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EFFECT OF METHYL JASMONATE ON Bt COTTON (*Gossipium hirsutam .L*) GENE EXPRESSION AND MORTALITY OF PINK BOLL WORM (*Pectinophora gossypiella)*

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ABSTRACT

Transgenic cotton was improved genetically by introducing bacterium *Bacillus thuringiensis* to produce Bt δ-endotoxins to kill the key pests of Lepidoptera. However, with passage of time low expression of *Cry1Ac* gene limited the glory of Bt technology. The phytohormones are key regulators of plant defense systems against the insect pests. It was noted that the defense systems of plants were significantly regulated by Salicylic acid (SA) and Methyl Jasmonate (MeJA). Particularly, Methyl Jasmonate induces proteinase inhibitors and interferes with insect digestion and feeding capability. The experiment was conducted to evaluate the effect of MeJA to induce proteinase inhibitors and *Cry1Ac* genes in Bt cotton cultivars and subsequently the mortality of three different populations of *Pectinophora gossypiella*. The two concentration of MeJA (0.25 mM and 0.5 mM) were sprayed exogenously foliar on five Bt and one non Bt cotton cultivars at the stage of bud and flower formation. The results of bioassay depicted that when plants were treated with a concentration of 0.5 mM MEJA, a significant mortality (66 to 78%) of early 3rd instar larvae of *P. gossypiella* was observed for field and resistant population whereas up to 89% mortality was noted for lab susceptible population as compared to very low mortality observed for not treated plants. Similarly, RT-PCR and ELISA tests showed the enhanced level of Cry1Ac gene expression and its protein (1.33-2.89 µg/g) in MeJA treated plants. In conclusion, the application of MeJA can be a suitable strategy to enhance natural defense system of plant as well as to improve the expression of Cry1Ac which cause higher mortality for the resistant and susceptible population *P. gossypiella.*

Keywords: Jasmonic acid, synergistic effect, Transgenic Cotton, *Pectinophora gossypiella*, *Cry1Ac*, Gene expression
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INTRODUCTION

Cotton is known as a natural fiber producing crop worldwide but in Pakistan it is also recognized as industrial crop. It is cultivated on a large area of 2.96 million hectares with a massive fabrication of 13.983 million bales of cotton (Pakistan Bureau of Statistics, 2014-15). One of the major threats to this profitable crop is bollworms, exerting 30 to 40% losses to cotton yield (Cororaton *et al*., 2008; Masood *et al*., 2011; Khan *et al*., 2012). The *Pectinophora gossypiella* is known as pest of great economic importance in many cotton-growing countries. Among the key lepidopteran pests *P. gossypiella* was observed as more damaging and exerts 2.8-61.9 % yield losses in cotton seed, 2.1-47.1 % loss of oil in seed cotton and 10.7-59.2 % delay in normal opening of cotton bolls (Patil, 2003).

The control of this notorious pest depends mainly on the pesticide applications, which results in the evolution of resistance. In this regard, to control the pest effectively, heavy pesticides applications are needed (Ahmad *et al*., 2002). The pesticide expenditure annually recorded in Pakistan was estimated US$300 million, from which 80% pesticide was applied on cotton specially to control the cotton bollworms (Arshad and Suhail, 2011). Bt technology proved to be environment friendly, provides effective control of bollworms and most importantly it provides target specificity (Mendelsohn *et al*., 2003; Wu and Guo, 2005). But in present era *P. gossypiella* has developed resistance against *Cry1Ac* gene (Fabrick *et al*., 2014; Ojha *et al*., 2014; Tabashnik *et al*., 2014).

Under natural environmental conditions a variety of plants habitually encounter various potential pathogens. All plants have consistently developed different mechanisms to resist with diverse biotic factors. As variety of crops has developed diverse resistant system that comprises of inducible and constitutive systems that provide safety to plants from hazardous insect pests (Bu *et al*., 2014: Eva *et al*., 2014). Plants also produce diverse range of signaling molecules which protects them from insect herbivory including Salicylic acid, Jasmonic acid and ethylene (Lorenzo and Solano, 2005: Balbi and Devoto, 2008). The two most importantly recognized phytohormones

Salicylic acid SA/BTH is naturally occurring integrative plant signaling hormone which normally performs various biochemical and physiological

functions of plants. It plays a huge part in plant resistance to biotic and abiotic factors (Wang *et al*., 2010; Rahat *et al*., 2011). It is also recognized that SA performs significant part in controlling the redox balance across plant membranes, in this manner neutralizing ROS negative effects generated by oxidative stress through enhancing the level of antioxidant enzymes. Remarkable Positive effects of that hormone on plant efficiency have been reported due to the abiotic stresses e.g Ozone, heat, UV-B, salt and osmotic stresses (El-Tayeb, 2005; Stevens *et al*., 2006; Eraslan *et al*., 2007; Gunes, 2007; Wang *et al*., 2010). The previous investigations revealed that SA regulates the functions of different genes in variety of crops under biotic and abiotic stress conditions, *GaRPL18* genes (Gong *et al*., 2017) *PR* genes (Jain *et al*., 2012: Hanafy *et al*., 2013: Hong and Hwang, 2005: Ding *et al*., 2002) *GST, GPX, GR, DHAR, and GSH* genes (Chen *et al*., 2011: Li *et al*., 2013: Kang *et al*., 2013) *MDHAR* gene (Sultana *et al*., 2012) *CAT* and *APX* gene (Ding *et al*., 2002: Duan *et al*., 2012).

Jasmonic acid or JA/MEJA acts as important signaling compound in various plant stress reactions and development. It is reported recognized that JA is involved in minimizing the harmful effects of abiotic stresses e.g ozone, salt, high temperature, cold, UV, heavy metal and drought stresses (Dar *et al*., 2015). Jasmonate treatment exerts positive effects on plant growth and development under salt stress conditions as documented in previous studies (Kang *et al*., 2005; Sheteawa, 2007; Dar *et al*., 2015). JA-inducible genes previously studied were VEGETATIVE STORAGE PROTEIN *2(VSP2/AT5G24770),* TYROSINE AMINO TRANSFERASE *(TAT1/AT4G23600)*, LIPOXYGENASE 2 *(LOX2/AT3G45140)* (Lorenzo *et al*., 2004; Lopukhina *et al*., 2001), Acyltransferases (Schilmiller *et al*., 2015; Fan *et al.,* 2016) and *BCKD-E_2, BCKD-E_3, FAE-3,* gene in tomato (Escobar-Bravo *et al*., 2016).

In Bt cotton plants there is slight understanding about the significance of SA/BTH and JA/MEJA signaling. (SA and JA play significant part in the initiation of defense systems of plants against insect pests. SA/BTH was mostly involved in mechanisms of resistance which are vigorous to pathogens of biotrophic levels. While JA involved in mechanisms of resistance to necrotrophic pathogens (Bari and Jones, 2009). In previously reported studies mostly JA and SA defense signaling pathways was found to work antagonistically in various plants which are dicotyledonous in nature (Niki *et al*., 1998; Koornneef and Pieterse, 2008). Recently, authors have conducted various scientific work regarding pest identification, management and genes expression analysis in Pakistan (Ahmad *et al*., 2017, 2018ab, 2019ab, 2020ab; Malik *et al*., 2020ab; Yaseen *et al*., 2020). The current work was performed to examine the effect of MEJA foliar application on the *Cry1Ac* gene expression in different Bt cotton cultivars to control *P. gossypiella* notorious pest of cotton.

MATERIALS AND METHODS

Plant source: Seeds of Bt cultivars (IUB-212, CIM 616, CIM 598 and Lalazar and FH-86) and Non Bt MNH 786 were sown at Learning Research Centre green houses, University of Agriculture Faisalabad for taking fresh samples of leaf for expression analysis of *Cry1Ac* and for insect bioassay. The samples from Bt and non Bt cultivars were taken at the age of 60 days. Three plants were selected randomly from each genotype to take the sample of leaf for bioassay. The three leaves from lower, middle and upper (at the height of 1/3rd from base, center and 1/3rd from top of plant respectively) part of individual Bt cotton cultivars and three leaves from non-Bt plant were also detached.

Treatment of Methyl Jasmonate: The Bt cotton plants were treated with solutions of 0.2 mM and 0.5 mM methyl Jasmonate (MeJa) Seedlings of cotton plants were grown in green house. They were treated by foliar application at the stages of bud and flower formation. The non-Bt cotton plants were kept as control and treated with water by maintaining the same pH levels.

Insect Rearing: *Pectinophora gossypiella* larvae were collected from cotton fields and then kept in controlled condition. *P. gossypiella* susceptible populations were developed at Dr, Jam Laboratory, Department of Entomology, University of Agriculture Faisalabad by following the established laboratory protocol for diet preparation and insect rearing. For diet preparation, the main ingredients of diet were purchased from Solarbio, China. Briefly, wheat germ meal (35g/kg), casein (30g/Kg), sucrose (10 g/Kg), brewer's yeast (5 g/Kg), alpha-cellulose (1 g/Kg), potassium sorbate (1.5 g/Kg), Nipalgin (0.5 g/Kg), Choline chloride (0.06 g/Kg), honey (2ml/L), and H20 (730 ml/L) were mixed in 330 ml of H2O for 25 minutes then decavitamin solution (0.01 ml/L) and maize oil (3.3 ml/L) was added in the mixture. In a separate beaker, Agar Technical (20 g/Kg; Oxoid, Korea) was dissolved in 400 ml dH20 for 5 min and placed at room temperature for normal cooling. Then mixture was poured and then diet was placed for solidification. The larvae were pupated in vials containing artificial diet. Some pupae were collected and placed in separate cages for adult emergence. The adults emerged from vials were provided with 10% honey solution by placing in separate cages/box caped with wire mesh and green paper for mating and egg laying purposes. Field populations were collected for comparative study against tested Bt genotypes/Cultivars. Two populations were also used for trials that were developed up to 6th generation; one susceptible (without Bt exposure) and 2nd resistant generation were developed

by feeding on Cry1Ac mixed with artificial diet. The population were reared at 25^0C ± 2^0C and 70% ± 5% Relative Humidity, with 14L:10 d hour photoperiod (Bot, 1966).

Bioassay for *Pectinophora gossypiella:* From each genotype three plants were selected randomly to take samples of flower and bolls for bioassay. The 3 flowers and bolls each from the lower, middle and upper (at height 1/3rd from base, plant center and 1/3rd from plant top respectively) portion of each transgenic line along with non-transgenic control line in triplicates were separated. The flower and bolls were covered in wet tissue after taking punches for ELISA to determine toxin level. Flowers were kept in glass Petri plates (6 inches diameter) and screw caped bottles with perforated lids. Twenty Five larvae of 3rd instar were weighed and placed in Petri Plates and vials having flowers and bolls for bioassay. The day on which flower and bolls were kept along with larvae was marked as 0 day bioassay (0 DOB). Mortality data and weight loss or gains by insects were recorded on 3 DOB.

***Cry1Ac* Protein Quantification:** A marketable quantification Kit (QuantiPlate™ Kit, EnvroLogix, Inc., Portland, ME) was used to measure the amount of *Cry1Ac* present in the leaves sample. This "sandwich" Enzyme-Linked Immuno-Sorbent Assay (ELISA) uses the step of color development where the intensity of production of color is directly proportional to the *Cry1Ac* level in the extract of sample. For all samples, optimal densities were plotted to a standard curve with calibrators supplied with Kit. The amount of *Cry1Ac* was calculated in ppm, which was corresponding to micro gram per gram fresh weight (µg/g) of the leaf.

Expression of Cry1Ac by Polymerase Chain Reaction (PCR)

RNA Extraction: The frozen samples of Bt and non Bt plants weighing 0.2g- 0.5g were ground in liquid nitrogen to power then subsequently total RNA was extracted by mixing 1ml of TriZol LS-Reagent® method (Invitrogen-USA) as described (Ahmad *et al*., 2013; 2014). The RNA was dissolved in 15 µl ddH20. Then, in a final volume of 50 µl following the manufacturer's instruction, 8µg to12µg of RNA from samples were treated with 5 units of RQ1 RNase- Free DNase (Promega, Madison, WI, USA) at 37 °C for 1 h in the presence of 1X reaction buffer (Promega, Madison, WI, USA). The concentration of RNA was determined by using spectrophotometer (pico200, UK) at λ =260 nm.

Reverse transcription: The RNA (1µg) treated with RQ1-DNase-treated was used to synthesize complementary DNA (cDNA) by using 5µM oligodT18 primer or gene specific reverse primers containing 200 units of Superscript® II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) (Ahmad *et al*., 2013; 2014) in a 25 µl reaction mixture (10 mM DTT, 20 µM dNTPs and 40 units of RNase Out™ (Invitrogen) following prescribed protocol except the mixture (RNA+oligodT18 or specific primer) was heat-denatured at 65 °C for 5 min before adding the reverse transcriptase and the other components (Ahmad *et al*., 2014). Controls, without reverse transcriptase, were used to verify the efficiency of the DNase treatment; as expected, no amplification was obtained.

RT-PCR amplification: RT-PCR amplification of Bt toxin (Cry1ac) genes was performed using genes specific primers and complementary DNA (cDNA) synthesized from treated and non-treated cotton plants. For, RT-PCR, 1µl of cDNA (1/10 diluted) RT reaction mixture with forward and reverse primers of proteinase inhibitors and Cry1Ac (2µM each) and 1.5 units of Taq DNA polymerase (Promega) was used in PCR machine (PeqStar, Germany) in the presence of primers *Cry1Ac:* Forward Primer: 5'-GAAGGAGTGGATGGAGTGGA-3'Reverse Primer: 5' GCGGTCTGGTAGGTGTTGAT-3'. The 34 PCR cycles consisted each of a 45 seconds denaturation step at 94 °C, 45 seconds annealing step at 56 °C and 45 seconds elongation steps at 72 °C. The final extension phase was prolonged to 10 minutes at 72 °C. Eight µl amplified PCR products of gene were run on 2% agarose gel along with 1kb ladder. All three gels were visualized under UV light and analyzed by 1.5 % agarose gel electrophoresis in 1X TAE buffer.

Statistical Analysis: The Student t-test was directed for all parameters of experiment including gene expression before and after the application of JA and insect mortality. Standard errors and mean values of quantitative data were analyzed by implementing analysis of variance test. The significance was tested from differences and p Values < 0.05 were deliberated as significant.

RESULTS

Mortality of different population of *Pectinophora gossypiella* on cotton cultivars: The two different population of *P. gossypiella* were used along with field collected population (Fig. 2). Mortality of field populations was high IUB-212 (53±4.40), CIM-616 (63±6.00), CIM-598 (60±3.17), Lalazar (55.67±2.86) and FH-183 (46.67±4.41) except Non-Bt MNH-786 (17.67±1.47) as compared to mortality of laboratory resistant population observed on same cultivars; IUB-212 (40±5.00), CIM-616 (35±2.89), CIM-598 (38±10.17), Lalazar (37.67±4.72) and FH-183 (31.67±6.00) except Non-Bt MNH-786 (6.17±1.45). The laboratory susceptible population depicts maximum mortality with IUB-212 (86.67±6.00), CIM-616 (85±7.64), CIM-598 (88±11.11), Lalazar (83.67±7.54) and FH-183

(81.67±10.93). The laboratory resistant population shows low mortality as Non-Bt MNH-786 (32.67±5.67), IUB-212 (86.66±6.00), CIM-616 (85±7.63), CIM-598 (88.00±11.00), Lalazar (83.67±7.53) and FH-183 (81.66±10.92).

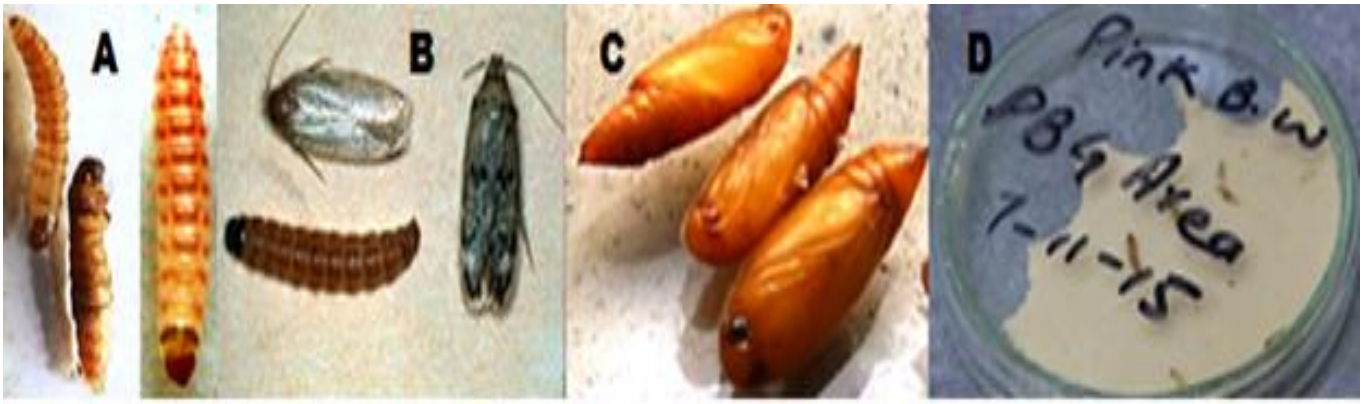

Figure 1. Rearing of *Pectinophora gossypiella* on artificial diet and experimentation; (A) dead larvae (left) and healthy (right), (B) male and female of *P.gossypiella* with healthy larvae, (C) pupae of *P.gossypiella*, (D) rearing of *P.gossypiella* on artificial diet.

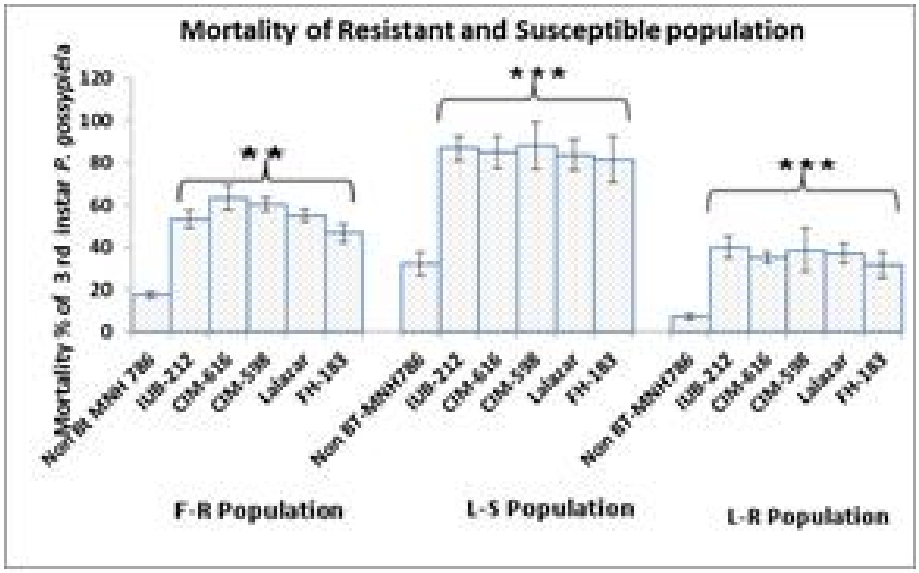

Figure 2. Percentage mortality of early 3rd instar larvae of *P. gossypiella* after application of MeJa; F-R Population: Field resistant population, L-S Population: Laboratory susceptible S6 population, L-R Population: Laboratory resistant R6 population (*P≤ 0.05,P≤ 0.01 and***P≤ 0.005)**

Mortality of *Pectinophora gossypiella* after application of MeJA: Significant mortality percentages were observed when early 3rd instar larvae of pink bollworm (*Pectinophora gossypiella*) were subjected to Methyl jasmonate (MEJA) treated Bt cotton plants. In case of non-treated cotton cultivars, mortality percentages were 6.67±1.67 (Non-Bt MNH-786), 45±2.89 (IUB-212), 55±2.89 (CIM-616), 52±7.57 (CIM-598), 43.67±4.09 (Lalazar) and 36.67±4.41 (FH-183) Bt cotton cultivars. When 0.2 mM MeJA applied, increased mortality was recorded in different cultivars including Non-Bt MNH-786 (20.67±2.60), IUB-212 (52.67±4.72), CIM-616 (59.33±3.78), CIM-598 (56.66±2.33), Lalazar (48.33±5.81) and FH-183 (46±4.50). While in case of 0.5 mM MEJA treatment, highly significant mortality of *P. gossypiella* was observed in Bt cultivars containing Non-Bt MNH-786 (33.67±2.96), IUB-212 (66.67±4.41), CIM-616 (75±7.64), CIM-598 (78.67±4.677), Lalazar (70.33±8.37) and FH-183 (70.66±3.48) as compared to not treated plants (Fig. 3)

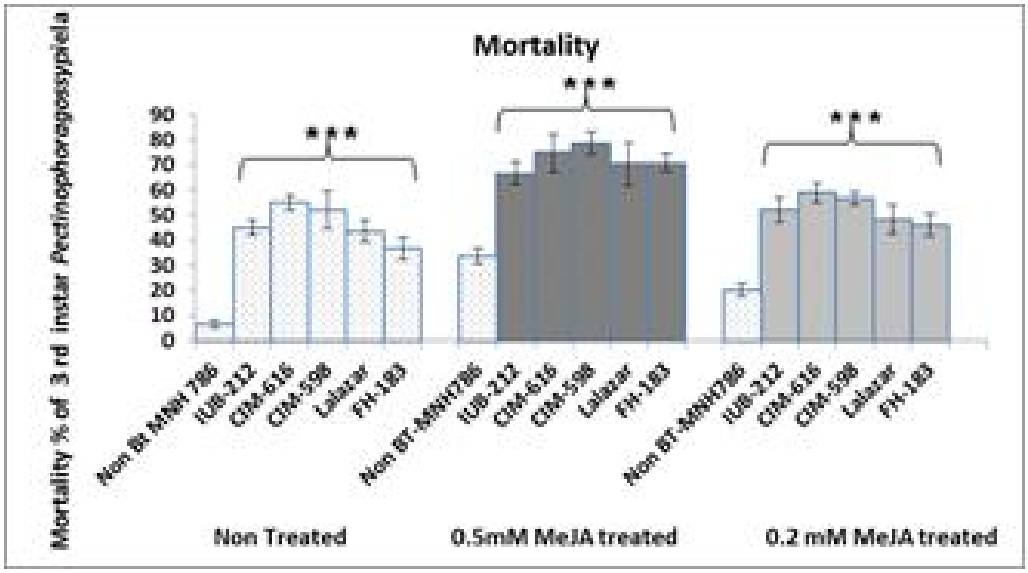

Figure 3. Percentage mortality rate of early 3rd instar larvae of field population of *P. gossypiella* before and after the treatment of two different concentration (0.5 mM and 0.2 mM) of phytohormones MeJA (*P≤ 0.05, P≤ 0.01 and*** P≤ 0.005)**

Effect of MEJA on Cry1Ac expression: Treated cotton plants with 0.02 mM and 0.5mM MEJA solutions were positively correlated with Cry1ac gene expression. In non-treated cotton cultivars, Cry1Ac gene quantification through ELISA (Fig. 4) recorded in cotton was as; Non-Bt MNH-786 (0±0.0), IUB-212 (1.40±0.24), CIM-616 (1.83±0.28), CIM-598 (2.34±0.54), Lalazar (1.75±0.35) and FH-183 (1.33±0.27). In case of 0.2 mM MeJA application, Cry1Ac was slightly enhanced as observed in all treated cultivars IUB-212 (1.68±0.18), CIM-616 (2.07±0.21), CIM-598 (2.21±0.31), Lalazar (1.94±0.24) and FH-183 (1.60±0.17) except Non-Bt MNH-786 (0±0.0). Whereas 0.5mM MEJA treated cotton cultivars significant gene expression was observed as Non-Bt MNH-786 (0±0.0), IUB-212 (2.39±0.24), CIM-616 (2.89±0.22), CIM-598 (2.98±0.16), Lalazar (2.64±0.22) and FH-183 (2.27±0.23). Using Cry1Ac specific primers in RT-PCR, Cry1Ac expression was shown (Fig.5) to be up-regulated in MeJA treated Bt cultivars as indicated in lanes 6-9 (IUB-212, CIM 616, CIM 598, Lalazar) by the enhanced bands intensity except lanes 10-11 in Non-Bt 786 cultivar as compared to non-treated (lanes 1-5) Bt cultivars (IUB-212, CIM 616, CIM 598, Lalazar and FH-86).

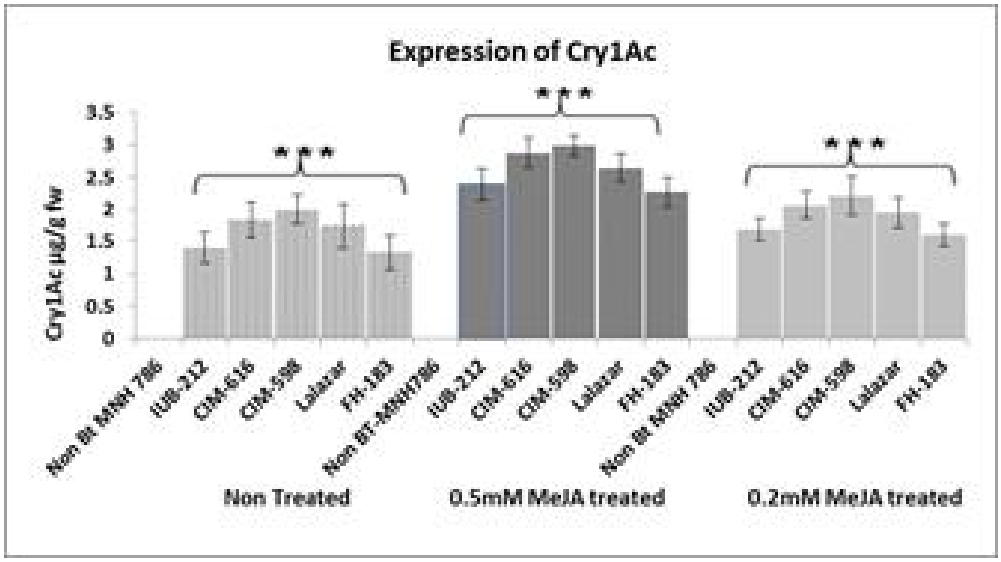

Figure 4. *Cry1Ac* expression in transgenic Bt cotton cultivars before and after the application of two different concentration (0.5 mM/0.2 mM) MeJA phytohormone (*P≤ 0.05, P≤ 0.01 and*** P≤ 0.005)**

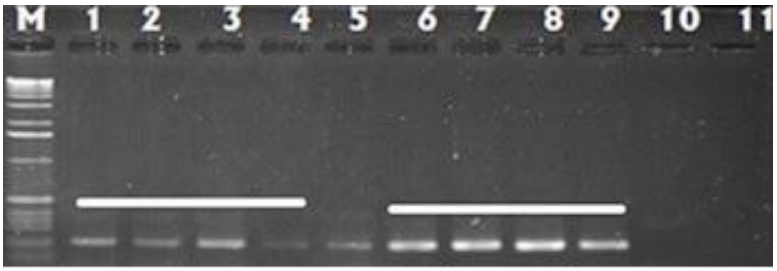

Figure 5. Gene expression through RT-PCR by using Cry1Ac specific primers in Bt cultivars. Non treated Bt plants; Lane 1-5 non treated Bt cultivars (IUB-212, CIM 616, CIM 598 and Lalazar and FH-86); Lane 6-9 MeJA treated Bt cultivars (IUB-212; CIM 616; CIM 598 and Lalazar); Lane 10-11 MeJa treated Non-Bt 786; M- 1kb molecular ladder (Invitrogen).

DISCUSSION

A variety of plant hormones are involved in plant's defence response against biotic and abiotic stresses. The hormonal activity of plant produces defense signals which deter herbivory and affect the insect pest's survival on different plants (Gordy *et al*., 2015). The effect of Jasmonic acid and other phytohormones has been explained extensively in previous publications by several researchers (Dicke and Hilker, 2003; Ahmad *et al.,* 2014). Treatment of plants with jasmonic acid or MeJA can enhance resistance development Rohwer and Erwin, 2008). Previously it was reported that low doses of BTH and MEJA under stress conditions more efficiently up regulate plant defense as compared with higher concentrations (Lee *et al*., 2010; Mustafa *et al.,* 2014; Salimi *et al.,* 2016).

Applications of JA and SA on different crops have repellent effect and reduce the herbivore population survival and growth rates populations of insect pests (Quiroz *et al*., 1997; Stuart *et al.,* 2000; Birkett *et al*., 2000; Bruce *et al*., 2003; Thaler *et al.,* 2002; Eva *et al.,* 2014). It was found on wheat that JA reduced populations reduced the population of aphid and thrips (Bruce *et al*., 2003; El-Wakeil *et al*., 2010). Similarly, application on tomato field reduced potato aphid (Cooper and Goggin, 2005). SA suppresses the population of midges and mosquitos (Stuart *et al*., 2000). The previously reported work shows that MeJA and SA are phytohormones which alert the plants about the insect pest attack as well as pathogens (Pettersson *et al*., 1996; Chamberlain *et al*., 2000; Slesak *et al*., 2001; Ahmad *et al*., 2014). In current research, Cotton plants were positively affected by plant elicitor MeJA applications (Fig.2-4). In the present study MeJA was found more effective against susceptible and field generation of *P. gossypiella* as compared to resistance strains developed in laboratory. It was previously reported that SA treatment exerts positive effects on carrot growth (Eraslan *et al*., 2007), improved shoot growth in Arabidopsis under salt stress (Jayakannan *et al*., 2013). Its application also increases and maintains the chlorophyll concentration in plants (Yildirim *et al*., 2008; Rady *et al*., 2013), protective role in plant tolerance (Aftab *et al*., 2010), enhance photosynthetic activity through stimulatory effects on Rubisco and increased CO_2 assimilation (Szepesi *et al.,* 2005; Namich *et al.,* 2007), enhanced the quantity of soluble sugar, reducing sugar, non-reducing sugar and prolines (Agamy *et al*., 2013;El-Beltagi *et al*., 2017), increased H_2O_2 levels which damage the insect pests (Peng *et al*., 2004). The present work was supported by previous investigations revealed that MeJA regulates the functions of different genes in variety of crops under biotic and abiotic stress conditions, *GaRPL18* genes regulation in cotton (Gong *et al*., 2017), *PR* genes in Peanut, Bean, Pepper and tomato (Jain *et al.,* 2012: Hanafy *et al.,* 2013: Hong and Hwang, 2005; Ahmad *et al*., 2014)), *GST, GPX, GR, DHAR, and GSH* genes in eggplant and wheat (Chen *et al*., 2011:Li *et al.,* 2013: Kang *et al.,* 2013), *MDHAR* gene in rice (Sultana *et al*., 2012), *CAT* and *APX* gene in tomato crops (Ding *et al.,* 2002: Duan *et al*., 2012). Similarly, JA-inducible genes previously studied were VEGETATIVE STORAGE PROTEIN *2(VSP2/AT5G24770),* TYROSINE AMINO TRANSFERASE *(TAT1/AT4G23600)*, LIPOXYGENASE 2 *(LOX2/AT3G45140)* (Lorenzo *et al*., 2004; Lopukhina *et al*., 2001). Acyltransferases (Schillmiller *et al.,* 2015; Fan *et al.,* 2016) and *BCKD-E_2, BCKD-E_3, FAE-3* genes in tomato (Escobar-Bravo *et al*., 2016).

We observed that *Cry1Ac* gene expression was up-regulated promptly by JA treatment, which reaches at peak after application in different Bt cotton cultivars as SA increases *GaRPL8* gene expression in transgenic cotton (Gong *et al*., 2017). In the same manner, MeJA treatment causes significant mortality of *P. gossypiella* that can be associated with the induced proteinase inhibitors in cotton plant interfering with digestion and feeding ability of *P. gossypiella* as observed resistance against phytoplasma in tomato (Ahmad *et al.,* 2013, 2014). The enhanced expression of *Cry1Ac* can also be linked with the improved health and reduced stress of plant upon phytohormones (MeJA) treatment. Application of phytohormones on stressed plants can also

up regulate the activities of resistant genes to deliver sustainable management of insect pests (Pieterse *et al*., 2009; Zhang *et al*., 2013). There are many genes which were up regulated by SA application in different crops *PR1*, *PR3*, *NPR1*, *AtGSTF6* and *WRKYs* (Ying *et al.,* 2010; Dubreuil-Maurizi *et al*., 2011; Manohar *et al.,* 2014; Ahmad *et al.,* 2013, 2014; Herrera-Vasquez *et al.,* 2015). The authors have also conducted Cry1Ac expression analysis in Bt cotton under abiotic stress and other related work in Pakistan (Ahmad *et al*., 2020cd). Furthermore, in current study, it is revealed that *Cry1Ac* perform better upon application of MEJA on transgenic and non-transgenic plants by increase transgenic plant resistance against *P. gossypiella* as compared to non-Bt plants. The present findings suggest that MeJA is effective and defense pathways may be linked with *Cry1Ac* functioning having strong synergistic working efficiency against *P. gossypiella*. The research on induction, identification and implementation of MeJA dependent defensive genes in cotton are under progress. The MeJA responsive genes will be very useful in future to produce Bt cotton with more resistance capability against *P. gossypiella*.

Conclusion: To the best of our knowledge, the present work is the first to understand the relations of *Cry1Ac* gene expression with the application of Jasmonic acid (MeJA) phytohormone. The study revealed that MeJA up regulate the *Cry1Ac* gene expression in cotton and causing more mortality of *P. gossypiella*. MeJA in (0.5mM concentration) exerts more significant effects on *Cry1Ac* gene regulation and mortality as compared to 0.2mM application. Further, it is suggested that resistance in Bt cotton against *P. gossypiella* can be managed by applying MeJA and producing cotton having proteinase inhibitors genes with Bt toxin.

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