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Productivity and endotoxin expression as influenced by nutrient levels and nitrogen doses application in *Bt* cotton under irrigation

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ABSTRACT: Pooled results over two years indicated that, significantly higher seed cotton yield/ha was recorded with MECH 162 *Bt* (2537 kg/ha), followed by RCH 2*Bt*. The lowest yield was observed in JK Durga *Bt* (2058 kg/ha). Among the nutrient levels, application of 200:100:100 N:P_2O_5:K_2O kg/ha recorded higher seed cotton yield (2515 kg/ha), as compared to other nutrient levels. Whereas in doses of nitrogen application, N @ 12.5 per cent as basal and at 30 , 90, 120 DAS and 50 per cent at 60 DAS with foliar spray of urea @ 2 per cent at 105 and 135 DAS produced significantly higher seed cotton yield (2397 kg/ha). JK Durga exhibited highest d-endotoxin concentration (2.33 mg/g) at 45 DAS as compared to other 3 genotypes than at 135 DAS (1.58 mg/g). Application of 200:100:100 N:P_2O_5:K_2O kg/ha recorded maximum d-endotoxin concentration(2.31 mg/g) at 45 DAS as compared to other nutrient levels. Whereas, among the doses of nitrogen application, nitrogen @ 12.5 per cent as basal and at 30, 90 and 120 DAS and 50 per cent N at 60 DAS with foliar spray of urea @ 2 per cent at 105 and 135 DAS produced higher d-endotoxin (2.22 mg/g) at 45 DAS as compared to other doses of nitrogen application.

Key words : *Bt* cotton, d-endotoxin expression, genotypes, mitrogen doses, nutrients levels, yield

Performance of *Bt* cotton varies from region to region with changing agro-climatic conditions, pest pressure and management practices. The cultivars of transgenic cotton, however, do not exhibit the same level d-endotoxin concentration and it may vary from genotype to genotype. Therefore, it is necessary to validate *Bt* cotton production technology for a location for exploiting complete potential of *Bt* hybrids. A difference in larval survival of lepidopteran pests has been correlated to differential expression of 'Cry 1Ac' in various plant parts among commercial varieties of *Bt* cotton during growth. Profiling 'season long expression of Cry 1Ac' in *Bt* cotton varieties has indicated that 'Cry 1 Ac' d-endotoxin level decreases as the plant ages. Possible alteration of cry protein levels with manipulation of management practices could be a sound practice to increase production, so also helps for further extension of cry protein levels at later stages of crop cycle when, the natural levels in the plant will decline. Furthermore, plant structures such as terminal leaves, express more 'Cry 1 Ac d-endotoxin compared to floral structures.

Cotton, particularly hybrids being exhaustive, draw plenty of soil nutrients and thus under continuous cropping pattern nutrient management assumes importance. Nutrient recommendation varies with crop response, soil condition and hence different levels of nutrients have to be used. In this context, present investigation was carried out to study on d-endotoxin expression as influenced by nutrient levels and doses of nitrogen applications in *Bt* cotton under irrigated condition in deep black cotton soil.

MATERIALS AND METHODS

A field experiment was conducted for two *kharif* seasons of 2007-2008 and 2008-2009 to study the d-endotoxin expression as influenced by nutrient levels and split application in *Bt* cotton under irrigation. The trial was laid out in split split plot design with 3 replications. The experiment consisted of 24 treatment combinations with genotypes in main plots (V_1: MECH 162 *Bt*,V_2: RCH 2 *Bt*,V_3: JK Durga, V_4: MRC 7201BG II), and nutrient levels in sub plots {(F_1: 120:60:60 (Recommended), F_2: 160:80:80 and F_3: 200:100:100 N: P_2O_5:K_2O kg/ha (Farmers' practice)} whereas doses of nitrogen application were assigned to sub sub plots (A: 50% N at basal + 50% N in three equal splits at 50, 80 and 110 DAS + foliar spray of urea @ 2% at 80 and 100 DAS and B: 12.5% N as basal and at 30, 90 and 120 DAS and 50% N at 60 DAS with foliar spray of urea @ 2% at 105 and 135 DAS). At sowing 100 per cent recommended P and K were applied to all the treatments and N was applied as per treatments. The soil of the site was deep black cotton soil. Enzyme Linked Immuno Sorbent Assay (ELISA) procedure was used to determine the d-endotoxin concentrations in cotton leaf samples taken at 45, 75, 105 and 135 DAS. The desiGen Cry 1 Ac plate is designed for quantification laboratory estimation of Cry 1 Ac protein in cotton leaf samples.

RESULTS AND DISCUSSION

Performance of *Bt* genotypes: Among the genotypes, significantly higher (2754 g/ha) seed cotton yield was recorded with MECH 162 *Bt* in 2007-2008 (Table 1) and it was *on par* with MRC 7201 BG II (2623 kg/ha) and RCH 2 *Bt* cotton genotype. MECH 162 *Bt* produced significantly higher seed cotton yield (2321 kg/ha) as compared to JK Durga (1830) and MRC 7201 (2092 kg/ha) during 2008-2009. Whereas in pooled results significantly higher seed cotton was recorded with MECH 162 *Bt* (2537 kg/ha) and the lowest yield was observed in JK Durga *Bt* cotton genotype (2058 kg/ha). Halemani *et al.,* (2004) reported that among the *Bt* cotton hybrids RCH 2*Bt* was top yielder, followed by RCH 144*Bt*, RCH 20*Bt* and MECH 184*Bt* at ARS, Dharwad Farm. Whereas Patil (2007), in his study observed that the performance of MRC 6322 was superior among the different *Bt* hybrids tested. These results are in conformity with the findings of Mayee *et al., (*2004). In the present study this higher yield in MECH 162 *Bt* was mainly attributed by higher yield/plant (148.7 g) as compared to JK Durga *Bt* (123.3 g) and MRC 7201 (141.7 g) and it was *on par* with RCH 2 *Bt* (140.7 g).

Effect of nutrient levels on yield : Among the nutrient levels, application of 200:100:100 N: P_2O_5:K_2O kg/ha registered higher seed cotton yield (2714 kg/ha) as compared to rest of other nutrient fertilizer levels (Table 1). Significantly lower seed cotton yield was noticed in 120:60:60 N: P_2O_5:K_2O kg/ha in 2007-2008. During 2008-2009, similar trend in yield was noticed in 200:100:100 N: P_2O_5:K_2O kg/ha (2315 kg/ha). The lowest yield was recorded in 120:60:60 N: P_2O_5:K_2O kg/ha (1952 kg/ha). The similar trend was also observed in pooled results, application of 200:100:100 N P_2O_5 K_2O kg/ha registered significantly higher seed cotton yield of 2515 kg/ha than the rest of other nutrient levels. Similar findings were also reported by Patil (2007). These results are also in line with reports made by Jagvirsingh *et al.*, (2003). In the present study, higher seed cotton yield with application of 200:100:100 N: P_2O_5:K_2O kg/ha was greatly influenced by higher seed cotton yield/plant (146.5 g) and boll weight (5.12 g) in 2007-2008. The similar trend was also observed in 2008-2009 with higher seed cotton yield/plant (125.1g) and boll weight (5.07 g). Similarly, the pooled data also revealed that, higher seed cotton yield/plant (135.8 g) and boll weight (5.09 g) with application of 200:100:100 N: P_2O_5:K_2O kg/ha. Whereas, lowest seed cotton yield with 120:60:60 N: P_2O_5:K_2O kg/ha was mainly due to low seed cotton yield/plant and boll weight (Table 1).

Effect of nitrogen doses application: Among the doses of nitrogen application, results during 2007-2008 revealed that, application of N @ 12.5 per cent each as basal and at 30, 90 and 120 DAS and 50 per cent N at 60 DAS with foliar spray of urea @ 2 per cent, registered significantly higher seed cotton yield (2607 kg/ha) as compared to recommended nitrogen dose application (2521 kg/ha). The similar trend in seed cotton yield of 2187 and 2397 kg/ha was recorded during 2008-2009 and pooled results, respectively. Patil *et al.,* (2004) reported that split application of fertilizers through drip with 90 per cent RDF in 19 equal splits at 5 days intervals

Table 1. Seed cotton yield and yield parameters as influenced by genotypes, nutrient levels and doses of nitrogen application in *Bt* cotton

Treatments	2007-2008			2008-2009			**Pooled**		
	Seed cotton yield (kg/ha)	Seed cotton yield (g/plant)	Boll weight (g)	Seed cotton yield (kg/ha)	Seed cotton yield (g/plant)	Boll weight (g)	**Seed cotton yield (kg/ha)**	**Seed cotton yield (g/plant)**	**Boll weight (g)**
Genotypes									
V_1 MECH 162 *Bt* (BG I)	2754 a*	148.7 a	4.94 a	2321 a	125.3 a	4.74 a	**2537 a**	**137.7 a**	**4.85 a**
V_2 RCH 2 *Bt* (BG I)	2592 a	140.7 a	5.01 a	2305 a	124.5a	4.84 a	**2448 ab**	**132.7 ab**	**4.92 a**
V_3 JK Durga (BG I)	2286 b	123.3 b	4.95 a	1830 c	98.8 c	4.82 a	**2058 c**	**111.1 c**	**4.89 a**
V_4 MRC 7201 *Bt* (G II)	2623 a	141.7 b	5.09 a	2092 b	112.9 b	4.90 a	**2358 b**	**127.3 b**	**4.99 a**
S Em ±	58	3.13	0.06	50	2.69	0.04	**43**	**2.26**	**0.06**
CD (p=0.05)	202	10.84	NS	172	9.32	NS	**147**	**7.82**	**NS**
Nutrient levels (N-P_2O_5-K_2O kg/ha)									
F_1 120:60:60 (Recommended)	2422 c	130.8 c	4.89 b	1952 c	105.3 c	4.49 c	**2187 c**	**118.0 c**	**4.69 c**
F_2 160:80:80	2566 b	138.5 b	5.00 ab	2143 b	115.8 b	4.92 b	**2355 b**	**127.1 b**	**4.96 b**
F_3 200:100:100 (Farmers' practice)	2714 a	146.5 a	5.12 a	2315 a	125.1a	5.07 a	**2515 a**	**135.8 a**	**5.09 a**
S Em ±	43	2.33	0.05	31	1.69	0.03	**27**	**1.46**	**0.04**
CD (p=0.05)	129	6.98	0.14	94	5.06	0.10	**82**	**4.38**	**0.13**
N split application									
A Basal (50% N) + (50% N) in 3 equal splits at 50, 80 and 110 DAS + foliar spray of urea (2%) at 80 and 100 DAS	2521 b	136.08 b	4.96 b	2087 b	112.8 b	4.74 b	**2304 b**	**124.42 b**	**4.85 b**
B N (12.5%) as basal and at 30, 90 and 120 DAS and N (50%) at 60 DAS with foliar spray of urea (2%) at 105 and 135 DAS	2607 a	141.11 a	5.04 a	2187 a	118.0 a	4.91 a	**2397a**	**129.58 a**	**5.01 a**
S Em ±	23	1.23	0.02	17	0.92	0.02	**18**	**1.01**	**0.02**
CD (p=0.05)	68	3.59	0.07	50	2.68	0.07	**53**	**2.95**	**0.07**
Interactions									
S Em ±	79	4.26	0.08	59	3.17	0.08	**63**	**3.50**	**0.06**
CD (p=0.05)	NS	NS	NS	NS	NS	NS	**NS**	**NS**	**NS**

* Means followed by same letters do not differ significantly

Table 2. Endotoxin (mg/g of leaf) as influenced by genotypes, nutrient levels and doses of nitrogen application in *Bt* cotton

Treatments	2007-2008 (DAS)				2008-2009 (DAS)				**Pooled (DAS)**			
	45	75	105	135	45	75	105	135	**45**	**75**	**105**	**135**
Genotypes												
$\mathbf{V_1}$ MECH 162 *Bt* (BG I)	2.12 b**	1.87 b	1.72 b	1.27 b	1.98 c	2.00 b	1.85 a	1.41 c	**2.05 c**	**1.94 b**	**1.78 a**	**1.34 c**
$\mathbf{V_2}$ RCH 2 *Bt* (BG I)	1.92 c	1.88 b	1.75 ab	1.11 c	1.73 d	2.07 ab	1.84 a	1.19 d	**1.83 d**	**1.98 b**	**1.79 a**	**1.15 d**
$\mathbf{V_3}$ JK Durga (BG I)	2.38 a	1.88 b	1.63 c	1.44 a	2.29 a	2.04 b	1.94 a	1.67 a	**2.33 a**	**1.96 b**	**1.78 a**	**1.58 a**
$\mathbf{V_4}$ MRC 7201 *Bt* (G II)	2.31 a	2.09 a	1.79 a	1.43 a	2.14 b	2.16 a	1.89 a	1.54 b	**2.22 b**	**2.12 a**	**1.84 a**	**1.48 b**
S Em ±	0.02	0.01	0.02	0.02	0.02	0.03	0.02	0.02	**0.02**	**0.02**	**0.01**	**0.02**
CD (p=0.05)	0.06	0.04	0.06	0.06	0.07	0.09	NS	0.06	**0.06**	**0.06**	**NS**	**0.06**
Nutrient levels (N-P_2O_5-K_2O kg/ha)												
$\mathbf{F_1}$ 120:60:60 (Recommended)	2.09 c	1.91 b	1.57 c	1.22 c	1.87 c	1.92 c	1.68 c	1.31 c	**1.97 c**	**1.92 c**	**1.62 c**	**1.26 c**
$\mathbf{F_2}$ 160:80:80	2.18 b	1.93 ab	1.68 b	1.32 b	2.06 b	2.03 b	1.84 b	1.47 b	**2.12 b**	**1.98 b**	**1.76 b**	**1.39 b**
$\mathbf{F_3}$ 200:100:100 (Farmers' practice)	2.31 a	1.95 a	1.92 a	1.43 a	2.17 a	2.25 a	2.12 a	1.57 a	**2.24 a**	**2.10 a**	**2.02 a**	**1.50 a**
S Em ±	0.03	0.01	0.02	0.02	0.03	0.02	0.02	0.02	**0.03**	**0.01**	**0.02**	**0.02**
CD (p=0.05)	0.08	0.03	0.06	0.06	0.09	0.06	0.06	0.06	**0.09**	**0.03**	**0.06**	**0.06**
N split application*												
A Basal (50% N) + (50 %) N in 3 equal splits at 50, 80 and 110 DAS + foliar spray of urea (2%) at 80 and 100 DAS	2.14 b	1.92 a	1.68 b	1.29 b	1.98 b	2.01 b	1.82 b	1.41 b	**2.06 b**	**1.97 b**	**1.75 b**	**1.35 b**
B N (12.5%) as basal and at 30, 90 and 120 DAS and N (50%) at 60 DAS with foliar spray of urea (2%) at 105 and 135 DAS	2.22 a	1.94 a	1.76 a	1.35 a	2.09 a	2.13 a	1.94 a	1.49 a	**2.15 a**	**2.03 a**	**1.85 a**	**1.42 a**
S Em ±	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.02	**0.02**	**0.01**	**0.01**	**0.02**
CD (p=0.05)	0.07	NS	0.04	0.06	0.07	0.03	0.06	0.06	**0.07**	**0.03**	**0.03**	**0.06**
Interactions												
S Em ±	0.08	0.02	0.04	0.07	0.08	0.04	0.06	0.07	**0.08**	**0.03**	**0.04**	**0.07**
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	**NS**	**NS**	**NS**	**NS**

* Days after sowing, **Means followed by same letters do not differ significantly

from 30 to 120 DAS and 10 per cent RDF as basal application to intra *hirsutum* hybrid cotton produced consistently higher yield as compared to conventional method of fertilizer application. It was also evident that increase in split number of application increased yield. Whereas, in an another study conducted by Mariyappan *et al.,* (2004), who reported that split application of N under recommended practice with 100 per cent P and K + 33 per cent N as basal and remaining N in two equal splits at 45 and 60 DAS was *on par* with skipping basal dose of NPK and supply of all dose as top dressing in two equal splits at 45 and 60 days after sowing produced highest cotton yield as compared to other treatments. In an another study conducted by Hosmath (2011), who opined seed cotton yield advantage was more with foliar application of KNO_3 @ 2 per cent, soil and foliar application of $MgSO_4$ @ 25 kg/ha and 1 per cent than recommended package. Fertigation studies made in Andhra Pradesh shown that 75 per cent of recommended N and K applied in five splits (10% as basal and remaining 90% applied from 30 to 120 DAS in 4 equal splits) produced economically viable yield as compared to recommended practice.

Interaction effects between genotypes, nutrient levels and N split application showed non-significant differences in the seed cotton yield (Table 1) in 2007-2008. Similar trend was also recorded during 2008-2009 and in pooled yield also.

d-endotoxin expression (ìg/g fw): Endotoxin expression in leaf of *Bt* cotton showed that maximum d-endotoxin was recorded with JK Durga *Bt* genotype (2.33 mg/g) compared to other three genotypes at 45 DAS. The similar trend was also noticed at 135 DAS (1.58 mg/g). Whereas, MRC 7201 BG II registered significantly higher endotoxin concentration of 2.12 and 1.84 mg/g at 75 and 105 DAS, respectively. d-endotoxin concentration increased with nutrient levels which could be due to increased nitrogen nutrient with each successive dose as reported by Pettigrew and Adamczyk (2006). It was revealed that the endotoxin/Cry protein content in *Bt* cotton genotypes changed significantly with the time, stage of growth and genotypes used. The protein content was higher in early stages and later declined steadily in later stages of growth with the advancement of season and plant age.

In the current investigation, among the nutrient levels, significantly higher d-endotoxin was produced with application of 200:100:100 N:P_2O_5:K_2O kg/ha (2.31 mg/g) as compared to other nutrient levels at 45 DAS (Table 2). The same trend was noticed at 75, 105 and 135 DAS. Doses of nitrogen application @ 12.5 per cent N each as basal and at 30, 90 and 120 DAS and 50 per cent N at 60 DAS with foliar spray of urea @ 2 per cent each at 105 and 135 DAS produced maximum d-endotoxin (2.22 mg/g) at 45 DAS (Table 2). The similar trend was also observed at 105 and 135 DAS except at 75 DAS. Readily available state of N enhances the activation of growth by activating protein synthesis in the plant. Anything that alters protein synthesis or metabolism potentially alters cry 1Ac expression At particular stage of the crop cry protein concentration changes with change in the level of N. Relationship exists between cry protein and leaf N concentration with application of N levels. So, nitrogen is to be managed in such a way that it should favour growth, production and enhancement of cry protein, an error in N management can impact crop through either deficiencies or excesses. Although deficiency is easy to correct, an excess N application is more difficult to detect and correct (Sawan *et al.*, 2006). The protein content was higher in early stages and later declined steadily with the advancement of season and plant age.

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