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Effect of tobacco streak virus, bacterial leaf blight, grey mildew diseases on the expression of Cry1Ac protein in Bt cotton

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Abstract

Transgenic Bt Cottons expressing Cry protein are proved to be toxic to bollworms played significant role in reducing crop losses. Besides Cry protein expression level, spatial and temporal expression of Cry protein plays a very crucial role in attaining hosts resistance. In the present investigation, we focused on the effect of four cotton diseases on Cry protein expression in JK Durga Bt cotton hybrids at 60 and 120 DAS (with Grey mildew), at 120 DAS (with Tobacco Streak Virus) and at 150 DAS (with Bacterial Leaf Blight). Experiment results revealed that there is no significant difference in the expression of cry protein between healthy Bt cotton hybrids and diseased hybrids. Thus, we conclude that there is no effect of disease incidence on the Cry protein expression which in turn conveys that the expression of Cry1Ac protein, responsible for resistance against insects is not influenced by other diseases on the plant.

Keywords: Bt cotton, Cry1Ac, tobacco streak virus, bacterial leaf blight, grey mildew

1. Introduction

Cotton is an economically and commercially important crop which is grown throughout the world. Cotton is as a source of natural fiber, food and feed. Globally India stands first in cotton acreage occupying about 34% of world cotton area [1]. Yet, there is only 12% of total cotton production from Indian due to less yield potentials and heavy damage by insect pests especially by boll worms [2]. Annually, about US$ 600 M are invested for pest management. Out of which nearly 50% uses are applied on cotton crops alone [3]. Though effectual use of insecticides contributed to certain level of suppression of pests, repeated pest outbreaks occurred which may due to pest resistance against pesticides. Furthermore environmental concerns called for a lasting solution.

At this point, genetic engineering led to development of transgenic Bt Cotton. Bt Cotton allowed sustainable growth in cotton production. Particularly in India area under Bt cotton has reached about 118.81 lakh/ha [4]. Furthermore, usage of Bt cotton reduced the use of pesticide use with higher effective yield [5-7]. The first generation transgenic cotton in India targeted to control bollworm complex with single gene construct. Later, to improve spectrum of activity and to increase efficacy, stacked or dual gene transgenic cottons were introduced. In India, a total of 809 cotton hybrids and a variety based on 6 events are under commercial cultivation [8]. Genes encoding cry group of endotoxins were inserted in cotton which enabled it to become highly toxic to insects. Insect safe cotton proved to be powerful in controlling lepidopteron pests and is exceptionally gainful to producer and environment by diminishing concoction insecticides and saving populace of advantageous arthropods.

For the sustainable development of Bt cotton, toxin protein is to be communicated in satisfactory amounts in proper plant parts at the essential time of the season to manage pests. But several studies revealed that the expression of Cry protein in different plant parts at a given point of time and different growth stages is not uniform. Stacked *Bt* cotton and *Bt* cotton cultivars control the bollworms up to 110 days and further the toxin expression level decreases as the plant age advances [9-11]. Results from work [12] revealed that Cry protein levels were high in leaves followed by squares, bolls and least in flowers. *Bt* gene does not allow the development of bollworm population because of the inherent toxicity of the *Bt* cotton against bollworms [13]. This leads to minimum usage of insecticides and is considered as one of the best tools of Integrated Pest Management against bollworm complex.

It has been proved eco-friendly in the management of bollworm complex [14-15]. In spite of several advantages of Bt cotton, several concerns have been raised. They include risk of out crossing through pollen drift, food safety, horizontal gene transfer, loss of susceptibility in target pests to Bt toxins, indirect or direct effect on soil biodiversity, ecosystem. In the view of importance of expression levels of Cry protein of Bt cotton in conferring resistance to host plant, the present investigation focuses on to explore the impact of plant diseases on insecticidal protein content in leaves in Cry1Ac *Bt* cotton.

2. Materials and Methods

Effect of diseases on the *Cry1Ac* expression in Bt cotton was evaluated for two consecutive seasons *i.e*. during *Kharif*, 2007-08 and *Kharif*, 2008-09 at Regional Agricultural Research Station, Lam, Guntur.

2.1 Plant Material and Reagents

A Bt cotton hybrid JK Durga *Bt with Cry1Ac* event was selected for the study. A bulk plot of 200 sq m each with JK Durga Bt cotton hybrid was raised both without protection for diseases and with protection from diseases for two seasons (*Kharif* 2007-2008 and 2008-2009) at RARS, Lam, Guntur by following recommended agronomic practices. Fungicides were applied at 15 days intervals against the diseases for plants grown with protection from diseases. Bt cotton plants grown with no plant protection were affected with Grey mildew disease, Tobacco Streak virus, and Bacterial Leaf Blight diseases. ELISA Test kit for Cry1Ac identification was acquired from Genei Bangalore, India.

2.2 Collection of Sample

Leaf samples from JK Durga Bt cotton hybrid, with protection were taken as control. Leaf samples from JK Durga Bt cotton hybrid raised without protection for diseases were collected at 60 and 120 DAS (affected with Grey mildew), at 120 DAS (affected with Tobacco Streak Virus) and at 150 DAS (affected with Bacterial Leaf Blight) were collected.

2.3 Estimation of Cry1Ac protein

Healthy and diseased leaf samples were collected and estimated for Cry protein levels in Bt referral laboratory at CICR, Nagpur, Maharashtra, India using ELISA technique A 96 wells microtiter plate, precoated with anti-Cry1Ac antibodies was used 50 µl of positive control of Cry1Ac and 50 µl negative control of Cry1Ac that are available in kit were added to ELISA plate. In the rest of wells, 50 µl of samples (both control and tested) were added followed by addition of 50 µl of Cry1Ac conjugate buffer given in kit. After mixing the contents gently, plate was kept for incubation at room temperature for 40 minutes. After the incubation, well was washed with working wash buffer. To each well, 100 µl of substrate solution was added and incubated for 20 minutes at room temperature followed by addition of 100 µl of stop solution (both available with kit). The absorbance of contents from each well was measured at 450 nm using UV/Vis Spectrophotometer (Shimadzu-1800).

2.4 Statistical Analysis

Each sample was analyzed individually as triplicate and the data were represented as mean and variance. Data were analyzed with *t*- test to determine the significance.

3. Results and Discussion

During *Kharif* 2007-08, at 60 DAS, Cry1Ac protein content in healthy leaves and Grey mildew diseased leaves of JK Durga *Bt* was 1.28 µg/g FWt and 1.10 µg/g FWt respectively. While, after 90 DAS in the same year Cry1Ac protein content in healthy leaves and diseased leaves was found to be 4.62 µg/g FWt and 4.12 µg/g FWt correspondingly. During *Kharif* 2008-09, Cry1Ac protein content in healthy leaves and Grey mildew diseased leaves after 60 DAS was slightly increased to 1.32 µg/g FWt and 1.28 µg/g FWt respectively. Whereas, in healthy leaves after 90 DAS, Cry1Ac protein was 5.28 µg/g FWt and in diseased leaves, protein content was found to 5.10 µg/g FWt (Table- 1, 2). There is no significant difference between the expression levels in healthy and diseased leaves in both the years. Interestingly the expression levels were high both in healthy and diseased leaves of *Kharif* 2008-2009 than *Kharif* 2007-2008 (Table- 1, 2). In both seasons, expression of Cry1Ac protein was faintly high from healthy leaves than that of from diseased samples and the difference is not significant. The present findings are in accordance with Govindappa *et al.* [16] who recorded less protein content in infected with *X. axonopodis* pv. *malvacearum* from 90 DAS to 120 DAS compared to healthy genotypes in the *Bt* cotton hybrids. Among several fungal diseases Grey mildew disease is predominant causing major yield losses. Grey mildew is said to be polycyclic disease as it can infect crop repeatedly in single cropping season [17].

Table 1: Cry1Ac protein expression (µg/g FWt) in Grey Mildew Disease affected and healthy samples of JK Durga *Bt*

Grey Mildew Disease (60 DAS) Cry1Ac µg/g FWt				
Season	***Kharif* 2007-2008**		***Kharif* 2008-2009**	
Samples	Healthy Sample	Diseased Sample	Healthy Sample	Diseased Sample
Mean	1.28	1.10	1.32	1.28
Variance	0.38	0.21	0.43	0.36
t–Test (p=0.05)	Non-Significant		Non-Significant	

Table 2: Differential Cry1Ac protein expression (µg/g FWt) in Grey mildew affected and healthy samples of JK Durga *Bt*

Grey Mildew (90 DAS) Cry1Ac µg/g FWt				
Season	***Kharif* 2007-2008**		***Kharif* 2008-2009**	
Sample	Healthy Sample	Diseased Sample	Healthy Sample	Diseased Sample
Mean	4.62	4.12	5.28	5.10
Variance	2.15	2.34	2.07	2.06
t–Test (p=0.05)	Non-Significant		Non-Significant	

Healthy and diseased leaves affected by Tobacco Streak Virus at 120 DAS were sampled and Cry1Ac protein was revealed to be 0.62 µg/g FWt and 0.40 µg/g FWt (Table- 3) respectively in Kharif 2007-08. In the consecutive year of Kharif 2008-09, Cry1Ac protein was found to be 0.60 µg/g FWt in healthy leaves and 0. 54 µg/g FWt in diseased leaves (Table- 3). Results convey a insignificant difference among expression profiles between healthy and diseased leaves between two consecutive years. Also, it is noteworthy to observe that, though there is least effect of disease on Cry1Ac protein expression, there was trivial decrease of protein content in diseased leaves. Further Ian [18] also observed reduced Cry1Ac expression in diseased and wilted plants. Several reports emphasized Tobacco Streak Virus as one of the predominant viral disease limiting yield in cotton [19].

Table 3: Cry1Ac protein expression (µg/g FWt) Tobacco Streak Virus in disease affected and healthy samples of JK Durga *Bt*

Tobacco Streak Virus Disease (120 DAS) Cry1Ac µg/g FWt				
Season	**Kharif 2007-2008**		**Kharif 2008-2009**	
Sample	Healthy Sample	Diseased Sample	Healthy Sample	Diseased Sample
Mean	0.62	0.40	0.60	0.54
Variance	0.06	0.02	0.06	0.12
t–Test (p=0.05)	Non-Significant		Non-Significant	

After 150 DAS, Cry1Ac protein was observed to be 3.38 µg/g FWt and 3.24 µg/g FWt in healthy and bacterial leaf blight diseased leaves respectively during kharif 2007-08 (Table- 4). During Kharif 2008-09, healthy and diseased leaves contained 3.40 µg/g FWt and 3.28 µg/g FWt Cry1Ac protein correspondingly Table- 4). There was an insignificant decrease in protein content in diseased leaves when compared with that of healthy leaves showing minimal effect of biotic stress on Cry1Ac protein expression. In contrast, Cry1Ac protein expression was reduced under stress [20].

Table 4: Cry1Ac protein expression (µg/g FWt) in Bacterial Leaf Blight Disease affected and healthy leaves of JK Durga *Bt*

Bacterial Leaf Blight Disease (150 DAS) Cry1Ac µg/g FWt				
Season	**Kharif 2007-2008**		**Kharif 2008-2009**	
Sample	Healthy Sample	Diseased Sample	Healthy Sample	Diseased Sample
Mean	3.38	3.24	3.40	3.28
Variance	2.48	2.36	1.36	1.50
t–Test (p=0.05)	Non-Significant		Non-Significant	

Cultivation of Bt transgenic crops have shown benefits in terms of noteworthy reduction in number and volume of insecticides, production cost, environmental contamination and improved crop yield [2]. In addition, Bt transgenic crops limit exposure of natural enemies to Bt toxins as Bt crops are highly selective to target pests, thereby minimizing negative effects on non- target organisms [21]. This is the first report studying the effect of other diseases on Cry1Ac protein expression. Results proposed no significant difference in expression of Cry1Ac protein in healthy and diseased leaf samples. This in turn conveys that the expression of Cry1Ac protein which is responsible for resistance against insects is not influenced by other diseases on the plant.

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