QUANTIFICATION OF CRY1AC PROTEIN AT DIFFERENT STAGES OF PLANT GROWTH IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract:

The present study was conducted at Central Cotton Research Institute, Multan, Pakistan during cotton growing season 2009-10. Nine cotton cultivars with Cry 1 Ac gene (Mon 531 event) selected for current experiment to characterize the toxin level of Cry1Ac protein in different Bt cotton cultivars and to record the variation in Cry1Ac protein at various plant growth stages. It was found that age of plant was having an influence on the expression of gene. Maximum level of endotoxin (0.373µg/g) was observed in genotype CIM-595 at 100 days of planting. While minimum value (0.166µg/g) was observed in genotype V-1 at 160 days of planting. Similarly different cotton genotypes showed different boll worm damage % at different growth stages. CEMB-2 was found to be most susceptible genotype showing 93.33 % boll worm damage at 160 days after planting. While V-5 proved to be most resistant showing no boll worm damage at 70 and 100 days after planting.

Key words: Bt cotton, Cry1Ac protein, plant age.

1. Introduction

Cotton being the cash and fiber crop of Pakistan contributing significantly to nation's economy by providing raw material for textile industry and earning valuable foreign exchange [1]. Pakistan is the fourth largest producer of cotton after China, USA and India [2]. Adverse growing conditions and pests are major causes of yield losses in cotton. Although chemical pesticides have been effective, yet their continuous usage has led to the development of resistance and environmental concerns [3]. Thus one option to reduce the use of toxic chemicals is the exploitation of transgenic cotton which has become an increasingly important tool for farmers around the world [4].

In Pakistan transgenic cotton is also rapidly replacing the conventional cotton. The cotton has gained about 80% share of the total cotton in Pakistan. The Bt cotton produces the Cry1Ac δ- endotoxin protein which has insecticidal effect. With the adoption of Genetically Modified cotton, increase in production and reduction in environmental pollution have been observed [5, 6, 7]. We can say this is the first step in the direction of organic cotton. Different cotton genotypes are under cultivation having different levels of resistance to the boll worms mainly *Helicoverpa armigera, Earias spps.* and pink bollworm (*Pectinophora gossypiella*) which is due to the production of different levels this endotoxin protein. The toxic protein levels are known to be influenced by the age of plant [8, 9, 10, 11].The prevailing temperature condition is also a factor to affect the synthesis of Cry 1Ac δ protein (in *Gossypium hirsutum* for resistance to *Heliothis virescens* and *Helicoverpa zea*) [12, 13].

Different level of performance of transgenic trait in cotton is reported in different regions of China and other countries [14, 15, 16, 17]. Expression levels of Bt protein have been correlated with the different survival levels of lepidopteron pests [11]. The reduction in efficacy to kill insect is associated with level of insecticidal protein [18, 12].

Transgenic plant must express toxin protein in a way that survivors are rare and heterozygous individuals are rendered functionally recessive. It is therefore required to quantify the expression level of Cry 1 Ac protein at different plant growth stages for proper pest control, prior to commercialize. The primary objectives of research reported here were to determine the toxin level of Cry1Ac protein of different Bt cotton cultivars and to record the variation in Cry1Ac protein at various plant growth stages.

2. Material and Methods

The current research was conducted at Central Cotton Research Institute, Multan, Pakistan during cotton growing season 2009-10. Nine cotton cultivars of *Gossypium hirsutum* L. containing Cry 1 Ac gene (Mon 531 event) selected for current experiment viz., Nelum -121, IR-1524, V-1, V-5, V-8, CIM-598, CIM-595, CEMB-2, and MG-6.

The seeds were sown on ridges and the sowing date was 19th May 2009. The plot size was 20 x 10 feet keeping row to row distance 30 inches and plant to plant distance 12 inches. The layout was RCBD with three replications. Water and fertilizer were applied as per normal recommendation. Three consecutive plants were selected from each genotype in every repeat and tagged after 30 days of germination. Selected plants were tested for their Bt studies. Agdia Immuno strips used for testing. Cry 1 Ac protein concentration was quantified in cotton leaves by ELISA technique using the procedure applied by [19]. After 70 days of sowing, leaf samples were collected and preserved in the liquated Nitrogen for ELISA test, fourth leaf from the top of each tagged plant was harvested for required testing. Tagged plants were quantified four times with interval of a month (70 DAP, 100 DAP, 130 DAP and 160 DAP). Each treatment is replicated three times. Average temperature of 7 days was calculated prior to every time of sampling Bt quantification data was analyzed by split plot design. Plant age was kept in main blocks while genotypes were kept in sub plots. The data on bollworms infestation was recorded from 3 randomly selected plants by counting sound and damage bolls and converted to percentage using the following formula;

$$Percent\ bolls\quad damage = \frac{Infested\quad bolls}{Total\quad bolls} x100$$

3. Results and Discussion

Analysis of variance of this split plot design (Table 1) showed non significant differences among the replications which indicates that the variation among the plants of single genotypes in different replication is negligible.

F-ratio for days after planting (DAP) which were kept in main block was highly significant. It is concluded that age of plant has great impact on the expression of gene Cry 1 Ac for production of endotoxin. Genotypes were kept in sub blocks also showed highly significant difference for production of toxin. It means that genotypes have different levels of expression for production of toxin. This highly significant difference in the genotypes under study may be due to the differences in their genetic makeup. F-ratio for DAP and genotype interaction was also highly significant.

It is very clear that maximum level of Bt protein was produce at hundred DAP in all cultivars (Table 2). CIM-595 showed maximum value (0.373µg/g) at hundred DAP followed by CIM-598 (0.357µg/g) also after hundred DAP. While minimum Bt protein was produce at 160 DAP in genotype V-1 (0.166µg/g). The genotypes V-1 showed the lowest level of Bt protein at 160 DAP. Very less deviation observed at 70 DAP between cultivars. Our results are in accordance of the findings of [20]. They also performed Cry 1Ac proteins using two cotton hybrids MECH-184 and RCH-2. They observed decline in protein levels with tissue maturity and senescence.

Table 1: Analysis of Variance (Split)

SOV	d.f	SS	MS	F-Ratio	S.E	C.D	
						5%	1%
Rep	2	0.0019058	0.00095	2.78 ^{NS}			
DAP	3	0.09717936	0.033239	94.47 **	0.005	0.01	0.02
Error (I)	6	0.00205739	0.00034				
Genotypes	8	0.07218719	0.00902	16.19 **	0.0096	0.02	0.03
DAP x Genotypes	24	0.03359422	0.0014	2.51 **	0.0193	0.04	0.05
Error (II)	64	0.03567348	0.00056				
	107	0.24259744		DAP x Genotypes (II)	0.0185	0.04	0.05

C.V (I) = 6.76% C.V (II)= 8.61

Table. 2 Bt value (µg/g)

Genotypes	Days after planting			
	70	**100**	**130**	**160**
IR-1524	0.255 (0.003)*	0.272 (0.004)	0.246 (0.0020)	0.234 (0.0032)
Bt-121	0.264 (0.0046)	0.322 (0.0439)	0.274 (0.0303)	0.248 (0.0359)
V-1	0.260 (0.00115)	0.281(0.0166)	0.194 (0.0297)	0.166 (0.0420)
V-5	0.259 (0.00577)	0.316 (0.0052)	0.214 (0.0277)	0.208 (0.0020)
V-8	0.265 (0.00577)	0.327 (0.0606)	0.270 (0.0327)	0.232 (0.0106)
CIM-598	0.256 (0.00153)	0.357 (0.0155)	0.306 (0.0072)	0.289 (0.0010)
CIM-595	0.265 (0.01136)	0.373 (0.0196)	0.304 (0.0115)	0.293 (0.0179)
CEMB-2	0.264 (0.003)	0.336 (0.0430)	0.289)0.0351)	0.284 (0.0423)
MG-6	0.273 (0.00289)	0.340 (0.0208)	0.276 (0.0227)	0.269 (0.0217)

* The values in bracket are the standard deviation values.

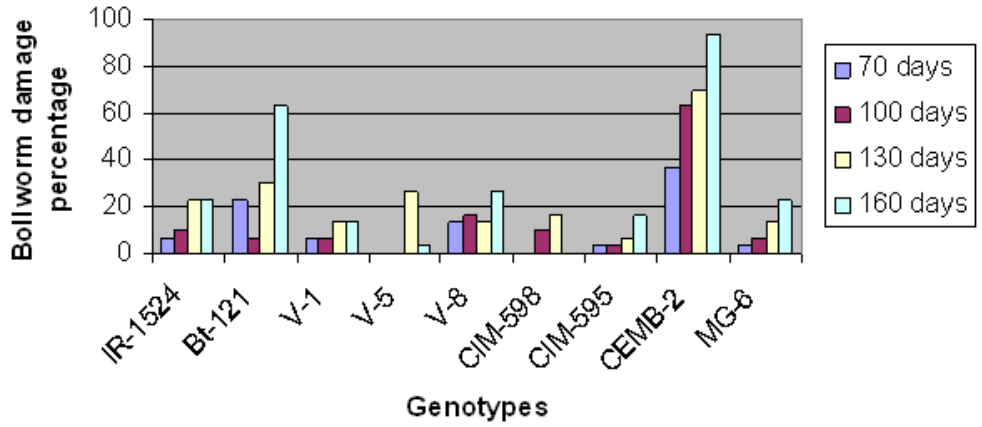

Figure 1: Boll worm damage %age in fruit on different stages of crop development

Figure 1 shows boll worm (*Helicoverpa armigera, Earias spps.* and *Pectinophora gossypiella*) damage percentage in fruits at different days after planting. Maximum boll worms damage (93.33%) was recorded in genotype CEMB-2 at 160 DAP. While genotype V-5 showed 0 % boll damage at 70 and 100 DAP. Similarly majority of genotypes showed maximum boll damage at 160 DAP followed by 130 DAP except for CIM-598 that showed 0 % bollworm damage at 70 and 160 DAP. Similar findings have been reported by [21] who reported that the efficacy of transgenic Bt cotton against targeted pests varies with plant age due to reduction in amount of endotoxin proteins in plant tissues. That resulted in greater boll damage with growing age of plant. It is therefore concluded from above that both genotypes and age of plant are critical factors as far as the level of Bt endotoxin gene is concerned.

4. References

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