J. Cotton Res. Dev. **30** (1) 84-89 (January, 2016)

Protection against cotton leaf curl disease by Jasmonic acid induced proteins

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ABSTRACT : In the present study role of Jasmonic acid induced proteins on American cotton cultivars namely Ankur 3028 BGII and a *desi* cotton variety LD 694 against cotton leaf curl disease (CLCuD) was studied. Cotton cultivars at four to six leaf stage were treated with 150 µM JA. Protein content was estimated from the leaf samples collected at 24, 48, 72, 96 hrs and a week after treatment with JA. Application of JA resulted in the induction of proteins 24 hrs after treatment. After a week interval at 150 µM concentration of JA maximum protein induction of 15.0 mg/g fr wt and 14.4 mg/g fr wt was recorded in Ankur 3028 BGII and LD 694 whereas in their respective control 7.5 mg/g fr wt, 4.0 mg/g fr wt Electrophoretic study of cotton cultivars treated with 150 µM of JA revealed the induction of proteins ranging from 15-45 kDa along with some other proteins as well. This application of JA was found to affect the CLCuD incidence and severity when inoculated with viruliferous whiteflies (*Bemesia tabaci*) in screen cages. At applied conc. of JA disease incidence was recorded to be 30% and disease index was found to be 40 per cent in Ankur 3028 BGII. The respective controls exhibited higher values for disease incidence and disease severity. This study signified that JA application resulted in the induction of PR proteins which provided protection against the disease.

Keywords: Cotton leaf curl disease, Jasmonic acid proteins

Plants are constantly attacked by various types of pathogen as a result they have evolved a plethora of wide variety of defence mechanisms which can be either local, systemic, constitutive or inducible. One particular inducible systemic response, is Systemic Acquired Resistance (SAR). SAR refers to a distinct signal transduction pathway that plays an important role in the ability of plants to defend themselves against pathogens by the induction of various types of proteins and metabolites. Application of novel plant protection chemicals that act by stimulating the plant's inherent disease resistance mechanisms by means of induction of various types of proteins thus could prove beneficial in disease management. It is found that certain natural and synthetic compounds *e.g.* Jasmonic acid (JA) and their structural analogues are capable of activating the defense mechanisms in plant and prove helpful in conferring tolerance/resistance against various pathogens (Wang *et al.,* 2005). Vanwees *et al.,* (2000) in his work signified the importance of SAR inducers like salicylates and jasmonates against broad spectrum of pathogens. Moreover, chemical inducers of plant resistance possess quite different mode of action as compared to fungicides. The latter products have direct toxic effect on pathogens; are noxious to environment; have narrow spectrum of defense; ensure short lasting protection (Kuc 2001). Thus, application of chemical inducers of resistance is an exciting new perspective to supplement the classical chemical means of disease control by providing both effective and ecologically-friendly plant protection. Present investigations were carried out to study the role of Jasmonic acid and Salicylic acid for the induction of resistance to cotton leaf curl disease CLCuD in cotton.

MATERIALS AND METHODS

Material used : Two cotton cultivars namely *Gossypium*. cultivar Ankur 3028 BGII (moderately resistant) and *G. arboreum* cultivar LD 694 (immune) having variable degree of susceptibility to CLCuD were selected for conduct of experiments. All these cultivars are the commercial cotton cultivars. The seeds of the cultivars LD 694 were obtained from Cotton Section, Department of Plant Breeding and Genetics, PAU, Ludhiana and Seeds of Ankur 3028 BGII were provided by Ankur Seeds Pvt. Ltd. Sowing of plant material was done in the earthen pots placed in the screen cages and twelve seeds of each cotton cultivar were sown in a pot.

Treatment of seedlings with JA : 150 µM of JA was sprayed with atomizer on to the seedlings of different cotton cultivars at 4 - 6 leaf stage. Water sprayed plants of corresponding genotypes served as control. Potted plants sprayed with JA and water (control) were kept separately in different screen cages. All the chemicals and solvents used in present investigation were of analytical grade. JA was purchased from Sigma-Aldrich**.**

Collection of plant tissue samples : For protein extraction periodical leaf sampling (in triplicate) was done at 24, 48, 72, 96 hrs and after one week of spray. Samples were brought to laboratory under refrigerated conditions and were stored at -80°C in deep freezer to prevent denaturation of proteins.

Extraction and estimation of total soluble proteins from leaf samples : 0.2 g of leaf tissue was weighed and was homogenized in 25 mM Tris HCl buffer (*p*H 8.0) in a pre cooled pestle and mortar on ice bath and centrifuged at 10,000 rpm for 25 minutes at 4^0C. Supernatant was used as protein extract. Soluble proteins in supernatant were estimated and expressed as mg/g fr wt.

SDS- PAGE : The electrophoretic protein profile of cotton leaves of different cotton cultivars was obtained by vertical slab gel SDS-PAGE. 5 per cent stacking gel was laid over the 10 per cent resolving gel. Protein samples were mixed with sample buffer (0.5 M Tris HCl, *p*H 6.8, SDS (10 %), Glycerol (0.1%) Bromophenol blue and 2-Mercaptoethanol (5%). Standard molecular marker was run along with the samples. Electrophoresis of the proteins was carried out at 1.5 mA current/cm till the end of run. The gel was taken out and stained overnight with staining solution containing Coomassie brilliant blue R 250. The stained bands were visualized by repeatedly placing the gel in destainer. The gels were preserved in 7 per cent acetic acid solution.

Statistical analysis : Statistical analysis of the experimental data was done to test the significance of treatment using factorial completely randomized design. Critical differences was tested at 5 per cent level of significance.

Inoculation and disease assessment : After 24 h of spray, potted plants of 4 to 6 leaf stage were exposed to viruliferous whiteflies. Six whiteflies/plant were released and plants were disturbed two times a day for uniform and overall inoculation of CLCuV. The colonies of viruliferous whiteflies, were reared and maintained on highly susceptible potted cotton plants in separate screen house. The whiteflies were allowed to develop and multiply on these plants. Newly developed adults were used for inoculation of cotton plants. CLCuD incidence and severity was calculated using the following formulae:

$$\textbf{Disease incidence (\%)} = \frac{Pi}{Pt} \times 100$$

Where, Pi = Number of infected plants
Pt = Total number of plants

Disease index (%) : The plants were graded according to revised CLCuD scale described in AICCIP (2008) as given in Table 1

$$\textbf{Disease index (\%)} = \frac{N1}{S1} \times \frac{S2}{N2} \times 100$$

Where,
N1= Number of plants in check
N2= Number of plants in test entry
S1= Sum of all infection grades in check
S2= Sum of all infection grades in test entry

RESULTS AND DISCUSSION

Effect of dose of JA *i.e.* 150 µM on total leaf protein content (mg/g fr wt) in different cotton cultivars at different time intervals.

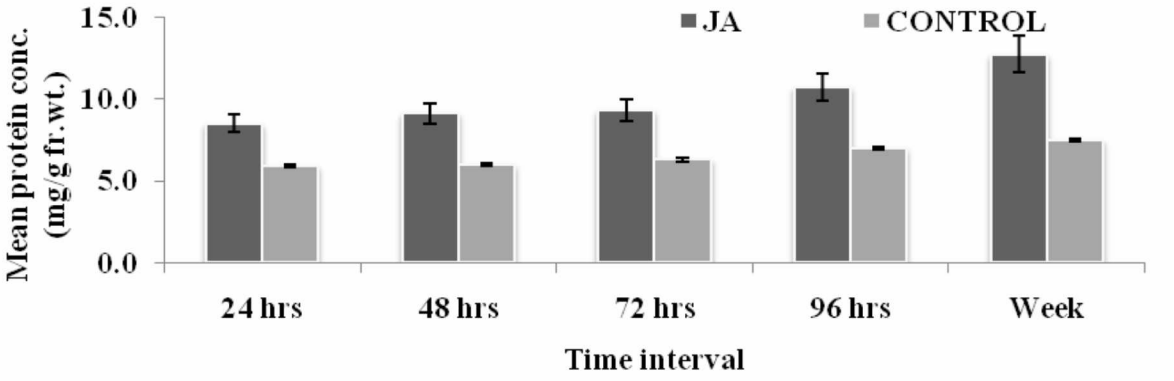
(I) *G. hirsutum* cultivar Ankur 3028 BGII : The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to 150 µM of JA revealed statistically significant differences in induced protein concentrations (Table 1). JA at 150 ìM resulted in mean maximum protein content ± SE (12.0 ± 0.9 mg/g fr wt) whereas in control plants value of protein was 6.5 ± 0.3 mg/g fr wt Amongst the treatments applied, JA caused 1.8 fold increase in protein content in leaves of cultivar Ankur 3028 BGII as compared to control thus completely signifying the positive response of JA in protein induction.

Table 1. Effect of 150 µM doses of JA on protein concentration (mg/g fr wt) ± SE of *G. hirsutum* cultivar Ankur 3028 BGII recorded at periodic intervals

Dose	Treatment	Time interval (h)					Treatment mean
		24	48	72	96	Week	
150 ìM	JA	9.6	10.8	11.1	12.6	15.0	12.0 ± 0.9
	Water	5.9	6.0	6.3	7.0	7.5	6.5 ± 0.3

Overall mean JA = 11.8 , **Water** = 6.5 **CD (0.05)JA (A)** = 0.141, **Water (B)** = 0.223**(A)(B)** = 0.315

The effect of treatment of JA at various time intervals on protein concentration (Fig 1) showed statistically significant differences for JA. JA was found to induce mean maximum protein of 8.5 ± 0.5, 9.1 ± 0.6, 9.4 ± 0.7, 10.7 ± 0.8 and 12.8 ± 1.1 mg/g fr wt at 24, 48, 72, 96 h and



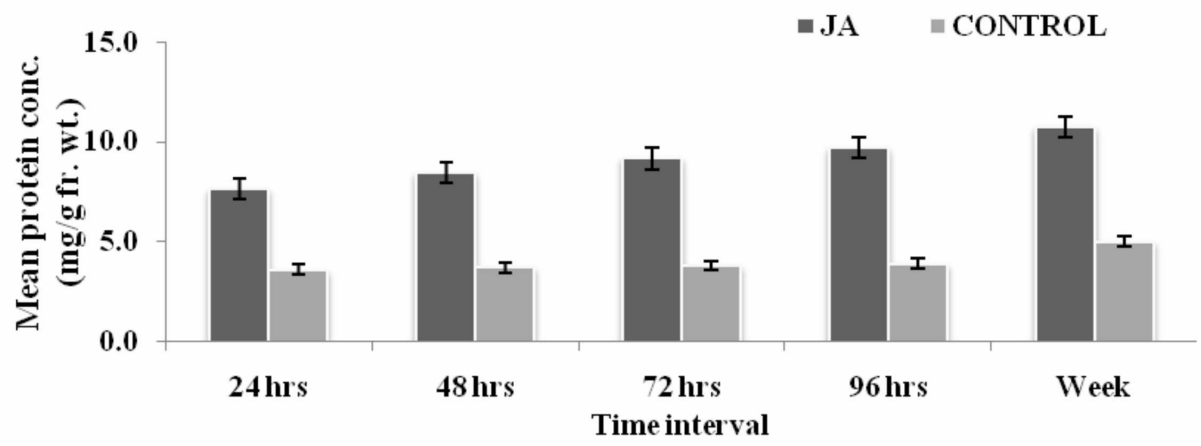
Each value is mean ± SE of values at different time intervals at respective doses of each treatment

Fig. 1. Effect of treatment of JA on protein concentration of *G. hirsutum* cultivar Ankur 3028 BGII at different time intervals

at a week interval respectively whereas mean minimum protein 5.9 ± 0.0, 6.0 ± 0.0, 6.3 ± 0.0, 7.0 ± 0.0 and 7.5 ± 0.0 mg/g fr wt was observed for control at all the corresponding time intervals.

(II) *Gossypium arboreum* cultivar LD 694 : The data pertaining to changes in protein concentration recorded at periodical interval of 24 h till a week in response to 150 µM of JA revealed statistically significant differences in induced protein concentrations (Table 2). JA at 150 ìM resulted in mean maximum protein content ± SE (11.5 ± 0.8 mg/g fr wt) whereas in control plants value of protein was 4.0 ± 0.3 mg/g fr wt Amongst the treatments applied, JA caused 2.9 fold increase in protein content in leaves of cultivar LD 694 in comparison to control indicating that JA is a better inducer of proteins. The effect of treatment of JA at various time intervals on protein concentration (Fig 2) showed statistically significant differences for JA JA was found to induce mean maximum protein of 7.6 ± 0.7, 8.4 ± 0.7, 9.2 ± 0.9, 9.7 ± 1.0 and 10.7 ± 1.4 mg/g fr wt at 24, 48, 72, 96 hrs and at a week interval respectively whereas mean minimum protein of 3.6 ± 0.0, 3.7 ± 0.0, 3.8 ± 0.0, 3.9 ± 0.0 and 5.0 ± 0.0 mg/g fr wt was observed for control at all the corresponding time intervals.

Protein Profiling : Extracted cotton leaf



Each value is mean ± SE of values at different time intervals at respective doses of each treatment

Fig. 2. Effect of treatment of JA on protein concentration of *G. arboreum* cultivar LD 694 at different time intervals

proteins were subjected to SDS-PAGE electrophoresis. Leaf samples of treated cotton cultivars were evaluated for protein profiling through SDS-PAGE. Total leaf proteins were resolved in molecular weights in the range 6-180 kDa with respect to standard protein marker. Specific bands falling in the range of 6-49 kDa were reported in treated samples as compared to their respective control as shown in Plate 1. It is known that PR proteins fall under the range of 15.8 kDa to 45 kDa Jishan *et al* (2011) also revealed the induced expression of defence related genes like PR 2, PR 3, PR 5, PR 10 and Ta JA2 which encode â,1-3 glucanase, chitinase, thaumatin-like protein, peroxidase etc. in different wheat cultivars- namely Chinese Spring, Pumai 9 and Zhoumai 18 with MeJA treatment.

Thus, it showed that exogenous application of JA and SA resulted in the induction of PR proteins of molecular size ranging from 15.8-45 kDa along with some other proteins. as well in plants under treatment.

Effect of 150 µM JA on disease incidence and severity of CLCuD : The data in

Table 2. Effect of 150 iM JA on protein concentration (mg/g fr. wt.) ± SE of *G. arboreum* cultivar LD 694 recorded at periodic intervals

Dose	Treatment	Time interval (h)					Treatment mean
		24	48	72	96	Week	
150 iM	JA	9.7	10.3	11.2	12.0	14.4	11.5 ± 0.8
	Water	3.6	3.7	3.8	3.9	5.0	4.0 ± 0.3

Overall mean JA = 11.5, **Water** = 4.0 **CD (0.05) JA (A)** =0.098, **Water (B)** = 0.155 **(A)(B)** = 0.219

Table 3 show the effect of JA applied at a concentration of 150 µM on the incidence and severity of CLCuD. At 150 µM of JA disease incidence and severity values were observed to be lower as compared to control in cultivars Ankur 3028 BGII. Disease incidence was 30% and disease index was 40 per cent which was 45 per cent and 63 per cent for disease incidence and disease index values in control and in case of cultivar Ld 694 no disease was observed. Results were found to be positively correlated with the amount of protein induced at this concentration as at above mentioned concentration higher protein induction was observed which could be responsible in lowering the disease. This decrease in disease parameters due to the induction of PR proteins and some other proteins is well supported by the findings of Yao and Tian (2005) who reported that application of 150-200 iM of MeJA not only induced resistance in peach fruit against *Monilinia fructicola* and *Penicillium expansum* but

Table 3. Effect of JA on disease incidence and severity of CLCuD.

Cultivar	Disease incidence (%) Dose @ 150 iM (DAS)						Disease index (%)	
	14*		21		28			
	Control	JA	Control	JA	Control	JA	Control	JA
Ankur 3028 BG II	0	0	28	20	45	30	63	40
LD 694	0	0	0	0	0	0	0	0

*DAS – Days after spray

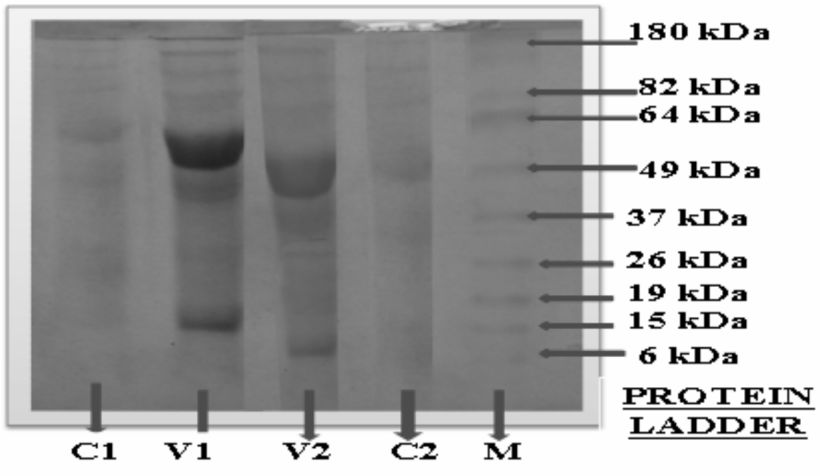


Plate 1. SDS-PAGE of leaf proteins of different cotton cultivars at 150 iM of JA, M- Protein Ladder (6 KDa- 180 KDa), V1- Ankur 3028 BGII and V2- LD 694; C1, C2 represent control of Ankur 3028 BGII and LD 694

also increased the population of biocontrol agent *Cryptococcus laurentii* (Kuffer) which altogether resulted in stronger disease resistance. Resistance against the pathogen was attributed to the induction of proteins belonging to PR-2, PR-4 and PR-8 family.

REFERENCES

Jishan N, Jing L, Wenben M, Qiao Y L, Zhengyang W and Dexian H. 2011. The relationship of methyl jasmonate enhanced powdery mildew resistance in wheat and the expressions of disease resistance related genes. *Agric Sci Tech* **12**: 504-08.

Kuc J. 2001. Concepts and direction of induced systemic resistance in plants and its application. *Eur J plant Pathol* **107**: 7-12.

Vanwees, De S.A.M., Van P.J.A., Van L. and Pieterse C.M.J. 2000. Enhancement of induced disease resisance by simultaneous activation of salicylate and jasmonate dependent defense pathways and in *Arabidopsis thalaina. Proc Natl Acad Sci* USA **97**: 8711-16.

Wang K., Weaver N. D., Kesarwani M. and Dong X. 2005. Induction of protein secretory pathway is required for systemic acquired resistance. *Science* **308**: 1036-40.

Yao H.J. and Tian S.P. 2005. Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. *J Applied Microbiol* **98**: 941-50.

Received for publication : August 1, 2014
Accepted for publication : February 19, 2015