^{a}	4.43^{a}	4.70^{a}	-	3.50^{a}	3.65^{a}	4.40^{a}	-	5.22^{a}	6.27^{a}	6.19^{a}	-	3062^{ab}	3067^{ab}	3730^{a}	-
	S.E.±		LSD (P=0.05)		S.E.±		LSD (P=0.05)		S.E.±		LSD (P=0.05)		S.E.±		LSD (P=0.05)	
Hybrid (G)	0.27		1.39		0.23		1.18		0.35		1.81		80.23		407.60	
Fertilizer (F)	0.23		NS		0.20		NS		0.30		NS		69.48		353.00	
Interaction (G x F)	0.47		2.41		0.40		2.05		0.61		3.14		138.96		705.90	

Note : F_1: 145:39:99 kg ha^{-1} NPK (2.0 t ha^{-1}) F_2: 181:49:124 kg ha^{-1} NPK (2.5 t ha^{-1}) , F_3: 217:59:148 kg ha^{-1} NPK (3.0 t ha^{-1}) NS: Not significant

Table 3 : Economics of cotton production as influenced by fertilizer levels

Treatments	Cost of cultivation (Rs. ha^{-1})	Gross return (Rs. ha^{-1})	Net return (Rs. ha^{-1})	B:C ratio
$G_1 F_1$	15598^{f}	68374^{ab}	52775^{ab}	3.07^{ab}
$G_1 F_2$	16644^{e}	68473^{ab}	51829^{ab}	3.11^{ab}
$G_1 F_3$	17454^{d}	83067^{a}	65620^{a}	3.76^{a}
$G_2 F_1$	15598^{f}	55405^{b}	38761^{b}	2.58^{bc}
$G_2 F_2$	16644^{e}	55853^{b}	40273^{b}	2.33^{bc}
$G_2 F_3$	17454^{d}	61940^{b}	44486^{b}	2.55^{bc}
$G_3 F_1$	19348^{c}	63842^{b}	44494^{b}	2.30^{bc}
$G_3 F_2$	20394^{b}	67132^{ab}	45901^{b}	2.16^{bc}
$G_3 F_3$	21204^{a}	73065^{ab}	53004^{ab}	2.59^{bc}
$G_4 F_1$	19348^{c}	57357^{b}	38009^{b}	1.96^{c}
$G_4 F_2$	20394^{b}	66946^{ab}	45741^{b}	2.15^{bc}
$G_4 F_3$	21204^{a}	68901^{ab}	48506^{ab}	2.38^{bc}
Mean	18440	65863	47449	2.60
S.E.±	1494	6331	6231	0.42
LSD	0.1312	15600	15520	0.85

Note : G_1: MRC-6322, G_2: MRC-6918, G_3: MRC-7351 and G_4: MRC-7201,
F_1: 145:39:99 NPK kg ha^{-1} (2.0 t ha^{-1}), F_2: 181:49:124 NPK kg ha^{-1} (2.5 t ha^{-1}) and F_3: 217:59:148 NPK kg ha^{-1} (3.0 t ha^{-1})

B:C ratio at all targeted yield levels. Similar results were reported by Patil *et al.* (2004 and 2005) who obtained higher economic returns with Bt genotypes.

Among the transgenic Bt cotton hybrids, delta endotoxin concentration was higher in Bollgard-II transgenic (MRC 7201, MRC 7351) compared to Bollgard-I and number of bad opened bolls were higher in Bollgard-I cotton hybrids. With increase in the fertilizer levels for higher targeted yield significantly increased the endotoxin concentration in cotton hybrids.

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