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Full Length Article

Temporal Expression of Cry1Ab/c Protein in Bt-Cotton Varieties, their Efficacy against *Helicoverpa armigera* (Lepidoptera: Noctuidae) and Population Dynamics of Sucking Arthropods on Them

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Abstract

Current study focused on expression profiling of locally bred Bt-cotton varieties through ELISA and were tested for their efficacy against survival of *Helicoverpa armigera* in control environment. The population dynamics of five sucking arthropods; whitefly, jassid, aphid, thrips and mites, along the growing season was also monitored and correlated with different meteorological factors. Expression levels of insecticidal protein Cry1Ab/c were found to vary significantly ($P<0.05$) among varieties and across different sampling dates. The highest mean expression was recorded in GN-31 and Sitara-008 and the lowest in FH-113 and MG-6 while across sampling dates the highest mean expression was recorded at 30 days after emergence (DAE) which decreased along the season with lowest mean at 120 DAE. A critical expression level of Cry1Ab/c in leaves was found at 770 ± 25 ng g^{-1}, for 95% control of the target insect pests. Sucking pest population was found to be variable among both the cultivars and the sampling dates. A positive correlation was found between rain/ humidity and sucking arthropods population in the sampled cotton plots. © 2014 Friends Science Publishers

Keywords: *Bacillus thuringiensis*; Cry1Ab/c; Bt-cotton; Expression; *Helicoverpa armigera*; Sucking arthropods

Introduction

Arthropod pests impose threat to cotton crop all over the world, causing serious yield and economic losses. Cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) had been regarded as the most vicious of the insect pests attacking on cotton crop in Pakistan during 1990s and early 2000s. The annual losses in cotton crop contributed towards insect pests in Pakistan were estimated up to 2.5 million bales during the latter half of 1990s (Arshad *et al.*, 2009; Karim, 2000). Farmers relied almost entirely on chemical pesticides to control this menace, most of which were found to be neurotoxic to humans and carcinogenic in nature. Furthermore, most of these chemicals persist in the environment causing pollution to land and water resources (Damalas and Eleftherohorinos, 2011). This strategy has resulted in development of resistance against most of these insecticides after repeated use over the years (Ishtiaq and Saleem, 2011; McCaffery, 1998).

Insect resistant, genetically modified (GM) crops have been developed and adopted by farmers in several parts of the world as a substitute to synthetic pesticides to fight insect-pests. According to an estimates, area under GM crops, has reached to 170m hectares over 30 countries by 2012 (James, 2012). Commercial release of insect resistant, transgenic crops transformed with genes from the soil born bacterium *Bacillus thuringiensis* has been one of the most successful developments in the area of genetic engineering during 1990s (Ferry *et al.*, 2006). Cry genes express crystalline proteins, already known for their insecticidal activity against the larvae of important lepidopteran pests, whether directly applied as biopesticide or indirectly through gene expression in plants. The larval mortality depends upon the insect species, larval age, time of exposure and the quantity of ingested Cry toxin (Ashfaq *et al.*, 2001; Halcomb *et al.*, 1996).

Cultivation of Bt-cotton in Pakistan was first reported in the year 2000 in Sindh province with the cultivation of unauthorized Australian cotton (Bollgard I - MON 531 event) which later on expanded to other provinces due to high tolerance of Bt varieties to the bollworm complex and by 2009 more than 60% of cotton area was covered by Bt cotton (USDA GAIN, 2010). After getting satisfactory results from Bt-cotton varieties and continuous requests from the farmers and the scientific community, the Government of Punjab province approved Bt cotton for general release and cultivation (Arshad *et al.*, 2007).

For sustainable defense against target insect pests, it is vital that the toxic protein expression should be sufficient in potentially vulnerable parts of the host plant and at the required growth stages. However, a number of reports from different cotton growing regions of the world have

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described variable amount of Bt protein measured in different cotton tissues when studied throughout the gowing season, and thus variable efficacy against lepidopteran insect pests (Dong and Li, 2007). Special emphesis has also been given to maintain a minimum level of protein required to control the target pest at any given time of the season called the critical expression level. Kranthi *et al.* (2005) in India have calculated a critical level of Cry1Ab/c (1.9µg g^{-1}) for effective control of *H. armigera,* below which the target pest had higher chances of survival.

Concerns are growing that the large-scale planting of Bt-cotton with inconsistency in the level of toxin expression, can limit the efficacy of the GM plants and also increases the chances of target insects becoming resistant to Bt-proteins (Kranthi *et al.*, 2006; Tabashnik *et al.*, 2008). These problems are of particular importance for Pakistan, where uncontrollably bred cotton varieties are grown instead of certified, high quality seeds used in developed countries.

The aim of this study was to evaluate Cry1Ab/c protein expression in the first batch of locally bred, approved, Bt-cotton varieties in Punjab, Pakistan and its efficacy against target insect pest *H. armigera*. The second objective was to examine population dynamics of sucking pests in Bt-cotton cultivars.

Materials and Methods

Plant Material and Experimental Design

Nine Bt cotton varieties IR-3701, Ali Akbar-802, IR-1524, Ali Akbar-703, FH-113, CEMB-02, Sitara-008, MG-6, Neelum-121, containing the Cry1Ac gene from *Bacillus thuringiensis* (event MON531), two hybrids GN-31 and GN-2085 expressing fusion gene Cry1Ac and Cry1Ab and a non-Bt control NIBGE-115 were planted within National Institute for Biotechnology and Genetic Engineering (NIBGE) premises. Presence of the Mon-531 event was confirmed by performing PCR, using gene specific primers (Forward; CAAAGGAGCCTGTTCA, Reverse; TTGAGGTGAGTCAGAATGTTGTTC') on extracted DNA from representative plants of each variety (Fig.1). All varieties were grown in the normal cotton growing season (May to November, 2010). Experimental plots consisted of 4 rows 1.0 m × 30 m each. Treatments were arranged in a randomized complete block design (RCBD). Varieties were replicated four times. All the agronomic practices such as irrigation, fertilizer application and intercultural operations were similar in all the treatments. No pesticide was sprayed until the last sampling of insect data.

Season-long Sampling of Bt Cotton Leaves for ELISA

The expression of the protein was first confirmed by immuno-strip assay, using Bt-Cry1Ab/c ImmunoStrip® STX 06200/0050 (Agdia, USA). Leaf samples were taken from confirmed plants, at 4 sampling dates throughout the season at 30, 60, 90, and 120 days after emergence (DAE). Since

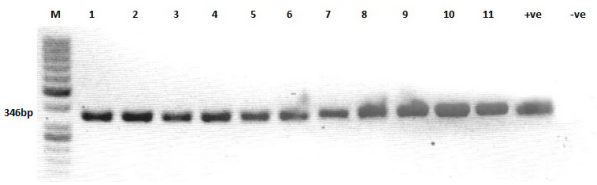

Fig. 1: Gell electrophoresis picture, showing Mon-531event. PCR was prformed on DNA extracted from replresntative plants of each Bt-cotton variety. Lane M, 50 bp marker; lane 1 to 11, Bt-cotton verities.

differential expression of Cry1Ac among different plant parts have been reported (Kranthi *et al.*, 2005), a single part was selected for quantification of expressed Bt protein. For each sampling date and for all varieties, a single main-stem fully expanded terminal leaf was randomly harvested from 10 plants $replication^{-1}$. Leaves were immersed in liquid nitrogen and immediately transported to the laboratory after collection. One leaf punch was taken from each sample using a 1.5 ml Eppendorf tube. The samples were weighed accurately to determine the initial mass of leaf tissue.

Quantification of Cry1Ab/c Protein

A commercial quantification kit (QuantiPlate™ Kit, EnviroLogix, Inc., Portland, ME) was used to quantify the amount of Cry1Ab/c present in each sample. This "sandwich" Enzyme-Linked Immuno-Sorbent Assay (ELISA) uses a color development step where the intensity of color production is proportional to the Cry1Ab/c concentration in the sample extract. The samples of Bt cotton leaves were ground in a pestle and mortar along with 1.5 mL Cry1Ab/c extraction buffer and then transferred into a 1.5 mL micro-centrifuge tube. The tubes were then centrifuged at 10,000 g for 2 min. For each sample, 20µL of supernatant was diluted 1:66 times with Cry1Ab/c extraction buffer. For all samples, optical densities were plotted against a standard curve with calibrators supplied with the kit. The amount of Cry1Ab/c was calculated as parts per billion (ppb), which corresponds to nano gram per gram fresh weight (ng g^{-1}) of leaf.

Detached Leaf Bioassays with *H. armigera*

Larvae hatching from field-collected eggs of *H. armigera* were reared on a chickpea based artificial diet (Armes *et al.*, 1992) individually in plastic cups (5cm diameter × 6 cm height), until pupation. Adults were kept in groups of 10, in glass jars (30 cm diameter × 40 cm height) and fed on 10% honey solution (Kranthi *et al.*, 2009). Small pieces of muslin cloth were placed on inner surface of the jars for oviposition. Less than 24 h old 1st instar larvae were used for the bioassays. Fresh cotton leaves from top canopy of the Bt-positive plants from each variety, at 30-40 DAE stage of growth, were collected and washed with distilled water.

Leaves were placed in Petri plates on moist filter papers. Five larvae of *H. armigera* were placed on each leaf. Petri plates were covered with lids and sealed with parafilm to avoid escape of larvae. Leaves were replaced with the fresh ones daily. Data for mortality was collected after every 24 h. Bioassays were conducted for 96 h with 30-40 larvae per treatment. All the bioassays were performed in controlled conditions at 26 ± 3ºC and 70 ±10 relative humidity and 16 hour photoperiod.

Recording of Sucking Pest Population

The incidence of sucking pests was recorded early in the morning at 15 day interval from June up to September, 2010, a total of 8 sampling dates. Number of live individuals, both nymphs and adults, from 20 randomly selected plants in each replication were recorded. Sucking pests, such as whitefly (*Bamisia tabaci*), jassids (*Amrasca devastans*), thrips (*Thrips tabaci*), mites (*Acarina spp.*) and aphids (*Aphis gossypii*) were counted on three leaves per plant, one from the top, middle and bottom layer of the plant. Average number of live individual leaf^{-1} (ipl) was calculated for each replication. Data for weather during the sampling time was retrieved from archives of meteorological laboratory, department of Agronomy, Ayyub Agricultural Research Institute (AARI), Faisalabad. Correlations among sucking arthropods population and different meteorological factors were calculated.

Statistical Analysis

Repeated measure analysis of variance ANOVA was performed for Bt protein concentration and sucking pest population using varieties and sampling date as factors. Means were separated by least significant difference (LSD) test. While the survival response of *H. armigera* larvae on different Bt cotton cultivars was compared using the Kaplan-Meier procedure and Log-rank test. All statistical analyses were performed on computer software SPSS (version 16.1, IBM Corporation, Armonk, NY, USA).

Results

Temporal Expression of Bt Protein

Season long expression of Bt protein varied significantly among different GM cotton varieties ($F=475.85$; $df=10$; $P<0.05$). The highest mean expression of Bt protein over the entire season was recorded in the varieties GN-2085 (829.45 ng g^{-1}) and Sitara-008 (675.27 ng g^{-1}) while the lowest being FH-113 (189.35 ng g^{-1}) and MG-6 (247.99 ng g^{-1}). Comparison of varietal means for Bt protein concentration by LSD analysis showed that all the varieties except GN-31, Ali Akbar-802 and CEMB-02 differed significantly from each other (Fig.2).

Expression analysis at different sampling dates (growth stages) clearly exhibited that the Cry1Ab/c protein expression in Bt-cotton decreased significantly ($F=1252.45$; $df=3$; $P<0.05$) with the age of the plant as the concentration of the protein at different sampling dates varied significantly (Fig. 3). Comparison of means for Cry1Ab/c expression by LSD also confirmed the significant variability among sampling dates with highest expression at 30 DAE stage and the lowest at 120 DAE (Fig. 3). The percent decrease in Cry1Ab/c expression was also calculated with lowest recorded decline of 32% in Neelum-121 and 33.03% in GN-2085 to as much as 67.68% in MG-6 and 58.90% in Sitara-008 with an overall mean decline of 45.32% from June 28 to September 26, 2010.

Detached Leaf Bioassay

Kaplan-Meier logrank test for survival response for survival of the *H. armigera* larvae fed on detached leaves show significantly higher survival in the larvae fed on NIBGE-115 (non-Bt cotton variety, control). Longevity in the control treatment was significantly higher than the average survival (logrank test = 54.5, $P< 0.05$) (Fig. 4). The survival of *H. armigera* fed on non-Bt control, 89%, was significantly higher than the average survival on Bt-cotton cultivars 7%. A highly negative correlation (-0.79) was calculated between Cry1Ab/c concentration and mean survival (h) and a regression curve with 60% accuracy was calculated through regression equation.

$$Y_{\text{(Average life in h)}} = 73.77 - 0.033X_{\text{(Cry1Ab/c in ng g-1)}}$$

The equation shows that increase in Cry1Ab/c by 1 ng g^{-1}, may reduce average life of *H. armigera* larvae by 0.033 h. Moreover, according to regression statistics, adjusted R^2 value explains that 60% variations in the average life of *H. armigera* larvae were due to Cry1Ab/c and 40% variations are due to other factors which were not considered during the experiment.

Nine Bt cotton varieties; GN-31, GN-2085, IR-3701, Ali Akbar-802, IR-1524, Ali Akbar-703, CEMB-02, Sitara-008 and N-121, showed more than 90% control of *H. armigera* larvae, while FH-113 and MG-6 showed 70% and 80% control after 96 h of feeding (Fig. 5). Correlation between percent mortality after 96 h and the Cr1Ab/c concentration was calculated to be 0.88, highly positive. A critical value (LC_{95}) of 770 ± 25 ng g^{-1} was deduced through regression estimation, using formula;

$$Y_{\text{(\% Mortality after 96 h)}} = 67.43 + 0.036\ X_{\text{(Cry1Ab/c ng g-1)}}$$

This formula shows that an increase in 1 ng g^{-1} of Cry1Ab/c increases mortality of *H. armigera* larvae by 0.036 %. Furthermore, adjusted R^2 value suggested that the influence of Cry1Ab/c protein on mortality is about 78%. The variation of 22% might be due to other factors not taken into account during the experiments.

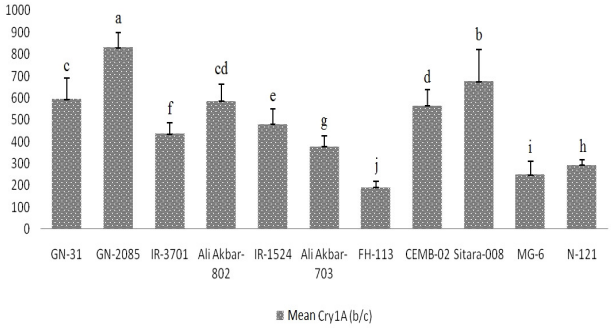

Fig. 2: LSD comparison of varietal means for Cry1Ab/c concentration in Bt-cotton leaves over the sampling period (ng/g+SE). Bars with different roman alphabets differ signifficantly from each other.

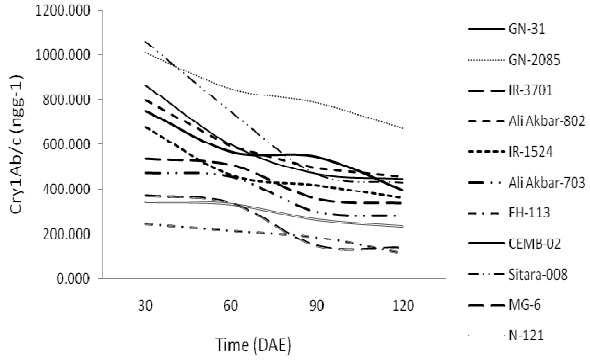

Fig. 3: Cry1Ab/c (ng g^{-1}) concentration in different Bt-cotton varieties along the season

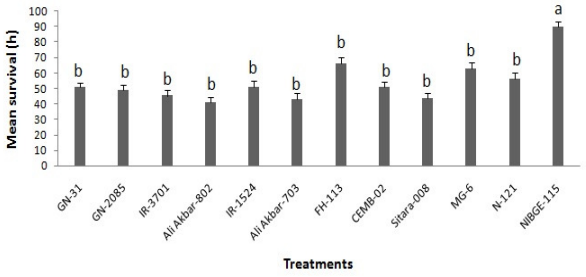

Fig. 4: Comparison of mean survival (h ± SE) of *H. armigera* larvae by logrank test . Each treatment is compared with the control (NIBGE-115). Treatments showing different alphabets (b) are signifficantly different from the control (a).

Population Fluctuation of Sucking Pests

Population of four sucking pest species varied significantly among all the Bt varieties [whitefly (F=4.46), thrips (F=4.46), aphids (F=4.89), and mites (F=8.14)] at $P< 0.05$, while in case of jassid it was non-significant $P = 0.1778$. The population of all recorded sucking pests was significantly different across sampling dates at $P <0.05$ [whitefly (F = 1551.57), thrips (F = 1925.05), aphids (F = 416.79), mites (F = 8.14) and jassid (F = 78.55)]. Pair-wise comparison of varietal means by LSD showed that sucking pest population in most of the Bt cotton cultivars was statistically in agreement to the non-Bt control in most cases. While pair-wise comparison of sampling date means by LSD showed that the sucking pest population varied significantly throughout the growing season of cotton (Fig.6). The correlation analysis between sucking arthropod population fluctuation during the growing season and weather factors gave positive correlations with rainfall and humidity while a negative correlation with temperature (Table 1).

Discussion

Several factors have been described to influence the expression of a transgenic protein in GM plant, including nucleotide sequence, copy number and insertion point of the transformed gene into the host DNA, and nature of the promoter. Internal cell atmosphere, parental background of the host plant and several external environmental factors have also been reported to play their role in expression (Bakhsh *et al.*, 2012; Guo *et al.*, 2001; Hobbs *et al.*, 1993; Mahon *et al.*, 2002). Resistance of Bt-cotton varieties against target insect pests have been reported to vary with varying expression of the toxin (Cry1Ab/c) in host plants and their vulnerable parts (Adamczyk *et al.*, 2001; Kranthi *et al.,* 2005).

Expression of Cry1Ab/c in first batch of approved Bt-cotton varieties in Punjab was found to vary significantly among each other (Fig. 3). Effect of hybrid vigour was witnessed on expression of the transgene as the ELISA results showed higher level of Cry1Ab/c in hybrid Bt-cotton varieties tested in current study. Expression level in locally bred varieties was observed to be lower as compared to previously published reports in similar studies from other parts of the world (Adamczyk *et al.*, 2009; Adamczyk and Sumerford, 2001; Kranthi *et al.,* 2005). This lower expression of the Cry1A (b/c) may be contibuted towards the genetic background of the local varieites in which the gene has been introgressed through backcross breeding (Hobbs *et al.*, 1993).

The Bt expression decreased significantly with the age of the plant. When the sampling date was considered as a linear trend the slope lines of Cry1Ab/c expression showed similar behavior, suggesting that the decrease in Cry1Ab/c protein level along the season was a behavior totally independent of the varietal background (Fig. 2).This trend followed the pattern of fall in the protien level with the age of the plant, conciling with most of the previous reports (Adamczyk *et al.,* 2009; Adamczyk and Sumerford, 2001; Kranthi *et al.,* 2005). Our results clearly demonstrated that protein expression continue to decrease throughout the growth cycle. These observation were in contrast to the

observations of Wan *et al.* (2005), who reported an increase in expression at the end of the season in one of his test varieties. This gradual decrease in transgenic protein expression has been correlated to the decline in mRNA production of the target gene and methylation of the promoter 35S in latter growth stages of the plant (Adamczyk *et al.*, 2009).

Efficacy Against *H. armigera*

The effectiveness of Bt-cotton has long been related to the amount of protein expressed in the plants at a given time, as it directly affects survival of the bollworm (*H. armigera*) when tested in laboratory conditions (Adamczyk *et al.*, 2001). Survival of the bollworm also depend upon the time of exposure to the toxin (Kranthi *et al.*, 2009). It was important to test this first batch of the approved, locally bred Bt-cotton varieties for their efficacy against the target insect-pest to guage their efficacy. Leaves were selected for the bioassays as it is the favoured site for ovipoistion (Patel *et al.*, 1974).

Kaplan-Meier logrank test confirmed that all the Bt-cotton varieties were significantly less susceptible to bollworm as compared to the non-Bt cotton control (Fig. 4), thus making a strong case for the use of Bt-cotton in future IPM technology and sustainable agriculture. All the signs of Bt toxicity like secession of feeding and restlessness were noticed in the larvae feeding on Bt-cotton leaves (Ali *et al.*, 2006). A highly negative correlation value of -0.79 between varietal means of Cry1Ab/c and survival gave a clear insight that higher expression of Bt toxin gave better control of insect pests as has already been reported (Adamczyk *et al.*, 2001; Kranthi *et al.*, 2005). The justification for 60% accuracy of the regression model may be contributed towards other factors like nutrition value of the feed, age of the larvae, tolerance level against the toxin, present in the population studied.

Results have shown the efficacy of Bt-cotton varieties providing more than 90 % mortality in 9 varieties within 96 h of exposure (Fig. 5). From bioassay results of this study a critical value (LC_{95}) of Cry1Ab/c, at any given time of the season, was deduced at 770 ± 25 ng g^{-1}. Below that concentration *H. armigera* had higher chances of survival, which also shows that the Bt-cotton varieties were more susceptible in their latter growth stages as the expression decreased considerably. This critical value is significantly lower than, the value of 1.9 ugg^{-1} calculated by Kranthi *et al.* (2005). This variation is due to different genetic background and physiomorphic characters of the cotton cultivars carrying the Bt genes. Furthermore, the insect populations used were entirely different, with different tolerance levels to the toxin. That means lower resistance against the toxin in Pakistani population of *H. armigera* used in this study as compared to the one used by Kranthi *et al.* (2005). The doubt factor of 22% in regression value of mortality might be due to different genetic backgrounds of

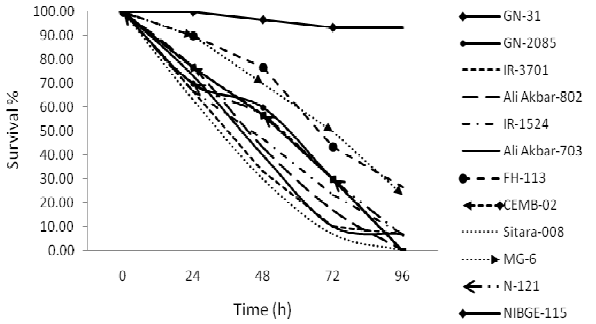

Fig. 5: Kaplan-Meier survival curve for *H. armigera* fed on different Bt-cotton varieties compared to non-Bt cotton (NIBGE-115)

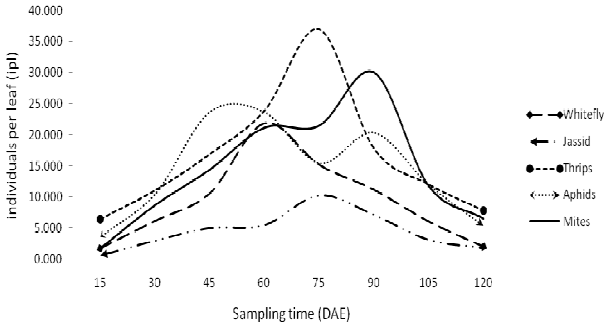

Fig. 6: Population dynamics of sucking pests along the season, in number of live individuals per leaf (ipl)

Table 1: Correlation between sucking arthropod population and different meteorological factors during the sampling period

Arthropod	Temperature	Humidity	Rainfall	Cry1Ab/c
Whitefly	-0.19	0.60	0.77	-0.64
Jassid	-0.44	0.70	0.81	-0.40
Thrips	-0.36	0.64	0.90	-0.52
Aphids	-0.14	0.55	0.67	-0.18
Mites	-0.37	0.78	0.55	-0.43

the varieties and physiomorphic characters of the host plants that might have influenced their defense mechanism and innate immunity against insect pests (Ali *et al* 2007; Kranthi *et al.*, 2005).

Population Fluctuation of Sucking Pests on Bt-cotton

The resistance against sucking pests is mainly due to many heritable morphological characters such as leaf color, hairiness, wax layer on the leaf epithelium and succulence of plant structures (Ahmad and Wakeel, 2000). Sucking arthropods appeared almost immediately after emergence. The population was not alarming during the early growing season but reached its maximum in July-August (Fig. 6). Population of sucking arthropods

increased exponentially with the vegetative growth of the plants and increase in humidity. This can be contributed to rain and subsequent increase in humidity of the environment that increased succulence of leaves containing high level of chlorophyll and phloem sap, the most cherished food of the sucking pests (Mari *et al.*, 2007).

ANOVA and LSD comparison showed that the varietal mean population of major sucking pests (whitefly, Jassid, aphid, thrips and mites) was statistically different among different cotton varieties. That was due to the different genetic background of the varieties. A loosely negative correlation was found between the mean Cry1Ab/c amount and mean sucking arthropods population (Table 1). That cannot be contributed towards the toxicity of Cry1Ab/c because its non-toxicity out of order Lepidoptera is well documented. That might have been due to better physiological traits of the varieties with higher Bt protein expression (Saleem *et al.*, 2012). These results are an evidence that Bt-cotton is not more attractive to the sucking arthropods as has earlier been reported (Sisterson *et al.*, 2004). Mean population of all the sucking pests varied highly significantly among sampling dates/ growth stages (Fig. 6). This rapid fluctuation in population at later stages of the plant growth might be a result of decreased nutritional quality of the maturing leaves (Trouve *et al.*, 1996).There were several other factors, not taken in to account, like presence of natural enemies in the field that might have played a role in reducing the population at latter stages of plant growth.

From this study we are of the view that the population of sucking pests depends upon environmental factors, plant age and physio-morphic characters of the plant (Ahmad and Wakeel, 2000), rather than on Cry1Ac/ Cry1Ab expression. Because, there has been no reliable data available for specific effects of a Cry toxin on non-target arthropods (Romeis *et al.*, 2008). Increase in sucking pest population in areas of Bt-cotton cultivation can be attributed towards the succulence of plant parts such as leaves and veins at that growth stage of the plant, combined with the conducive weather conditions. The decrease in population at later sampling dates can be attributed to the maturity of the plant and adverse weather conditions. Therefore, it is evident that the previous reports of increase in sucking arthropods in Bt-cotton growing fields was most likely due to the reduced use of broadspectrum insecticides on Bt cotton (Arshad and Suhail, 2010; Men *et al.*, 2005). However, it is hereby recommend to the breeders that they should evolve varieties with better Bt protein expression and superior physiological traits to have improved control of the target lapidopteran insect pests.

Current study was first of its kind in Pakistan and has given several lessons to enhance our understanding of the expression pattern of Cry1Ab/c toxin in locally bred Bt-cotton varieties. Expression profiling of Cry1Ab/c δ-endotoxin in locally bred Bt-cotton cultivars containing MON531 event has revealed that the expression of the protein varied considerably among different cultivars and at different growth stages of plant. It could be a result of genetic background of host plants and several environmental factors. The survival of *H. armigera* is negatively affected by higher expression of the Cry1Ab/c, in nine of the tested eleven Bt-cotton varieties the control of insect pest was above 90 % within 96 h of exposure to the detached leaves. The critical value of Cry1Ab/c at any given time is found to be 770 ± 25 ng/ gm for the control of Pakistani population of *H. armigera*. This value will help breeders and agricultural biologists in setting a bench mark for expression of Cry1Ab/c, while developing insect resistant, transgenic plants. The sufficient amount of toxic protein in potentially vulnerable plant parts and at appropriate growth stages must be ensured to get efficient control of the target arthropod pests. Hence, it is recommended that the genetic engineers and plant breeders, engaged in developing insect resistant transgenic crops, must emphasize on bringing together genotypes and promoter sequences having reputation for higher expression. Minimum standard must be set for Bt-protein expression in host plants, their potentially vulnerable tissues and at all growth stages. Post release monitoring of Bt-cotton varieties for toxin expression and resistance development in target pests is also recommended.

Current study further emphasizes on the fact that Bt-cotton expressing Cry1Ab/c toxin has no direct impact on the population dynamics of sucking arthropods. Therefore, increased sucking arthropod's population in Bt-cotton growing areas is mainly due to reduced utilization of broad spectrum synthetic insecticides and low innate immunity of cultivars. Since, many physio-chemical factors like chlorophyll content, level and number of phenolic compounds also play a major role in plant defence machenism against arthropods. Researchers must focus to identify genotypes with superior agronomic and physiological traits that promise better resistance towards sucking arthropods. This way we can also decrease our dependance on chemical insecticides, and ultimately reaching environmental stability. Moreover, surveys and further field trials must be conducted to build a long term integrated pest management (IPM) strategy that includes Bt technology. Furthermore, different available models including use of biological control agents and cultural practices must be devised and tested in Bt-cotton growing areas for effective control of sucking arthropods.

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References

Adamczyk Jr, J.J., and D.V.Sumerford, 2001. Potential factors impacting season-long espression of Cry1Ac in 13 commercial varieties of Bollgard® cotton. 6 pp. *J. Insect Sci.*, 1:13. Available online: insectscience.org/1.13 Available online: insectscience.org/1.13

Adamczyk, J., D. Hardee, L. Adams, D. Sumerford, 2001. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A (c) δ–endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.,* 94: 284–290

Adamczyk, J.J., O. Perera, W.R. Meredith, 2009. Production of mRNA from the cry1Ac transgene differs among Bollgard® lines which correlates to the level of subsequent protein. *Transgenic Res.,* 18: 143–149

Ahmad, F. and W. Wakeel, 2000. Role of biochemical components in varietal resistance of cotton against sucking insect pests. *Pak. Entomol.,* 22: 69–71

Ali, M., R. Luttrell,S. Young III, 2006. Susceptibilities of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) populations to Cry1Ac insecticidal protein. *J. Econ. Entomol.,* 99: 164–175

Armes, N.J., Bond, G., Cooter, R., 1992. *The Laboratory Culture and Development of Helicoverpa armigera*, p: 22. Bulletin–Natural Resources Institute. 57: Natural Resources Institute, Chatham, UK

Arshad, M., A. Suhail, 2010. Studying the sucking insect pests community in transgenic Bt cotton. *Int. J. Agric. Biol.,* 12: 764–768

Arshad, M., A. Suhail, M.J. Arif, M.A. Khan, 2009. Transgenic–Bt and Non–transgenic cotton effects on survival and growth of *Helicoverpa armigera*. *Int. J. Agric. Biol.,*11: 473–476

Arshad, M., A. Suhail,M. Asghar, M. Tayyib and F. Hafeez, 2007. Factors influencing the adoption of Bt cotton in the Punjab, Pakistan. *J. Agric. Soc. Sci*., 3: 121–124

Ashfaq, M., S. Young, R. McNew, 2001. Larval mortality and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) reared on a transgenic *Bacillus thuringiensis*–cotton cultivar expressing CryIAc insecticidal protein. *J. Econ. Entomol*., 94: 1053–1058

Bakhsh, A., S. Siddique, T. Husnain, 2012. A molecular approach to combat spatio–temporal variation in insecticidal gene (*Cry1Ac*) expression in cotton. *Euphytica,*183: 65–74

Damalas, C.A., I.G. Eleftherohorinos, 2011. Pesticide exposure, safety issues, and risk assessment indicators. *Int. J. Environ. Res. Public Health,* 8: 1402–1419

Dong, H., W. Li, 2007. Variability of endotoxin expression in Bt transgenic cotton. *J. Agron. Crop Sci.,* 193: 21–29.

Ferry, N., M. Edwards, J. Gatehouse, T. Capell, P. Christou, A.M. Gatehouse, 2006. Transgenic plants for insect pest control: a forward looking scientific perspective. *Transgenic Res.,* 15: 13–19

Guo, W.Z., J. Sun, Y.F. Guo, T.Z. Zhang, 2001. Investigation of different dosages of inserted Bt genes and their insect–resistance in transgenic Bt cotton. *Acta Genet. Sin.,* 28: 668

Halcomb, J., J. Benedict, B. Cook, D. Ring, 1996. Survival and growth of bollworm and tobacco budworm on nontransgenic and transgenic cotton expressing a CrylA insecticidal protein (Lepidoptera: Noctuidae). *Environ. Entomol.*, 25: 250–255

Hobbs, S.L., T.D. Warkentin, C.M. DeLong, 1993. Transgene copy number can be positively or negatively associated with transgene expression. *Plant Mol. Biol.,* 21: 17–26

Ishtiaq, M., M.A. Saleem, 2011. Generating susceptible strain and resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) against some conventional and new chemistry insecticides in Pakistan. *J. Econ. Entomol*.,104: 1343–1348

James, C., 2012. *Global Status of Commercialized Biotech/GM Crops: 2012*. ISAAA BriefNo. 44. ISAAA: Ithaca, New York, USA

Karim, S., 2000. Management of *Helicoverpa armigera*: a review and prospectus for Pakistan. *Pak. J. Biol. Sci.,* 3: 1213–1222

Kranthi, K., C. Dhawad, S. Naidu, K. Mate, G. Behere, R. Wadaskar, S. Kranthi, 2006. Inheritance of resistance in Indian *Helicoverpa armigera* (Hübner) to Cry1Ac toxin of *Bacillus thuringiensis*. *Crop Prot.,* 25: 119–124

Kranthi, K.R., S. Naidu, C. Dhawad, A. Tatwawadi, K. Mate, E. Patil, A. Bharose, G. Behere, R. Wadaskar, S. Kranthi, 2005. Temporal and intra–plant variability of Cry1Ac expression in Bt–cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). *Curr. Sci.*, 89: 291

Kranthi, S., C. Dhawad, S. Naidu, A. Bharose, A. Chaudhary, V. Sangode, S. Nehare, S. Bajaj, K. Kranthi, 2009. Susceptibility of the cotton bollworm, *Helicoverpa armigera* (Hubner)(Lepidoptera: Noctuidae) to the *Bacillus thuringiensis* toxin Cry2Ab before and after the introduction of Bollgard–II. *Crop Prot.*, 28: 371–375

Mahon, R., J. Finnergan, K. Olsen, L. Lawrence, 2002. Environmental stress and the efficacy of Bt cotton. *Aust. Cotton Grow.,* 23: 18–21

Mari, J., S. Nizamani, M. Lohar, 2007. Population fluctuation of sucking insect pests and predators in cotton ecosystem. 8th African Crop Science Society Conference, El–Minia, Egypt, 27–31 October 2007. *Afri. Crop Sci. Soc.*, pp: 929–934

McCaffery, A.R., 1998. Resistance to insecticides in heliothine Lepidoptera: a global view. *Phil. Trans. Royal Soc. London*. *Series B: Biol. Sci.*, 353: 1735–1750

Men, X., F. Ge, C.A. Edwards, E.N. Yardim, 2005. The influence of pesticide applications on *Helicoverpa armigera* Hübner and sucking pests in transgenic Bt cotton and non–transgenic cotton in China. *Crop Prot.,* 24: 319–324

Patel, R.C., R.M. Patel, B.V. Madhukar, R.B. Patel, 1974. Oviposition behaviour of *Helicoverpa armigera* on cotton hybrid–4. *Curr. Sci.,* 588–589

Romeis, J., M. Meissle, A. Raybould, R. Hellmich, 2009. Impact of insect–resistant transgenic crops on above–ground nontarget arthropods. Environmental impact of genetically modified crops. CABI, Wallingford, UK, pp. 165–198

Saleem, M.W., S. Ahmed, W. Wakil, S.T. Sahi, 2012. Resistance to Thrips (*Thrips tabaci*) in Bt Cotton Genotypes in Punjab, Pakistan, Based on Population Dynamics and Plant Morpho–chemical Properties. *Int. J. Agric. Biol.,* 15: 331–336

Sisterson, M.S., R.W. Biggs, C. Olson, Y. Carrière, T.J. Dennehy, B.E. Tabashnik, 2004. Arthropod abundance and diversity in Bt and non–Bt cotton fields. *Environ. Entomol.,* 33: 921–929

Tabashnik, B.E., A.J. Gassmann, D.W. Crowder, Y. Carrière, 2008. Insect resistance to Bt crops: evidence versus theory. *Nat. Biotechnol.,* 26: 199–202

Trouve, C., S. Ledee, J. Brun, A. Ferran, 1996. Biological control of the hop aphid. A review of three years of tests in northern France. *Phytoma,*486: 41–44

USDA GAIN, 2010. *Pakistan cotton: annual production and products*. Foreign Agricultural Services. Available at: http://www.thecropsite.com/reports/?id=3633

Wan, P., Y. Zhang, K. Wu, M. Huang, 2005. Seasonal expression profiles of insecticidal protein and control efficacy against *Helicoverpa armigera* for Bt cotton in the Yangtze River valley of China. *J. Econ. Entomol.,* 98: 195–201

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