International Journal of Agriculture Innovations and Research
Volume 6, Issue 2, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received: 25/09/2017 | Accepted on: 03/10/2017 | Published: 16/10/2017

Induced Defense in Cotton Upon Insect Herbivory and Exogenous Application of Jasmonic Acid

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***Abstract* – Induced defense responses as a result of induction through feeding by *Spodoptera litura* and *Aphis gossypii*, as well as due to mechanical stimulation and topical application of insecticidal soap solution and Jasmonic Acid were studied in cotton varieties viz., MCU7, LRA 5166, MECH184-BT, MECH 184 non-BT and SVPR 2. The activities of the oxidative enzymes polyphenol oxidase (PPO), peroxidase (POX) and lipoxygenase (LOX) and proteinase inhibitors (PIs) were used as a measure of induced response. Differences in induction were evident among the five different varieties of cotton studied. The maximum induction of PPO, POD, LOX and PI was observed in the variety MCU 7 and the least in SVPR 2. The build-up of resistance in induced plants was confirmed by a lower feeding preference of S. litura recorded on induced plants in comparision to the non-induced control plants on all the varieties studied. Results on the induction upon exogenous application of Jasmonic Acid indicated high defensive response in MCU 7 and LRA 5166 for PPO and the least on SVPR 2 variety, while POD and LOX induction were more or less similar for all the varieties upon JA treatment. The weight gain of S. litura larvae was minimum in both the control and induced plants of Bt-cotton, when compared with the other remaining varieties indicating the influence of Bt toxin, but the reduction in weight gain due to induced resistance was maximum for MCU 7 and LRA 5166 varieties. Hence induced defense responses can be exploited for developing crop cultivars as one of components of integrated pest management for sustainable crop production.**

***Keywords* – Induced Plant Defense in Cotton; Herbivory, Jasmonic Acid; Oxidative Enzymes and Proteinase Inhibitor.**

I. Introduction

Plant defenses have generally been assumed to be constitutive, being always expressed in the plant. Recently it has come to be realized that induced responses reduce insect survival, reproductive output as well as the performance of the pest, so that the plant benefits from such responses. It is an important component of integrated pest management, which can be triggered by biotic or abiotic elicitors, can be used very effectively when combined with selective pesticides and induced resistance technique. Induced response in plants is one of the important components of pest control in agriculture, and has been exploited for regulation of insect herbivore population. Plants often increase their resistance to herbivores by locally increasing the production of defensive compounds at the site of damage, as well as systemically on undamaged leaves (Karban and Baldwin, 1997).

Plants respond to physical and chemical changes associated with insect feeding through the accumulation of phenolic compounds and in accordance with the kind and degree of damage, diverse phenols are induced, which involve oxidative enzymes such as PPO and POD. Plant lipoxygenase, has been also considered as an indicator of induced resistance against insect pests (Bi *et al.*, 1994; Felton *et al.*, 1994). Different types of mechanical injury/abrasion also induce different levels of resistance in plants. Wounding of plants results in a cascade of enzyme-characterized events, resulting in the gene expression coding for the production of proteinase inhibitors. Jasmonic acid (JA), a ubiquitous, damage-inducible compound elicits a diverse suite of plant defense responses is a product of the lipoxygenase pathway, also called the octadecanoid pathway. Induction of the jasmonate pathway by herbivore feeding or artificial treatment with JA leads to the synthesis of defensive proteins such as proteinase inhibitors (PIs) and oxidative enzymes, which are linked to a decreased performance of generalist herbivores on plants. Hence activities of PPO, POD, LOX and PI were used as markers to study the induction of resistance in cotton. This study pertains to the varietal screening of cotton plants for induced responses upon herbivory (*S. litura* and *A. gossypii*), mechanical injury and topical application of JA.

II. Materials and Methods

Plant Material and Induction Treatments

Five different varieties of cotton (MECH 184 Bt, MECH 184 non-Bt, MCU 7, LRA 5166 and SVPR 2) were taken to study the induction of four foliar proteins *viz.,* PPO, POD, LOX and PI against four different types of damage *viz., S. litura, A. gossypii,* mechanical and insecticidal soap solution. The plants were grown in 4l pots and watered as necessary and fertilized once at first true leaf stage. *S. litura* feeding damage: Third instar *Spodoptera litura* was allowed to feed on (third leaf) the varieties at 30 days old plants using clip cages (Stout *et al*., 1996). After 24 hrs, cages and insects were removed and the plants were left for an additional two days. As the induction of PPO and PI are systemic, the terminal leaf was incised and assayed for PPO and PI while the caged leaf was incised for assaying the POD and LOX as their induction is localized upon damage treatments. *A. gossypii* feeding damage: Approximately 50 nymphs and adults of aphids were transferred from heavily infested plants to the third leaf of the experimental plant and confined to that leaf by using clip cage around the petiole of the leaf. Aphids were allowed to feed for two days after which the clip cages and insects were removed and the plants kept in green house for another two days. Subsequently, the plants were taken to laboratory and enzyme activity was estimated. Mechanical damage: Two wounds were made perpendicular to the midvein on the third leaf of the plants with forceps. The size of the wound was approximately 2 cm long and 0.5 cm wide, and the wounding did not sever the leaf. Plants were assayed after 48 hr wounding for enzyme activities. Insecticidal soap immersion treatment: The entire third leaf was immersed for approximately five seconds in a 5% (v : v) solution of safer insecticidal soap (Safer, Inc. USA). Plants were maintained in a greenhouse for 48 hr after dipping and were then taken to the laboratory and assayed for protein activities. Control leaves of plants of similar size and age were dipped in distilled water.

The experiment was repeated twice with five replications per trial. The difference in enzyme activity between control and damaged plants for individual enzymes was subjected to two-way ANOVA analysis to study the response of different varieties to different damage treatments and to assess their interaction effect. Five replicates were maintained for each variety of cotton.

Jasmonic acid (Sigma) (1.5 mM) was applied to the top and sides of plants (Thaler *et al*., 1996) and the terminal leaf from the JA treated plants and control plants were incised and assayed for the foliar enzyme (PPO, POD, LOX and PI) activities. The difference in activity between the treated and the control plants was subjected to two-way ANOVA and Tukey's multiple pair wise comparision test using Sigmastat.

Effect of previous herbivory and JA application on feeding preference of S. litura

Plants with six to eight true leaf stage (one month) were used in all the bioassays McAuslane *et al.,* (1997). Two second instar of *S. litura* were weighed and placed on the third leaf and enclosed in perforated zip-cages and control plants devoid of insects were also maintained. Larvae and cages were removed after 24 hrs and the plants were left free for three days to facilitate the induction of resistance. The fully opened terminal leaf from induced plants were cut and the total area of these leaves were measured and a single third instar larva was weighed and allowed to feed on the leaf for 24 hrs. Larvae were then removed from all the replications and the leaf area was again measured. The amount of leaf area consumed was expressed in mm^2. Larvae were further allowed to feed for three days with the leaves from induced plants and then reweighed. The weight gain was calculated for each larva on a fresh weight basis. The plants were left for an additional 3 days after JA treatment. The terminal leaf from the JA treated plants and control plants were incised and were taken for the feeding bioassay through no choice experiments. Data on the amount of leaf area (mm^2) consumed in feeding bioassay was subjected to one-way ANOVA with all pairwise multiple comparision procedures (Tukey Test) using Sigmastat software. The difference between the leaf area consumed on induced (damaged) and control plants were analysed with one-way ANOVA. Similarly one-way ANOVA was used to study the difference between the varieties with respect to the weight gain of the larvae.

III. Results and Discussion

Induction of Resistance in Different Cotton Varieties

Study on the effect of different varieties upon different damage treatments indicated maximum induction of the defense enzymes *viz.,* PPO, POD, LOX and PI in the variety MCU 7 and LRA 5166 upon the damage treatment *S. litura* followed by *A. gossypii* damage treatment. Significant difference in induction of above said defense enzymes was recorded between *S. litura* and *A. gossypii* damage treatments, but difference in induction of PPO and POD between mechanical and insecticidal soap treatment was non-significant in all the varieties (Table 1). The comparision of mean in cotton varieties in terms of the induction of defense enzymes upon different damage treatments showed highest induction in the variety MCU 7 and no significant difference was observed between the varieties MECH 184 non-Bt and SVPR 2 indicating both the varieties as least performing in relation to the induction of defense enzymes (Table 2). Similarly varietal influence on pattern of biochemical changes with special reference to infestation by thrips, *Retithrips syriacus* on five different cotton cultivars was reported by. Ananthakrishnan(1990). Srinivasan and Uthamasamy (2004) reported significant induction of ß-1, 3-glucanases level upon *B. tabaci* feeding and increase in ß-1,3-glucanases, total phenol and tannins against *H. armigera* feeding damage in variety COTLCVRH 4 suggesting induction of resistance vary among the different varieties of the crop.

Induction of plant defense enzymes by topical application of Jasmonic Acid

Jasmonic acid (JA), a ubiquitous regulator of the wound response in plants, is recognized as a part of a long distance defense signalling pathway. The influence of exogenous application of JA spray on foliar defense enzymes *viz.,* PPO, POD and LOX and PI was therefore studied and their induction in control and JA treated plants were compared following t-test (Table 3). The pooled results of all the cotton varieties revealed that JA treatment significantly increased the levels of the PPO and PI when compared with their corresponding controls at <0.001 level and for POD and LOX at <0.05 level three days after the spray of JA. Pair wise Tukey's comparision for the difference in mean values of enzyme induction among the cotton varieties indicated high induction but not significantly different among varieties MCU 7 and LRA 5166 for PPO, PI and POD followed by Bt and non-Bt cotton and the least induction on SVPR 2 variety. LOX induction was significantly high in variety MCU 7, but was more or less similar for all the remaining varieties upon JA treatment (Table 4). The results are in agreement with the findings of many researchers who reported that jasmonic acid is produced by the plant after herbivore damage and results in increased production of compounds involved in resistance (Constabel *et al.,* 1995, Thaler *et al.,* 1996) and such an induction could be brought about by topical application. This natural plant hormone plays a crucial role in signaling plant defense responses against both insects and microorganisms (Lyon *et al.,* 1995; McConn *et al*., 1997). Moore *et al*., (2003) reported JA as a part of a long distance defense signalling pathway and when applied exogenously induces several defense related responses in many species including the activation of proteinase inhibitor proteins and defense-related metabolites. Jasmonic acid treatment of 4-, 6-, and 8-week-old tomato plants resulted in the induction of peroxidase and polyphenol oxidase activity and reduced the relative growth rate of first-instar *Manduca sexta* larvae fed treated leaves (Cipollini *et al.,* 1999).

Effect of previous herbivory and JA application on feeding preference of S. litura

Feeding by *S. litura* resulted in the induction of defense enzymes in *G. hirsutum*. Such induced plants were used for feeding bioassay experiments, to determine the resistance build-up by quantifying the area of leaf consumed. The results indicated a significant reduction in the consumption of the induced plants of all the varieties (Table 5). In the undamaged control plants, minimum leaf consumption was recorded for MECH 184 Bt cotton followed by MCU 7, LRA 5166 and no significant difference was observed between non-Bt cotton and variety SVPR 2. A similar trend in consumption was recorded in all the induced plants. However the difference in mean values of leaf consumption between control and induced plants showed a significant influence of induction on the feeding by *S. litura* consumption except for Bt-cotton and SVPR 2. Maximum reduction in leaf area consumption was observed for MCU 7 followed by LRA 5166, non-Bt and Bt cotton which indicate MCU 7 as a best performing variety against *S. litura.*

In both the control and induced plants of Bt-cotton, the weight gain of *S. litura* larvae was minimum when compared with the other remaining varieties indicating the influence of Bt toxin. But the reduction in weight gain due to induced resistance was maximum for MCU 7 and LRA 5166 varieties followed by non-Bt cotton and SVPR 2 (Table 6). These results indicate that constitutive level of resistance is high in Bt-cotton, but induced resistance was recorded maximum in variety MCU 7 and LRA 5166. The results are corroborative with the findings of McAuslane and Alborn (1998) who reported that the foliar terpenoids in cotton deter feeding by leaf chewing insects such as *S. exigua*. The present study confirms that resistance has been induced in all the varieties of cotton, but the quantity of induction vary in proportion to the feeding response of *S. litura.* The defensive function of protease inhibitors is attributed to their ability to suppress insect digestive enzymes, which then leads to a reduction in amino acid assimilation by insects (Shulke and Murdock, 1983; Broadway and Duffey, 1986). Heil and Bostock (2002) reported the decrease in spotted bollworm population on JA treated plants where in the JA serve as a signal for expression of a number of defense-related compounds, such as proteinase inhibitors, oxidative enzymes and numerous phenolics, against insects and pathogens.

Feeding preference of *S. litura* larvae on JA treated cotton plants of different varieties was assessed following no-choice feeding bioassay experiments (Table 7). On all the varieties of cotton there was significant reduction in feeding, the maximum difference from the control being for the variety MCU 7. A similar trend of influence was observed on weight gain by *S. litura* on all the cotton varieties indicating the influence of induced resistance by JA Table 8. Maximum decrease in weight gain was recorded for the variety MCU 7 and LRA 5166. The effects of induced resistance to cotton aphids *Aphis gossypii* Glover, two-spotted spider mites *Tetranychus urticae* Koch, and western flower thrips *Frankliniella occidentalis* in cotton plants were investigated using applications of the natural plant inducer, jasmonic acid and the preference was reduced by more than 60% for aphids and spider mites, and more than 90% for thrips compared with control leaves (Karban, 1999, Lyon and Newton 1999 and Nabil, 2003). Similar induced resistance was demonstrated in tomato plants against corn earworm, *Helicoverpa zea* Boddie, and beet armyworm, *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) using the elicitor Jasmonic acid (Thaler *et al.*, 1999). Lu *et al*., (2004) used jasmonic acid application to induce resistance in Chinese cabbage (*Brassica campestris*) to the diamondback moth, *Plutella xylostella* L.

CONCLUSION

In conclusion, plants defend themselves from insect attack through a variety of mechanisms and stimulated by many different biotic inducers. Results showed that *S. litura* and *A. gossypii* feeding induced biochemical defense responses in the cotton varieties. Induction of PPO, POD, LOX and PI was observed maximum in the variety MCU 7 and the least in SVPR 2. The resistance developed was confirmed by a lesser feeding preference of *S. litura* recorded on induced plants in comparision to the non-induced control plants on all the varieties studied. Exogenous application of Jasmonic Acid indicated high induction but not significantly different among varieties MCU 7 and LRA 5166 for PPO, while POD and LOX induction were more or less similar for all the varieties. Similarly reduction in consumption and weight gain of *S. litura* upon JA treatment was recorded. Hence the accumulation of defense molecules in resistant and susceptible genotypes has to be exploited to utilize the defense mechanisms in integrated pest management.

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Table 2. Comparative analysis of different cotton varieties in relation to the mean induction of foliar proteins

Variety	PPO	POD	LOX	PI*
MCU 7	0.44a	0.483a	0.407a	1.376a
LRA 5166	0.394b	0.459b	0.382b	1.305b
MECH184-BT	0.342c	0.457b	0.388c	1.365a
MECH 184 non-BT	0.328c	0.447c	0.349d	1.336c
SVPR 2	0.322c	0.442c	0.353d	1.297c

Columns followed by same letter(s) are not significantly different at P<0.05 (Tukey's pairwise comparison);Values represent difference between control and treated plants [ΔOD log10(x+1) transformed] of PPO, POD, LOX.
* difference in % Inhibition of chymotrypsin activity [Sqr. Root(x+1) transformation] between control and treated

Table 3. Effect of JA on induction of foliar enzymes (t-test between control and treatment)

Enzyme	Control	Jasmonic Acid	Difference
PPO	3.818	13.54	9.722**
POD	2.200	6.721	4.527*
LOX	6.670	11.226	4.556*
PI	17.667	78.400	60.733**

* Significant at 0.05 level ** significant at 0.001 level Values represent mean enzyme activity (Δ OD) pooled across varieties.

Table 4. All pair wise comparison of enzyme activity among varieties of cotton upon Jasmonic Acid treatment

Variety	PPO	POD	LOX	PI
MCU 7	1.216a	0.91a	0.926a	1.883a
LRA 5166	1.094ab	0.816a	0.807ab	1.819ab
MECH 184-Bt	0.925bc	0.644c	0.629cd	1.758bc
Mech 184-non Bt	0.988b	0.663c	0.704bc	1.768b
SVPR 2	0.789c	0.587c	0.546d	1.701c

Columns followed by same letter(s) are not significantly different at P<0.05 (Tukey's pairwise comparison).
Values represent difference in enzyme activity (Δ OD log10[x+1] transformed) between control and treated plants.

International Journal of Agriculture Innovations and Research
Volume 6, Issue 2, ISSN (Online) 2319-1473

Table 5. Consumption of S. litura on different varieties of resistance induced cotton

Variety	Control	Induced	*Difference*
MCU 7	68.33b	64.67b	3.67*
LRA 5166	74.00c	71.33c	2.67*
MECH 184-Bt	62.33a	61.00a	1.33
Mech 184-non Bt	77.67d	75.33d	2.33*
SVPR 2	80.33d	78.67e	1.67

Columns followed by same letter are not significantly different at 0.05 level.
*significant at 0.05 level. Values represent mean leaf area consumption in mm^2

Table 6. Weight gain of S. litura fed on resistance induced cotton varieties

Variety	Control	Induced	*Difference*
MCU 7	0.269b	0.227a	0.042**
LRA 5166	0.283c	0.248a	0.035**
MECH 184-Bt	0.194a	0.185b	0.009
Mech 184-non Bt	0.354d	0.333c	0.021*
SVPR 2	0.378e	0.359d	0.019*

Columns followed by same letter are not significantly different at 0.05 level.
*significant at 0.05 level; **significant at 0.01 level, Values represent mean weight gain in grams

Table 7. Consumption of S. litura on Jasmonic Acid treated cotton varieties

Variety	Control	Induced	*Difference*
MCU 7	68.33b	51.67a	17.00**
LRA 5166	74.00c	58.70b	15.30**
MECH 184-Bt	62.33a	51.00a	11.33**
Mech 184-non Bt	77.67d	64.52c	13.15**
SVPR 2	80.33d	72.67d	8.00*

Columns followed by same letter are not significantly different at 0.05 level.
*significant at 0.05 level, values represent mean leaf area consumption in mm^2

Table 8. Weight gain of S. litura fed on Jasmonic Acid treated cotton varieties

Variety	Control	Induced	*Difference*
MCU 7	0.269b	0.141a	0.128**
LRA 5166	0.283c	0.184b	0.099**
MECH 184-Bt	0.194a	0.132a	0.062**
Mech 184-non Bt	0.354d	0.281c	0.073**
SVPR 2	0.378e	0.324d	0.054**

Columns followed by same letter are not significantly different at 0.05 level.
*significant at 0.05 level; **significant at 0.01 level, Values represent mean weight gain in grams

Table 1. Enzyme activity in different varieties of cotton against different damage types

Damage treatment x variety **(Interaction)**	**PPO**					**POD**					**LOX**					**PI***				
	Varieties					**Varieties**					**Varieties**					**Varieties**				
Damage treatments	MCU 7	LRA 5166	MECH 184-Bt	MECH 184 non-Bt	SVPR 2	MCU 7	LRA 5166	MECH 184-Bt	MECH 184 non-Bt	SVPR 2	MCU 7	LRA 5166	MECH 184-Bt	MECH 184 non-Bt	SVPR 2	MCU 7	LRA 5166	MECH 184-Bt	MECH 184 non-Bt	SVPR 2
S. litura	1.074a	0.932a	0.804a	0.775a	0.771a	0.649a	0.633a	0.629a	0.619a	0.614a	0.593a	0.574a	0.577a	0.552a	0.557a	1.770a	1.739a	1.781a	1.730a	1.728a
A. gossypii	0.632b	0.607b	0.535b	0.509b	0.493b	0.605b	0.586b	0.581b	0.577b	0.572b	0.393b	0.350b	0.355b	0.288b	0.292b	1.476b	1.342b	1.473b	1.420b	1.328b
Mechanical	0.037c	0.027c	0.022c	0.023c	0.019c	0.339c	0.313c	0.309c	0.303c	0.295c	0.342c	0.315c	0.326c	0.282b	0.285b	1.350c	1.275c	1.316c	1.318c	1.277b
Soap	0.019c	0.010c	0.006c	0.007c	0.005c	0.339c	0.304c	0.307c	0.291c	0.286c	0.298d	0.288d	0.294d	0.276b	0.279b	0.908d	0.862d	0.891d	0.876d	0.857c

Columns followed by same letter(s) are not significantly different at P<0.05 (Tukey's pairwise comparison)
Values represent difference between control and treated plants [ΔOD log10(x+1) transformed] of PPO, POD, LOX.
*difference in % Inhibition of chymotrypsin activity [Sqr. Root(x+1) transformation] between control and treated