RESPONSE OF COTTON GENOTYPES TO JASMONIC ACID IN RELATION TO INDUCTION OF RESISTANCE AGAINST COTTON LEAF CURL DISEASE

Thesis

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
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MASTER OF SCIENCE
in
PLANT PATHOLOGY
(Minor Subject: Entomology)

By

Ritu Raj
(L-2011-A-90-M)

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CERTIFICATE I

This is to certify that the thesis entitled, **"Response of cotton genotypes to Jasmonic acid in relation to induction of resistance against cotton leaf curl disease"** submitted for the degree of M.Sc. in the subject of **Plant Pathology** (Minor subject: **Entomology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ritu Raj (L-2011-A-90-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled, **"Response of cotton genotypes to Jasmonic acid in relation to induction of resistance against cotton leaf curl disease"** submitted by **Ritu Raj (L-2011-A-90-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of M.Sc in the subject of **Plant Pathology** (Minor subject: **Entomology**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same, in collaboration with an external examiner.

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Abstract

The present study was conducted to check the response of different American cotton cultivars namely RS 921, LH 2076, PIL 8, Ankur 3028 BGII and a desi cotton variety LD 694 to Jasmonic acid (JA) and Salicylic acid (SA) in induction of resistance against cotton leaf curl disease (CLCuD). Different cotton cultivars were grown in pots under screen cages. At four to six leaf stage potted plants of different cotton cultivars were treated with different concentration of JA and SA i.e. 50 µM, 100 µM, 150 µM, 200 µM respectively and water sprayed plants of corresponding genotypes served as control. Quantification of proteins was done using spectrophotometric method from the leaf samples collected at 24, 48, 72, 96 hrs and a week after treatment with JA and SA. Application of JA and SA resulted in the induction of proteins 24 hrs after treatment. After a week interval at 150 µM concentration of JA maximum protein induction of 19.9 mg/g fr. wt., 16.7 mg/g fr. wt., 19.6 mg/g fr. wt., 15.0 mg/g fr. wt. and 14.4 mg/g fr. wt. was recorded in RS 921, LH 2076, PIL 8, Ankur 3028 BGII and LD 694 respectively whereas, in case of SA 200 µM concentration showed maximum protein induction i.e 10.6 mg/g fr. wt., 9.3 mg/g fr. wt., 9.9 mg/g fr. wt., 9.0 mg/g fr. wt., 8.1 mg/g fr. wt. in RS 921, LH 2076, PIL 8, Ankur 3028 BGII and LD 694 respectively. In the induction of proteins JA was found to be more effective than SA. Electrophoretic study of cotton cultivars treated with 150 µM of JA and 200 µM of SA revealed the induction of Pathogenesis-related (PR) proteins ranging from 15-45 kDa along with some other proteins as well. JA and SA applied at different concentrations affected the CLCuD incidence and severity when inoculated with viruliferous whiteflies (*Bemesia tabaci*) in screen cages. Lower disease incidence as well as disease index was observed with JA when applied @ 150 µM and SA @ 200 µM. Disease incidence was 37%, 30%, 30% and disease index was 48%, 40%, 40% in RS 921, LH 2076, Ankur 3028 BGII at 150 µM concentration of JA whereas, at 200 µM SA disease incidence was 48%, 36%, 34% and disease index was 57%, 50%, 50% respectively in above mentioned cultivars and in their respective control values for disease incidence and disease severity were quite high. So, JA at 150 µM and SA at 200 µM concentration was found to be most effective in lowering the disease. Latent carry over detection of symptomless plants treated with 150 µM of JA and 200 µM of SA through PCR amplification using DNA β specific primers confirmed the presence of virus in all the tested cotton cultivars except LD 694 which signified that PR proteins does not eliminate virus. JA and SA application resulted in imparting tolerance with the induction of PR proteins but does not lead to complete resistance against the disease.

Key words: Cotton, Cotton leaf curl disease (CLCuD), Jasmonic acid (JA), Salicylic acid (SA) and Protein

Signature of the Major Advisor **Signature of the Student**

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ਸਾਰੰਸ਼

ਇਹ ਤਜ਼ਰਬੇ ਨਰਮੇ ਦੀਆਂ ਵੱਖ-2 ਕਿਸਮਾਂ ਜਿਵੇਂ ਕਿ ਆਰ ਐਸ 921, ਐਲ ਐਚ 2076, ਪੀ ਆਈ ਐਲ 8, ਅੰਕੁਰ 3028 ਬੋਲਗਾਰਡ II ਅਤੇ ਦੇਸੀ ਕਪਾਹ ਦੀ ਕਿਸਮ ਐਲ ਡੀ 694 ਉਪਰ ਜੈਸਮੋਨਿਕ ਐਸਿਡ (ਜੇ ਏ) ਅਤੇ ਸੈਲਸਿਲਕ ਐਸਿਡ (ਐਸ ਏ) ਦਾ ਬਿਮਾਰੀ ਰੋਕੀ ਪ੍ਰਭਾਵ ਪਤਾ ਕਰਨ ਲਈ ਕੀਤਾ । ਜਦੋਂ ਨਰਮੇ ਦੇ ਬੂਟਿਆਂ ਨੇ 4-6 ਪੌਦੇ ਕੱਢ ਲਏ ਤਾਂ ਉਹਨਾਂ ਉਪਰ ਜੇ ਏ ਅਤੇ ਐਸ ਏ ਦੀਆਂ ਵੱਖ-ਵੱਖ ਮਾਤਰਾ ਜਿਵੇਂ ਕਿ 50, 100, 150 ਅਤੇ 200 ਮਾਈਕਰੋਮੋਲਰ (ਮ: ਮੋਲਰ) ਦੀ ਤਾਕਤ ਦੀ ਸਪਰੇ ਕੀਤੀ ਅਤੇ ਬਿਨ੍ਹਾਂ ਸਪਰੇ ਵਾਲੇ ਬੂਟਿਆਂ ਤੇ ਇਕੱਲਾ ਪਾਣੀ ਸਪਰੇ ਕੀਤਾ । ਨਰਮੇ ਦੇ ਪੱਤਿਆਂ ਵਿੱਚ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ ਦੀ ਜਾਣਕਾਰੀ ਲਈ ਪੱਤਿਆਂ ਦੇ ਸੈਂਪਲ 24, 48, 72, 96 ਘੰਟੇ ਅਤੇ ਇੱਕ ਹਫਤੇ ਤੇ ਲਏ ਗਏ । ਇਨ੍ਹਾਂ ਦੀ ਪ੍ਰੋਟੀਨ ਮਾਤਰਾ ਦੀ ਜਾਣਕਾਰੀ ਸਪੈਕਟਰੋਫੋਟੋਮੀਟਰੀ ਰਾਹੀਂ ਕੀਤੀ ਗਈ । ਪੱਤਿਆਂ ਵਿੱਚ 24 ਘੰਟਿਆਂ ਬਾਅਦ ਹੀ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ ਵੱਧਣੀ ਸ਼ੁਰੂ ਹੋ ਗਈ । ਇੱਕ ਹਫਤੇ ਤੇ ਜਿੰਨ੍ਹਾਂ ਬੂਟਿਆਂ ਤੇ 150 ਮ: ਮੋਲਰ ਜੇ ਏ ਦੀ ਸਪਰੇ ਕੀਤੀ ਗਈ ਸੀ ਉਹਨਾਂ ਵਿੱਚ ਵੱਧ ਤੋਂ ਵੱਧ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ 19.9, 16.7, 19.6, 14.4 ਅਤੇ 15.0 ਮਿਲੀਗ੍ਰਾਮ/ਗ੍ਰਾਮ ਫਰੈਸ਼ ਵੇਟ ਕ੍ਰਮਵਾਰ ਆਰ ਐਸ 921, ਐਲ ਐਚ 2076, ਪੀ ਆਈ ਐਲ 8, ਅੰਕੁਰ 3028 ਅਤੇ ਐਲ ਡੀ 694 ਵਿੱਚ ਪਾਈ ਗਈ। ਐਸ ਏ ਦੀ 200 ਮ: ਮੋਲਰ ਤੇ ਸਪਰੇ ਕਾਰਨ ਵੱਧ ਤੋਂ ਵੱਧ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ 9.8, 9.3, 10.6, 8.1 ਅਤੇ 9.0 ਮਿਲੀਗ੍ਰਾਮ/ਗ੍ਰਾਮ ਫਰੈਸ਼ ਵੇਟ ਇਸ ਤਰ੍ਹਾਂ ਉਪਰੋਕਤ ਦਰਸਾਈ ਕਿਸਮਾਂ ਦੀ ਤਰਤੀਬਵਾਰ ਪਾਈ ਗਈ । ਸੋ ਤਜ਼ਰਬਿਆਂ ਦੇ ਆਧਾਰ ਤੇ ਜੇ ਏ ਦਾ ਪ੍ਰਭਾਵ ਐਸ ਏ ਨਾਲੋਂ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ ਵਧਾਉਣ ਵਿੱਚ ਵੱਧ ਦੇਖਿਆ ਗਿਆ। ਤਜ਼ਰਬਿਆਂ ਅਧੀਨ ਕਿਸਮਾਂ ਦੇ ਪੱਤਿਆਂ ਦਾ ਇਲੈਕਟ੍ਰੋਫੋਰੇਸਿਜ ਵਿਸ਼ਲੇਸ਼ਣ ਕਰਨ ਤੇ ਦੇਖਿਆ ਗਿਆ ਕਿ ਇਨ੍ਹਾਂ ਵਿੱਚ ਪੈਥੋਜੈਨਿਸਸ ਸਬੰਧਤ ਪ੍ਰੋਟੀਨ ਜਿਨ੍ਹਾਂ ਦਾ ਮੌਲੀਕੂਲਰ ਭਾਰ 15-45 ਕਿਲੋ ਡਾਲਟਨ ਹੁੰਦਾ ਹੈ 150 ਜੇ ਏ ਅਤੇ 200 ਮ: ਮੋਲਰ, ਐਸ ਏ ਦੀ ਸਪਰੇ ਨਾਲ ਪਾਇਆ ਗਿਆ । ਜੇ ਏ ਦੀ ਵਰਤੋਂ ਦਾ ਬਿਮਾਰੀ ਦੀ ਤੀਬਰਤਾ ਅਤੇ ਬਿਮਾਰੀ ਦੇ ਇਨਡੈਕਸ ਨੂੰ ਘੱਟ ਕਰਨ ਵਿੱਚ ਰੋਲ ਸਾਬਤ ਹੋਇਆ ਕਿਉਂ ਜੋ ਬਿਮਾਰ ਬੂਟਿਆਂ ਦੀ ਪ੍ਰਤੀਸ਼ਤਤਾ ਆਰ ਐਸ 921, ਐਲ ਐਚ 2076 ਅਤੇ ਅੰਕੁਰ 3028 ਵਿੱਚ 37, 30, 30, ਅਤੇ ਬਿਮਾਰੀ ਦਾ ਇਨਡੈਕਸ 48, 40, 40, ਪ੍ਰਤੀਸ਼ਤ ਕਰਮਵਾਰ ਪਾਇਆ ਗਿਆ ਅਤੇ 200 ਮੈ: ਮੋਲਰ ਐਸ ਏ ਤੇ ਬਿਮਾਰੀ ਦੀ ਤੀਬਰਤਾ 37, 30, 30, ਅਤੇ ਬਿਮਾਰੀ ਦਾ ਇਨਡੈਕਸ 48, 40, 40, ਪ੍ਰਤੀਸ਼ਤ ਪਾਇਆ ਗਿਆ । ਬਿਨ੍ਹਾਂ ਸਪਰੇ ਵਾਲੇ ਇਨ੍ਹਾਂ ਤਿੰਨ ਕਿਸਮਾਂ ਦੇ ਬੂਟਿਆਂ ਵਿੱਚ ਬਿਮਾਰੀ ਦੇ ਦੋਹੇ ਲੱਛਣ ਬਹੁਤ ਜ਼ਿਆਦਾ ਸੀ । ਜੇ ਏ ਅਤੇ ਐਸ ਏ ਤਜ਼ਰਬੇ ਅਧੀਨ ਬੂਟਿਆਂ ਜਿੰਨ੍ਹਾਂ ਵਿੱਚ ਬਿਮਾਰੀ ਦੇ ਕੋਈ ਲੱਛਣ ਨਹੀਂ ਸਨ ਉਨ੍ਹਾਂ ਵਿੱਚ ਵਾਇਰਸ ਦੇ ਛੂਪੇ ਪ੍ਰਭਾਵ ਦੀ ਸਮੀਖਿਆ ਬੀਟਾ ਪ੍ਰਾਇਮਰ ਵਰਤ ਕੇ ਪੋਲੀਮੇਰੇਜ ਲੜੀਬੱਧ ਪ੍ਰਕ੍ਰਿਆ ਰਾਹੀਂ ਕੀਤੀ, ਜਿਸ ਵਿੱਚ ਬੀਟਾ ਵਾਇਰਸ ਦੀ ਲੁੱਕੀ ਪਹਿਚਾਣ ਲੱਭੀ ਗਈ । ਪ੍ਰੋਟੀਨ ਦੀ ਵਧੀ ਮਾਤਰਾ ਬਿਮਾਰੀ ਦੇ ਵਾਇਰਸ ਦਾ ਖਾਤਮਾ ਕਰਨ ਵਿੱਚ ਅਸਮਰੱਥ ਸੀ ਪਰ ਇਸ ਨੇ ਬੂਟਿਆਂ ਦੀ ਬਿਮਾਰੀ ਰੋਕਣ ਦੀ ਸਮਰੱਥਾ ਵਿੱਚ ਵਾਧਾ ਜਰੂਰ ਕੀਤਾ ।

ਮੁੱਖ ਸ਼ਬਦ: ਨਰਮਾ, ਪੱਤਾ ਮਰੋੜ ਰੋਗ, ਜੈਸਮੋਨਿਕ ਐਸਿਡ (ਜੇ ਏ), ਸੈਲੇਸਿਲਕ ਐਸਿਡ (ਐਸ ਏ) ਅਤੇ ਪ੍ਰੋਟੀਨ ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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Chapter I

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is also known as "white gold". It is the most important fibre crop in the world. Grown in countries on five continents, cotton is a major crop in each of the five top producing countries including China, USA, the countries of the former Soviet Union, Pakistan and India (Hillocks 1992). It substantially, adds to export revenue and gross domestic product (GDP) of the country. The Indian textile industry consumes the cotton, as principal raw material, which contributes about 4 per cent to the GDP. The by-product of seed cotton i.e. cotton seed also adds value in other industries viz. oil extraction, seed cake, manufacturing of wooden articles, cotton linters, high currency note, animal feed etc. Both cotton and textile exports account for nearly one-third of total foreign exchange earnings of the country, crossing Rs. 60,000 crores. Therefore, growth and development of cotton and cotton industry have a vital bearing on overall development of Indian economy (Anonymous 2011).

India is the only country in the world where all the cultivated species of genus *Gossypium* namely *G. arboreum, G. herbaceum*, *G. hirsutum* and *G. barbadense* are grown on fairly commercial scale.The States of Punjab, Haryana and Rajasthan includes 16 per cent of the total cultivated land where cotton is grown and these states contribute 20 per cent of the total cotton grown in India (Rajagopalan *et al* 2012). Among these, four domesticated species of cotton, *G. hirsutum* L., the upland cotton, is most widely grown and contributes about 80 per cent of the total production in Asia. During 2011-12, cotton was cultivated on an area of 121.91 lakh ha with a production of 371.20 lakh bales and productivity of 481.23 kg/ha in India whereas in Punjab, it was cultivated on area 5.60 lakh ha with total production 19.50 lakh bales and productivity of 591.96 kg/ha during 2011-2012 (Anonymous 2011).

The cotton leaf curl disease (CLCuD) has become a major limiting factor for successful cotton cultivation in North India. Cotton leaf curl disease was first reported by Farquharson (1912) and Tarr (1951) on *Gossypium barbadense* L. from Nigeria and subsequently from Tanzania in 1926 (Jones and Mason 1926, Kirkpatrick 1931). In Pakistan CLCuD was first reported in 1967 (Hussain and Ali 1975) and became a minor sporadic problem until 1986. It then became an epidemic spreading to almost all cotton growing regions of Pakistan and into adjoining areas of India, in subsequent years. Losses to the economy of Pakistan were enormous, estimated at US$5 billion between 1992 and 1997 (Briddon and Markham 2000).

In India, the disease was first recorded from Sriganganagar in Rajasthan during 1993 (Singh *et al* 1994), while in Punjab, it was observed at a few locations in Hindumal Kot area of Abohar during 1994 (Kapur *et al* 1994). During 1997, it assumed serious proportion and was observed in almost all the cotton growing areas of North India with a disease intensity

touching as high as 98 per cent (Chopra and Randhawa 1997).

The cotton leaf curl disease is known to be caused by cotton leaf curl virus (CLCuV) which is a Gemini virus and it interacts in a persistent circulative manner with its vector, whitefly (*Bemisia tabaci* Genn.). Briddon *et al* (2001) identified that cotton leaf curl disease is a causative complex comprising DNA-A and DNA-β molecules of CLCuV and which is responsible for the production of typical symptoms of this disease.

Genetic resistance to CLCuD is considered to be most viable, environmentally safe, ecologically sound and less expensive approach for the management of this menace. However, breeding CLCuD resistant varieties pose many challenges to plant breeders and plant pathologists, since there are certain complications due to various factors like (i) CLCuV is reported to be causative complex, (ii) involvement of vector i.e. whitefly (iii) weather conditions (iv) wide host range of vector and virus and (v) latent carry over of virus (vi) new aggressive variants of CLCuV.

Moreover, in 2011 all previously known resistant cotton varieties began to show the typical symptoms of CLCuD, indicating that the resistance had been overcome. During *Kharif* 2009, regular survey of cotton belt in Punjab was carried out by PAU, Ludhiana and it was found that CLCuD had appeared in epidemic form in Ferozepur district on RCH 134 BG II and non-descript susceptible genotypes resulting in considerable yield losses (Anonymous 2009).

Breakdown of resistance to CLCuD and non-availability of effective chemical control necessitate the search for new means of disease control. Systemic acquired resistance (SAR) inducing chemicals can provide novel benefits to our existing strategies of disease control. It is known that plants defend themselves from pathogen infection through wide variety of mechanisms for e.g. employing systemic acquired resistance by resulting in the synthesis of defense enzymes, accumulation of pathogenesis-related (PR) proteins etc SAR is a mechanism of induced defense that confers long lasting protection against broad spectrum of pathogens (Edreva 2004). It is based on expression of latent genetic information and is thus biologically safe also. SAR requires the signaling molecules namely Jasmonic acid (JA), Salicylic acid (SA) etc. which induce resistance by the induction of proteins against various pathogens. These chemicals when applied activate defense related activities in different crops and are found to be effective (Kessmann *et al* 1994, Durrant and Dong 2004).This area of research still needs to be explored in case of *G. hirsutum* and CLCuD. Keeping in view the role of these signaling molecules in combating the diseases of various crop plants by inducing natural defense mechanism without causing any hazard to our environment, present investigations were carried out with the following objective:

- To study the role of Jasmonic acid and Salicylic acid for the induction of resistance to CLCuD in cotton.

Chapter II

REVIEW OF LITERATURE

Cotton is the most important *kharif* cash crop of North Indian states of Punjab, Haryana and Rajasthan. CLCuD has emerged as a serious threat to successful cotton cultivation in this region. Reports about the breakdown of resistance in established genotypes like LHH 144, MRC 6304 Bt, MRC 6301Bt and Ankur 651 against CLCuD has been repoted from cotton belt of Punjab in All India Coordinated Cotton Improvement Project Annual Report (Anonymous 2009). During *kharif* 2009, regular survey of cotton belt in Punjab carried out by PAU, Ludhiana and it was found that CLCuD had appeared in epidemic form in Ferozepur district on RCH 134 BG II and non- descript susceptible genotypes resulting in considerable yield losses. The breakdown of resistance to CLCuD in cotton is a known phenomenon in India. Therefore it would be demanding to evolve new strategies for CLCuD management. So, present investigations entitled as "Response of cotton genotypes to Jasmonic acid in relation to induction of resistance against cotton leaf curl disease" were undertaken and review of literature is being presented under following subheads

2.1 History of occurrence CLCuD

This disease was earlier known as African leaf curl of cotton. For the first time, it was observed on *Gossypium peruvianum* and *G. vitifolia* in Nigeria (Farquharson 1912). In Asia, it was first observed in Multan district of Pakistan in 1967 where it became prominent during 1973 (Hameed *et al* 1994). Afterwards, in Pakistan the disease appeared in an area of 14,000 ha during 1991 which further increased to 8,89,000 ha in 1993 and assumed an epidemic form (Ali *et al* 1995). In India, the disease was first reported from Sriganganagar in Rajasthan during 1993 (Singh *et al* 1994). Later on disease was observed in almost all the cotton growing areas of North India with a disease intensity touching as high as 98 per cent at some locations (Chopra and Randhawa 1997).

2.2 Symptoms of CLCuD

The initiation of disease is characterized by small vein thickening on the young upper leaves of the plants. Upward curling of leaves occurs because of the uneven growth of the veinal tissues on the abaxial side of the leaves. Veins of the leaves become thickened which are more pronounced on the underside. Affected leaves become thick, leathery, brittle and more green than healthy leaves. Later, formation of cup shaped or leaf laminar out growth called "enation" appear on the underside of the leaf. In severe cases and in plants affected at early age, reduction in internodal length leading to stunting and reduced flowering/fruiting is observed (Narula *et al* 1999). In some cases upward as well as downward curling of the leaves in the infected American cotton plants has been observed (Khalid and Shah 1999, Khalid and Masood 1999, Iqbal *et al* 2003). Hameed *et al* (1994), Ali *et al* (1995) and Chopra and Randhawa (1997) also gave a similar description of symptoms caused by CLCuD.

2.3 Causative complex associated with CLCuD/ Etiology of CLCuD

The disease was believed to be caused by cotton leaf curl geminivirus (Mansoor *et al* 1993). Earlier, only CLCuV was considered to be responsible for causing cotton leaf curl disease, but later investigations suggested the involvement of a virus complex in causing the disease. Mansoor *et al* (1999) isolated and identified a novel circular single stranded DNA (DNA-1) associated with CLCuD in Pakistan. Similarly, Briddon *et al* (2001) identified that cotton leaf curl disease is a causative complex comprising DNA-A and DNA- β molecules of CLCuV and DNA-β which is responsible for the production of typical symptoms of this disease.

2.3.1 DNA 1/DNA A associated with CLCuD

Mansoor *et al* (1999) isolated and identified a novel circular, single-stranded DNA (DNA-1) associated with cotton leaf curl disease which resembled DNA A component of bipartite geminiviruses. The begomoviruses associated with CLCuD, like all geminiviruses, have geminate (twinned) particles. It is 2.75 kb in size and encodes genes required to perform functions like replication, control of gene expression and encapsidation.

CLCuV DNA A is systemically infectious in cotton but is only able to induce mild symptoms and yellowing in this host rather than downward leaf curling, vein swelling and greening, and the sporadic production of leaf-like structures that are typical symptoms of the disease (Briddon *et al* 2000). It was, thus, suggested that begomovirus was not sole cause of CLCuD.

2.3.2 DNA β associated with CLCuD

DNA β was initialy found associated with *Ageratum* yellow vein disease in south-east Asia (Briddon *et al* 2001 and Saunders *et al* 2000). It is a single stranded, circular, satellite DNA molecule of about 1.3 Kb size that induces typical symptoms of cotton leaf curl disease including leaf curling, vein swelling, vein darkening and enations on the underside of leaves.

CLCuV relies on this helper begomovirus for replication, encapsidation, movement within plants and transmission between plants (Mansoor *et al* 2003). The CLCuV/ DNA-1/DNA-β complex represents members of an entirely new type of infectious disease causing agents. Similarly, Briddon *et al* (2003) proposed the first report that CLCuD is associated with multiple begomoviruses supported by single stranded DNA β.

2.4 Breakdown of resistance in cotton against CLCuD

Mansoor *et al* (2003) reported the breakdown of resistance in cotton to cotton leaf curl virus disease in Pakistan and attributed it to the appearance of new virus strain. Simultaneously reports regarding breakdown of resistance in established resistant genotypes like MRC 6304 Bt, MRC 6301 Bt, Ankur 651 etc were published in India (Anonymous 2006). During *Kharif* 2009, survey of cotton belt in Punjab carried out by PAU, Ludhiana reported appearance of CLCuD in epidemic form in Ferozepur district on RCH 134 BG II and non-descript susceptible genotypes in mild form on resistant recommended and cultivated BGII hybrids like MRC 7017 and MRC 7031 as well as on LHH 144 (Anonymous 2009).

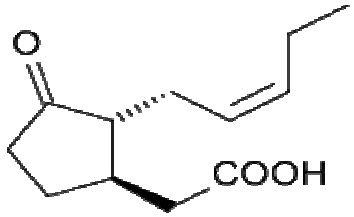
2.5 Role of SAR inducing chemicals in imparting resistance

Plants are known to defend themselves from pathogen infection through a wide variety of mechanisms that can be either local, systemic, constitutive or inducible (Dixon 1986; Ryals *et al* 1994). One particular inducible systemic response, is Systemic Acquired Resistance. SAR refers to a distinct signal transduction pathway that plays an important role in the ability of plants to defend themselves against pathogens. Durrant and Dong (2004) defined SAR as a mechanism of induced defense that confers long-lasting protection against broad spectrum of pathogens. Although SAR is interesting as a paradigm for signal transduction, it may have practical value as well. An understanding of the biochemical changes leading to the resistance/tolerance through the application of novel plant protection chemicals that act by stimulating the plant's inherent disease resistance mechanisms (Ryals 1996).

Kessmann *et al* (1994) and Wang *et al* (2005) reported that in addition to various types of pathogens certain natural and synthetic compounds eg. JA and SA etc. are capable of triggering similar plant responses which activate the defense mechanisms in plant and prove helpful in conferring tolerance/resistance against various pathogens. Sticher *et al* (1997) and Vanwees (2000) in their work signified the importance of SAR inducers like salicylates and jasmonates which are capable of providing effective and long lasting resistance 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. But, no such work has

been carried out on CLCuD and *G hirsutum* interaction in India and abroad.

2.5.1 Jasmonic acid



JASMONIC ACID

Jasmonates were first detected in essential oils of *Jasminum grandiflorum*. JA is derived from the fatty acid linolenic acid by a lipoxygenase (LOX)-mediated oxygenation process (Hamberg and Gardener 1992). It is a member of the jasmonate class of plant hormones. In plants jasmonates move easily in both liquid and vapor phase. Methyl ester of Jasmonic acid (MeJA) is especially volatile suggesting that it might act in gaseous form (Farmer and Ryan 1992). Major function of JA and its various metabolites is regulation of plant responses to biotic and abiotic stresses as well as plant growth and development. It has an important role in response to wounding of plants and SAR (Delker 2006).

Treatment with jasmonates was shown to induce various responses including the accumulation serine proteinase inhibitors (Farmer and Ryan 1990), leucine aminopeptidase and threonine deaminase (Hildmann *et al* 1992) phenylalanine ammonia-lyase, thionin (Anderson *et al* 1992), ribosome- inactivating protein (Chaudhary *et al* 1994) and number of secondary metabolites were also shown to accumulate in cultured cells of various plant species upon treatment with JA (Gundlach *et al* 1992) which resulted in the induction of defense responses in plants. JA and MeJA have been reported to induce number of pathogen responsive plant defense genes (Anderson *et al* 1992).

Publication presented by Cohen *et al* (1993) showed that foliar spray of JA and MeJA protected tomato and potato plants against *Phytophthora infestans* by enhancing the production of proteinase inhibitors (PI) and certain acid soluble proteins. SDS-PAGE analysis revealed the induction of proteins of size 24 kDa, 29 kDa, and 30 kDa which fall under various PR protein families. Schweizer *et al* (1993) reported that in barley *Hordeum vulgare* L. application of Jasmonic acid effectively protected it against subsequent infection of *Erysiphe graminis* f.sp. *hordei* by resulting in the formation of JA induced proteins (JIP) and Pathogenesis related proteins (PR). Two prominent groups of proteins of molecular size 25 kDa and 10 to 12 kDa size were induced. Exogenously applied MeJA imparted protection against *Alternaria brassicicola* in *Arabidopsis* by the induction of PDF1.2 PR-3 and PR-4 gene (Thomma *et al* 1998).

Kolowski *et al* (1999) conducted experiments which showed that jasmonates were

effective in inducing local and systemic protection against *Pythium ultimum* in Norway spruce. Kauss *et al* (2000) reported that treatment with MeJA resulted in the elicitation of defense responses in parsley.

Ding *et al* (2002) reported that pre-treatment of tomato fruit with MeJA induces the synthesis of some PR proteins such as PR-2b, PR-2a, PR-3b etc. which lead to increase chilling tolerance and resistance to pathogens, there by decreasing the incidence of decay. Work done by Walters *et al* (2002) proved that treatment of barley (cv. Golen promise) seedlings with MeJA imparts systemic protection against powdery mildew caused by *Blumeria graminis* f.sp. *hordei.* Application of 100 mM of Methyl Jasmonate (MJ) induced anatomical reactions related to defense in Norway spruce which enhanced resin flow and increased resistance against blue stain fungus *Ceratocystis polonica* (Franceschi *et al* 2002).

JA and MeJA when applied in case of soybean resulted in the expression of different PR genes which resulted in the induction of resistance against *Phytophthora sojae* (Graham *et al* 2003). Thaler *et al* (2004) studied resistance mediated through Jasmonates against various pathogens in tomato plants. Jasmonates were found to be successful in imparting protection against *Pseudomonas syringae, Xanthomonas compestris, Fusarium oxysporum* and *Botrytis cinerea.* Buji *et al* (2004) in his studies found that treatment of melon seeds with MeJA protected melon seedlings from gummy stem blight and white mould caused by *Didymella bryoniae* and *Sclerotinia sclerotiorum.* MeJA treatment was found to enhance the induction of various PR proteins etc.

Yao and Tian *et al* (2005) reported that application of 200 µM of MeJA not only induced resistance in peach fruit against *Monilinia fructicola* and *Penicillium expansum* but 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.

JA was not only found effective in controlling fungal, bacterial, viral diseases but also found to induce defense against root knot nematode in tomato plants as well. Cooper *et al* (2005) studied the effect of JA against root knot nematode in tomato. Results indicated that JA induced systemic defense response which reduced nematode reproduction on susceptible tomato plants. Studies conducted by Yuan Jiao *et al* (2007) indicated the induction of chemical defense system by the external application of JA in Bt Corn (*Zea mays*). Application of JA resulted in the expression of PR-2a, PR-1 genes and also enhanced the Bt protein concentration in the treated plants.

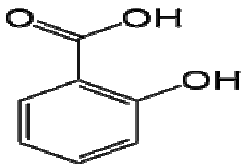
Jing Shi *et al* (2010) studied the role of GhMPK 7 gene which is known to play a role in plants multiple defense pathways. In transgenic *Nicotiana benthamiana* it was found that exogenous application of MeJA induced the expression of GhMPK7 gene which provided significant resistance to virus PVY and to fungus *Colletotrichum nicotianae*. Application of

MeJA was reported to induce partial resistance in *Medicago truncata* against the charcoal rot pathogen, *Macrophomina phaseolina* (Gaige *et al* 2010).

Four week old sterile seedlings of *Nicotiana tabaccum* plants when treated with MeJA resulted in the expression of different genes which were found to be involved in various defense related pathways (Fammartino *et al* 2010). Haggag *et al* (2010) reported that MeJA application on sugarbeet plants against Beet mosaic virus (BtMV) decreased disease severity and when 3µg/ml of MeJA was applied it entirely subdued BtMV protein accumulation. MeJA resulted in the accumulation of total soluble proteins, chitinases etc which belong to various PR families and thus, proved that MeJA plays an important role in the induction of defense mechanisms against BtMV infection.

Studies conducted by Jishan *et al* (2011) revealed the induction of resistance against powdery mildew in wheat (cv. Chinese Spring, Pumai 9 and Zhoumai 18) with MeJA treatment. The induced resistance was positively correlated with the induced expression of defense related genes like PR 2, PR 3, PR 5, PR 10 and Ta-JA2 which encode β,1-3 glucanase, chitinase, thaumatin-like protein, peroxidase etc. Yamada *et al* (2012) reported that treatment of rice plants with jasmonates conferred resistance against bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae.* It was attributed to the induction of OsJAZ8 protein.

2.5.2 Salicylic acid (SA)



SALICYLIC ACID

SA is synthesized via phenyl propanoid metabolism from cinnamonic acid and benzoic acid. It accumulates intracellularly at a specific receptor or binding protein (Chen *et al* 1993). The first hint that SA might be involved in plant defense was provided by White (1979) who found that exogenous application of SA onto tobacco plants enhanced resistance to subsequent infection by TMV by resulting in the accumulation of PR proteins. Van Loon *et al* (1982) and Uknes (1992) reported induction of same set of genes by SA that were expressed following biological SAR induction. Van Loon (1983) also first raised the possibility of a link between SA and SAR. Kombrink and Somssich (1997) also stated the involvement of SA in SAR which activated gene expression of various defensive factors such as induction of PR proteins. Exogenous application of SA was shown to be mimicking certain aspects of a pathogen infection, resulting in both SAR gene expression and induction of SAR (Vernooij *et al* 1995). Various workers have put forward their work which signifies the role of exogenous application of SA in inducing defense in plants.

Spraying of tobacco plants with SA induced both the synthesis of PR proteins and

resistance to viruses that can induce necrotic lesions. Spraying Samsun NN tobacco with SA induced the production of PR-1 and inhibited the systemic multiplication of alfalfa mosaic virus (A1MV). Application of SA was also found to induce the synthesis of PR proteins in bean plants (Huijsduijnen *et al* 1986).

Sunflower leaf discs floated on a solution containing aspirin (Acetylsalicylic acid) produced a set of new proteins extractable at pH 5.2 and excreted into the intercellular space. More than 80 per cent of the proteins found in the intercellular fluids of induced leaf discs were identified as pathogenesis-related (PR) proteins by their immunological relationship with tobacco PR proteins. This finding by Jung *et al* (1993) signifies the importance of SA in the induction of PR proteins. Ryal *et al* (1996) reported that SA or its functional analogue Benzothiadiazole (BTH) were found to be affective against various pathogens.

Maize seeds when applied with BTH (Functional analogue of SA) resulted in the induction of PR-1 and PR-5 genes which caused reduction in Downy mildew Caused by *Peronosclerospora sorghi* (Morris *et al* 1998). Spletzer *et al* (1999) investigated the role of exogenous application of SA in activating SAR genes against *Alternaria solani* in tomato plants. It was reported that with the addition of 200 µM of SA to the root system elevated foliar SA level along with the induction of PR-1B gene expression Thus, activated SAR against the pathogen.

Berbert *et al* (2000) carried out an analysis of genes expressed in barley after the application of SA and observed the induction of BC1 genes incoding lipoxygenase, thionin etc (which are PR proteins homologue) which are involved in various disease resistance pathways. Tolerance to various abiotic and biotic stress induced by the application of SA and Acetyl Salicylic acid (ASA) in tomato and bean plant was studied by Senaratena *et al* (2000).Seeds imbibed in aqueous solution (0.1-0.5 mM) of SA and ASA enhanced tolerance of tomato and bean plants to various types of stress.

SA and its structural analogue BTH are successfully used in inducing protection against various plant viruses like Tobacco mosaic virus (TMV) in tobacco, Turnip crinkle virus (TCV) in *Arabidopsis* (Lawton 1996), and Cucumber mosaic virus (CMV) in tomato (Anfoka 2000).

In cowpea Chandra *et al* (2001) showed that foliar spray of 0.02 per cent of SA twice at a week interval caused reduction in root rot disease caused by *Rhizoctonia solani* by enhancing the production of proteins. In another study by Guleria *et al* (2001) in peas (*Pisum sativum*) accumulation of PR proteins was observed by the application of SA these PR proteins are known to provide resistance against various pathogens. Similar results were reported by Mosa (2002) which showed significant reduction in the severity of plant diseases of rice after treatment with SA.

Faheed *et al* (2006) reported that exogenous application of 0.1, 0.5 and 1mM SA on two

week old plants of *Phaseolus vulgaris* induced partial inhibition in the accumulation of virus and elevated the induction of various total soluble proteins as compared to untreated plants which resulted in the induction of resistance against TNV. Anand *et al* (2008) investigated the effect of SA on crown gall disease in *Nicotiana benthamiana* against *Agrobacterium tumefaciens*. Plants treated with SA showed decreased susceptibility to *A. tumefaciens.*

Foliar application of SA was tested by Esmailzadeh (2008) against tomato stem canker. SA pretreated plants showed decrease in blighted area and discoloration as compared to non treated plants. In *Brassica juncea* (var. RLM 619) Sharma *et al* (2008) found that activities of various defense related enzymes got enhanced with the application of SA which provided resistance against Alternaria blight caused by *Alternaria brassicae*. SA foliar application induced resistance in potato against black scurf and stem canker caused by *Rhizoctonia solani* (Khalil 2008). Vimala *et al* (2009) reported induction of resistance against *Erysiphe cichoracearum* in Okra with the foliar application of SA which resulted in the induction of increased enzymatic activity.

2.6 Latent carry over of CLCuD

Sekhon and Chopra (2002) gave the first report of latent carry over of CLCuV in cotton which is quite common in *hirsutum* cotton. Similarly, Sabhiki *et al* (2004) confirmed the presence of latent infection of CLCuV in apparently disease free plants and healthy plants of resistant genotypes.

Chapter III

MATERIAL AND METHODS

The field experiments of the study "Response of cotton genotypes to Jasmonic acid in relation to induction of resistance against cotton leaf curl disease" were conducted under artificial conditions in screen cages at Cotton Research Area, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana during the *Kharif* season of 2012. The laboratory work for quantification of proteins and SDS-PAGE profiling was carried out at Biochemistry Lab, Department of Biochemistry Punjab Agricultural University, Ludhiana and for detection of latent carry over of viral particles employing Polymerase chain reaction (PCR) based molecular work was carried out in the Central Molecular Biology Lab, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The details of the material and methods used for conducting all the experiments are discussed as follows:

3.1 Experiment I - Standardization of concentration of Jasmonic acid and Salicylic acid for the induction and over expression of proteins in CLCuD tolerant and susceptible cultivars of cotton

The material and methods used for this experiment are as follows:

3.1.1 Selection of *Gossypium hirsutum* cultivars/hybrids

Five cotton cultivars having variable degree of susceptibility to CLCuD were selected for conduct of experiments.

a) *G. hirsutum* cv RS 921 CLCuD highly susceptible cultivar
b) *G. hirsutum* cv LH 2076 moderately resistant cultivar
c) *G. hirsutum* cv PIL 8 Male parent of LHH 144, a resistant hybrid; having a high degree of resistance against CLCuD
d) Ankur 3028 BGII (Moderately resistant to CLCuD): A Bt hybrid recently released for general cultivation in Punjab
e) *G. arboreum* cv LD 694: Desi cotton variety which shows immune reaction towards CLCuD

The seeds of all varieties/hybrids was obtained from Cotton section, Department of Plant Breeding and Genetics, PAU, Ludhiana but seeds for Ankur BGII was provided by Ankur Seeds Pvt. Ltd.

3.1.2 Sowing of crop

For raising different cotton cultivars, the earthen pots having 30 cm diameter were filled with loamy soil and then DAP (Diammonium phosphate) was added to it and the pots were watered and placed in the screen cages. Twelve seeds of each cultivar were sown in each pot. The sowing was done in second week of April 2012 (Plate 1).

3.1.3 Treatment of young seedlings with different concentrations of JA and SA

Different doses of JA and SA i.e 50 µM, 100 µM, 150 µM, 200 µM were sprayed with the help of atomizer on young seedlings (4 - 6 leaf stage) of different cultivars. Water sprayed plants of corresponding genotypes served as control. Potted plants sprayed with specific doses of JA, SA and water (control) were kept separately under different screen cages. All the chemicals and solvents used in present investigation were of analytical grade.

3.1.4 Collection of plant tissue samples

For protein extraction preodical leaf sampling (in triplicate) was done after 24, 48, 72, 96 hrs and at a week interval. Samples were brought to laboratory under refrigerated conditions and were stored at -80°C in deep freezer to prevent denaturation of proteins.

3.1.5 Extraction and estimation of total soluble proteins from leaf samples

Reagents

a) 25 mM Tris HCl buffer (pH 8.0): 0.786 g of Tris HCl was dissolved in 100 ml distilled water. The pH was adjusted to 8.0 with 1N NaOH and volume was made to 200 ml with distilled water and was kept in refrigerator.

Extraction

0.2 g of leaf tissue was weighed and was homogenized in 25 mM Tris HCl buffer (pH 8.0) in a precooled pestle and mortar on the ice bath and centrifuged at 10,000 rpm for 25 minutes at 4^0C. Supernatant was used as protein extract.

Estimation

Protein content of supernatent was estimated by the method of Lowry *et al* (1951).

Reagents

a) 2% Sodium carbonate in 0.1N sodium hydroxide: 2 g of sodium carbonate was dissolved in 100 ml of 0.1 N NaOH (Reagent A).

b) 0.5% Copper sulfate in 1% sodium potassium tartarate: Dissolved 1 g of sodium potassium tartarate in 80 ml distilled water and made the final volume to 100 ml. To this added 0.5 g of copper sulfate ($CuSO_4.5H_2O$)(Reagent B).

c) 50 ml of reagent A was mixed with 1 ml of reagent B just before use (Reagent C)

d) 1 N Folinciocalteau's phenol reagent (Reagent D)

Procedure

To 0.1 ml of the protein extract added 0.9 ml of distilled water. 5 ml of reagent C was added and mixed well. After an interval of 10 min, 0.5 ml of reagent D was added, mixed and kept for 30 min at room temperature. The intensity of blue color developed was then read at 520 nm against a reagent blank. Bovine serum albumin (BSA) standards (20-100 µg) were also run along with the test samples and the concentration of protein was calculated from the standard curve of BSA (Fig 3.1).

PLATE 1: Cotton experiments being conducted in screen cages

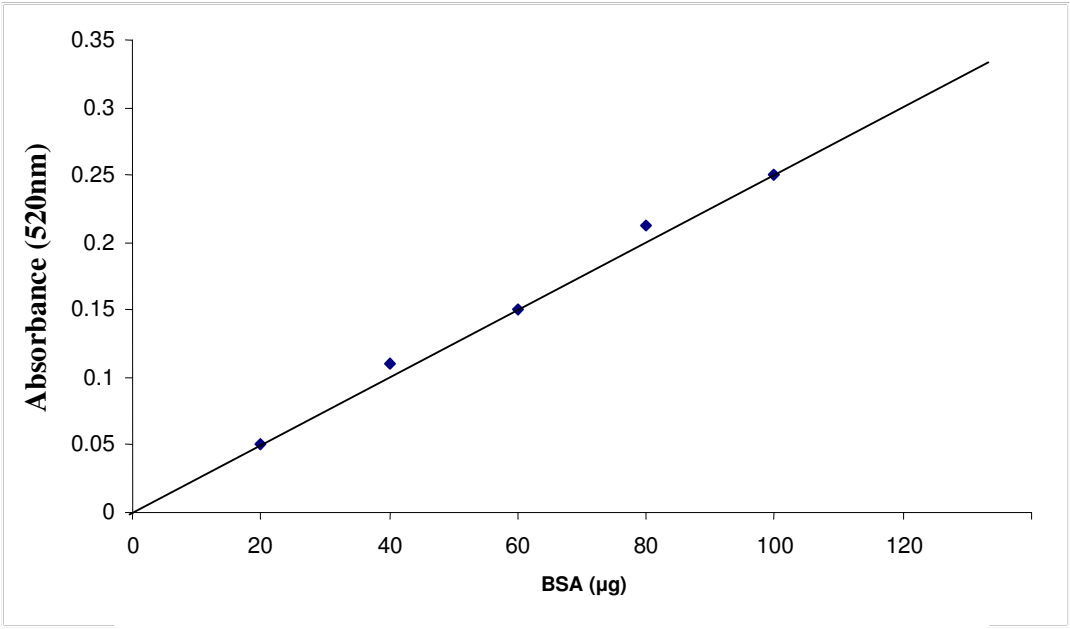


Fig 3.1 Standard protein curve (BSA 20-100 µg)

3.1.6 Protein profiling by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Proteins were fractionated by Vertical Slab SDS-PAGE (Walker 1996).

(I) Preparation of stock reagents

a) 30% Acrylamide-bisacrylamide solution: Dissolved acrylamide and N, N, N', N' – methylene bisacrylamide in the ratio of 30:0.8 in distilled water to prepare 100 ml solution. The mixture was filtered and stored at 4° C in dark brown bottle.

b) Resolving gel buffer: 1.5 M Tris- HCl buffer (pH 8.8); Dissolved 9.085 g of Tris-base in 25 ml of distilled water. The pH was adjusted to 8.8 with 3N HCl and made the final volume to 50 ml with distilled water. The solution was stored at 4° C.

c) Stacking gel buffer: 0.5 M Tris- HCl buffer (pH 6.8); Dissolved 3.963 g of Tris-HCl in 25 ml distilled water. The pH was adjusted to 6.8 with 1N NaOH and volume was made to 50 ml with distilled water and solution was stored at 4° C.

d) 10% (w/v) ammonium persulphate (APS): 10 mg of APS was dissolved in 100 µl distilled water. Fresh solution was prepared every time before use.

e) 10% SDS – 1g of SDS was dissolved in distilled water with gentle stirring and volume was made to 10 ml.

f) TEMED – N, N, N', N' tetramethylene diamine

g) Sample buffer

Table 3.1 Composition of sample buffer

REAGENT	CONCENTRATION
0.5M Tris – HCl (pH 6.8)	2.5 ml
Sucrose	2.5 g
SDS	0.25 g
2-mercaptoethanol	0.12 ml
Bromophenol blue (0.1 % in distilled water)	2.5 ml

Final volume was made 25 ml with distilled water.

h) Reservoir buffer (pH 8.3): 3.0 g of Tris – base, 14.4 g glycine and 0.5 g SDS were mixed and total volume made to 500 ml with distilled water. The solution was stored at 4°C.

i) Staining solution: Dissolved 0.1 g Coomassie brilliant blue R-250 in 50 ml methanol, 10 ml glacial acetic acid and 40 ml of distilled water.

j) Destaining solution: Prepared by mixing methanol, glacial acetic acid and distilled water in the ratio of 100: 70: 830.

(II) Cleaning of the electrophoresis apparatus

The surfaces of the plates were washed firstly with detergent and dried. The dried glass plates were clumped together using spacers and fixed in gel casting apparatus. The assembled plates were checked for leakage with distilled water.

(III) Preparation of resolving gel and stacking gel

Table 3.2 Composition of resolving and stacking gel

Reagents	Stacking gel (5%)	Resolving gel (10%)
Acrylamide : Bisacrylamide	0.5 ml	3.0 ml
Tris – HCl	0.6 ml (6.8 pH)	1.6 ml (8.8 pH)
Distilled water	2.0 ml	4.2 ml
SDS	75.0 µl	0.1 ml
APS	30.0 µl	60.0 µl
TEMED	8.0 µl	15.0 µl

The resolving gel and stacking gel was prepared using the composition as mentioned in Table 3.2. The resolving gel solution was immediately poured into the gel mould upto the level of 1 cm below the position of sample wells. The surface of polymerizing gel was overlaid with 0.5 ml of water using micropipette. The separating gel was left to polymerize for 15-30 mins. The water was poured off from the top of separating gel. After which the mould was filled with the stacking gel solution upto the top of the plates and the comb was inserted. The satcking gel was left to polymerize. After about one hour, the comb was carefully

removed without disturbing the wells. Wells were washed twice with the reservoir buffer. Care was taken to remove bits of polymerized gel present in the wells.

(IV) Sample preparation and loading of samples

Protein samples containing 50-100 µg of protein in 0.1-0.2 ml of saline were mixed in equal volume of sample buffer and heated for 2 min to ensure complete dissociation and optimum SDS-binding. Place the gel containing plates in vertical slab gel electrophoresis apparatus containing running buffer in such a way so that no air bubbles are formed at the bottom of the gel. The upper reservoir was filled with running buffer. Sample containing 50-100 µg of protein was loaded in each well with the help of micropipette or syringe. After loading the samples, the electrodes were connected to DC powerpack and current was adjusted to 1.5 mA per cm. Electrophoresis was continued until the dye (bromophenol blue) reached near the bottom of the gel. Molecular markers were run as standard proteins.

(V) Staining and destaining of proteins

After the termination of electrophoresis, the gel plates were removed and carefully separated from each other. Made a cut on the gel corner for marking the position of samples. The gel was then placed in staining solution as mentioned in (i) for 4-5 hours and destained with several changes of destaining solution as mentioned in (j). The gels were preserved in 7 per cent acetic acid solution.

3.1.7 Statistical analysis

Statistical analysis of the experimental data was done to test the significance of treatments using factorial completely randomized design. The significance of difference was tested at 5 per cent level of significance.

3.2 EXPERIMENT II – Investigation of role of different doses of JA and SA in inducing tolerance/resistance to CLCUD

The materials and methods used for this experiment are as follows:

3.2.1 Sowing and spraying of crop

Sowing and spraying of crop was done in a same way as discussed in 3.1.2 and 3.1.3. The sowing was done in second week of April 2013.

3.2.2 Maintenance of whitefly population

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.

3.2.3 Inoculation of potted cotton plants with viruliferous whiteflies

After 24 hrs of spray potted plants at four to six 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 CLCuD.

3.2.4 Observations

Observations on the disease development (per cent disease incidence and per cent disease index) were recorded starting from June till August 2013.

(I) Disease incidence (%)

The per cent disease incidence was worked out using the formula:

$$\text{Disease Incidence (\%)} = \frac{Pi}{Pt} \times 100$$

Where, Pi = Number of infected plants Pt = Number of total plants

(II) Disease index (%)

The plants were graded according to revised CLCuD scale described by AICCIP (Anonymous, 2008) as given in Table 3.3.

Table 3.3 Cotton leaf curl disease rating scale

Rating scale	Symptoms
0	Plants free from CLCuD
1	Thickening of small veins, only few upper leaves affected
2	Thickening of veins, curling and cupping of leaves
3	Thickening of veins, curling and cupping of leaves, enation development on underside of leaves
4	Thickening of veins, cupping, enations, stunting of plants and few bolls

Per cent disease index	Reaction
0	Highly resistant (HR)
0.01-5	Resistant (R)
5.1-25	Moderately Resistant (MR)
25.1-50	Moderately Susceptible (MS)
>50	Susceptible (S)

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

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

(III) Phytotoxicity

Presence/absence of different parameters namely epinasty, hyponasty, vein clearing, yellowing, necrosis and wilting was checked 14, 21, 28, 35 days after treatment with different

doses of JA and SA to determine phytotoxicity in the treated plants.

3.2.5 Detection of presence/absence of viral DNA in symptomless plants

Leaf samples from symptomless plants of different cotton cultivars which were earlier treated with 150 µM of JA and 200 µM of SA and inoculated with viruliferous whiteflies were collected to detect the presence/absence of satellite β DNA through PCR amplification using β primer.

3.2.5.1 Total genomic DNA extraction

Total genomic DNA from symptomless cotton plants of different cotton cultivars was isolated using the CTAB (Cetyl trimethyl ammonium bromide) method as reported by Shaghai-Maroof *et al* (1984). Cotton leaves from symptomless plants were harvested, placed in the polythene bags and stored in -80 °C freezer until isolation. The leaves were ground to fine powder in liquid nitrogen by constant crushing using sterilized pre-chilled pestle and mortar, so as to make the leaves brittle and to reduce the DNase activity. Fine tissue powder was transferred immediately to a 50 ml autoclaved polypropylene centrifuge tube (Oakridge tube) containing about 20 ml of pre-warmed (65°C) CTAB extraction buffer. The composition of CTAB extraction buffer is given in Table 3.4.

Table 3.4 Composition of CTAB extraction buffer

Components	Final concentration
CTAB	1.5%
Tris HCl (pH 8.0)	100 mM
NaCl	1.4 M
EDTA (pH 8.0)	20.0 mM
β-Mercaptoethanol	2%
Polyvinylpyrolidone (PVP)	2%

The tissue powder was suspended in the extraction buffer thoroughly by inverting and rotating the tubes. The homogenate was incubated at 65°C for one hour in water bath. The samples were mixed occasionally while maintaining at 65°C. After incubation, 15ml of chloroform: isoamyl alcohol (24:1) was added in each tube. Then, tubes were swirled, till it made a dark green emulsion. The Oakridge tubes were placed on a rotary shaker for 30 minutes. After that the tubes were centrifuged for 20 minutes at 4,000 rpm in centrifuge. The supernatant was then transferred to clean steriled 50 ml Falcon tube. 2µl RNase (10mg/ml of RNase, ready to use) was added to each tube and incubated at 37°C in water bath for 1 hour. This was an optional step and could be performed after purification steps. Chloroform: isoamyl alcohol extraction and centrifugation step was repeated after RNAse treatment, if required. A two third volume of chilled isopropyl alcohol was added and then tubes were inverted gently several times. DNA formed white cotton like precipitate and good quality DNA floated atop. The floating DNA was hooked out using a sterile hooked Pasteur pipette. If

the DNA was not hookable, it was pelleted by centrifugation. The hooked or pelleted DNA was transferred into a clean sterile 2.0 ml microfuge tubes and was rinsed with 70 % ethanol for five minutes so as to remove any residual salts followed by re-centrifugation.

The final pH of the buffer was adjusted to 8.0. PVP and β-Mercaptoethanol were added just before DNA extraction was carried out. Pellet was collected and the leftover ethanol was dried up completely by turning down microfuge tubes on a blotting paper and was allowed to air dry (at room temperature) for few hours. Then 500 µl volume of 1X TE (Tris EDTA buffer–10 mM Tris HCl, 1 mM EDTA, pH 8.0) was added. The tubes were left for few hours at room temperature to allow DNA to dissolve in 1X TE buffer. The DNA was stored at 4°C until used. The freshly isolated cotton DNA contains certain impurities like, polyphenolics, proteins, polypeptides etc. Hence it is necessary to purify the DNA before PCR analysis is performed. The DNA samples were thawed to room temperature and equal volume of Tris-saturated phenol: chloroform (1:1) was added. The mixture was mixed thoroughly and centrifuged for about 5 minutes at 12000 rpm. The upper phase was pippeted out in a fresh tube. As described before chloroform: isoamyl alcohol (24:1) extraction step was performed twice. The mixture was centrifuged at 10,000 rpm for 10 minutes in each step. The aqueous phase was again pippeted out and 0.1 volume of 3M sodium acetate and 2.5 times the total volume of chilled ethanol were added to it. The DNA got precipitated as the contents were mixed gently. The precipitated DNA was spooled out. Two washings of 70% ethanol at 10,000 rpm for 5 minutes were given to precipitated DNA to remove excess salts. The pellet of DNA was dried at room temperature. Then, pellet was dissolved in appropriate volume of 1X TE buffer. The DNA samples were dissolved at room temperature and stored at -20° C until used.

3.2.5.2 Assessment of quality and quantity of DNA

The quality and quantity of DNA was assessed using Nano-drop spectrophotometer. (Thermo Scientific Nano-drop™ 1000 Spectrophotometer) in the Department of Vegetable Science, Punjab Agricultural University, Ludhiana. For this, 1-2 µl DNA sample was pipetted onto the end of a fiber optic cable (the receiving fiber). A second fiber optic cable (the source fiber) was then brought into contact with the liquid sample causing the liquid to bridge the gap between the fiber optic ends. A pulsed xenon flash lamp provided the light source and a spectrometer utilizing a linear silicon CCD array was used to analyze the light after passing through the sample. Spectrophotometer was controlled by PC based software, and the data were logged in an archive file on the PC. The Nano-drop Spectrophotometer ideally suited for measuring nucleic acid concentration and purity of nucleic acid samples up to 3700 ng/ul (dsDNA) without dilution ratio of sample absorbance at 260 and 280 nm. The ratio of absorbance at 260 and 280 nm was used to assess the purity of DNA. A ratio of ~1.8 was generally accepted as “pure” for DNA. If the ratio was appreciably lower in either case, it

might indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. The 260/230 value was a secondary measure for "pure" nucleic acid are often higher than the respective 260/280 values. The ratio 260/230 ranging from 1.8 to 2.2 indicated good quality DNA samples. If the ratio is appreciably lower, this may indicate the presence of co-purified contaminants. The quantification of most of the DNA samples ranged between 400-1500 ng/µl.

3.2.5.3 Selection of primers

Beta specific primers from NCBI (Accession no. AY083590.1) as mentioned in Table 3.5 was selected for *in-vitro* PCR based amplification/identification of CLCuD DNA β.

Table 3.5 List of Beta- based primer pair used in the detection of CLCuV

Source	Primer	Base sequence (5'-3')
AY083590.1	β 1 (F)	ACCGTGGGCGAGCGGTGCCCGAT
	β 1 (R)	CACGTGTAATACGTCTCCATCGTC

3.2.5.4 PCR amplification

Table 3.6 Stock and final concentration of different components used in PCR

Components	Stock concentration	Volume (µl)	Final concentration
Water	----	3.3	----
PCR buffer	10X*	4.0	1X
$MgCl_2$	25.0 mM	1.5	15.0 mM
dNTPs	1.0 mM	4.0	200.0 µM
Primer Forward	5.0 µM	1.0	0.5 µM
Primer Reverse	5.0 µM	1.0	0.5 µM
Taq Polymerase	5 U/µl	0.2	1 Unit
DNA template	10.0 ng/µl	5.0	50.0 ng
Total		20.0	----

*10X PCR buffer: 10 mM Tris HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % Gelatin.

Table 3.7 Temperature profile used in PCR

Step	Temperature (°C)	Time (minutes)	No of cycles
Initial denaturation	94	2	1
Denaturation	94	1	
Annealing*	56	1	40
Elongation	72	3	1
Final extension	72	10	
Hold	4		

*For β1 primer - 56°C

In vitro amplification using PCR was performed in a 24 well microtiter plate or in PCR tubes in Eppendorf Master Cycler using 50 ng genomic DNA of each genotype in a final volume of 20µl per reaction. The stock and final concentration of different components used in PCR is given in Table 3.6. Amplification was performed using temperature profile mentioned in Table 3.7.

3.2.5.5 Agarose gel electrophoresis

The PCR products obtained were run on 2.5% agarose gel made in 1X TBE buffer to visualize the DNA β hence amplified. For this 2.5 gram agarose was added to 100 ml of 1X TBE buffer and was allowed to heat till a transparent solution was obtained i.e. agarose was completely mixed in the buffer. It was then cooled to around 60°C and 5 µl of ethidium bromide was added. The solution was then poured into the gel mould and was allowed to solidify at room temperature. On solidification, the PCR product was loaded into the wells. A standard ladder (Fermentas 1500 bp) was also loaded to check the size of the PCR product. The gel was run at 5V/cm and visualized under UV light and photographed using UV gel documentation system (Alpha Innotech Corp. Suite 100).

Chapter IV

RESULTS AND DISCUSSION

The results of the present investigation entitled, "Response of cotton genotypes to Jasmonic acid in relation to induction of resistance against cotton leaf curl disease" have been presented and discussed under the following heads:

4.1 Changes in protein concentration (mg/g of fresh wt) in different cotton cultivars at various time intervals in response to different doses of JA and SA

4.1.1 *Gossypium hirsutum* cv. RS 921 (HS)

4.1.2 *G. hirsutum* cv. LH 2076 (MR)

4.1.3 *G. hirsutum* cv. PIL 8 (R)

4.1.4 *G. hirsutum* cv. Ankur 3028 BGII (MR)

4.1.5 *G. arboreum* cv. LD 694 (Immune)

4.1.6 Comparative analysis of induction of proteins in different cultivars w.r.t to different doses of JA and SA

4.2 Electrophoretic study of different cotton cultivars treated with JA and SA

4.3 Effect of different doses of JA and SA on CLCuD incidence, disease severity and phytotoxicity

4.4 Detection of latent carry over of CLCuD

4.1. Changes in protein concentration (mg/g of fresh wt) in different cotton cultivars at various time intervals in response to different doses of JA and SA

4.1.1 *Gossypium hirsutum* cv. RS 921

The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to various doses of JA and SA i.e 50 µM, 100 µM, 150 µM and 200 µM revealed statistically significant difference amongst the various doses applied (Table 4.1). Mean maximum overall protein induction was observed at 150 µM i.e 9.8 mg/g fr. wt. followed by 8.6 mg/g fr. wt. at 200 µM and mean minimum protein 7.5 mg/g fr. wt. at 50 µM irrespective of treatment applied. Amongst the treatments (JA and SA) applied JA caused two fold increase in protein content in leaves of cv RS 921 whereas SA resulted in 1.3 fold increase in protein concentration w.r.t to control indicating that JA is a better inducer of proteins.

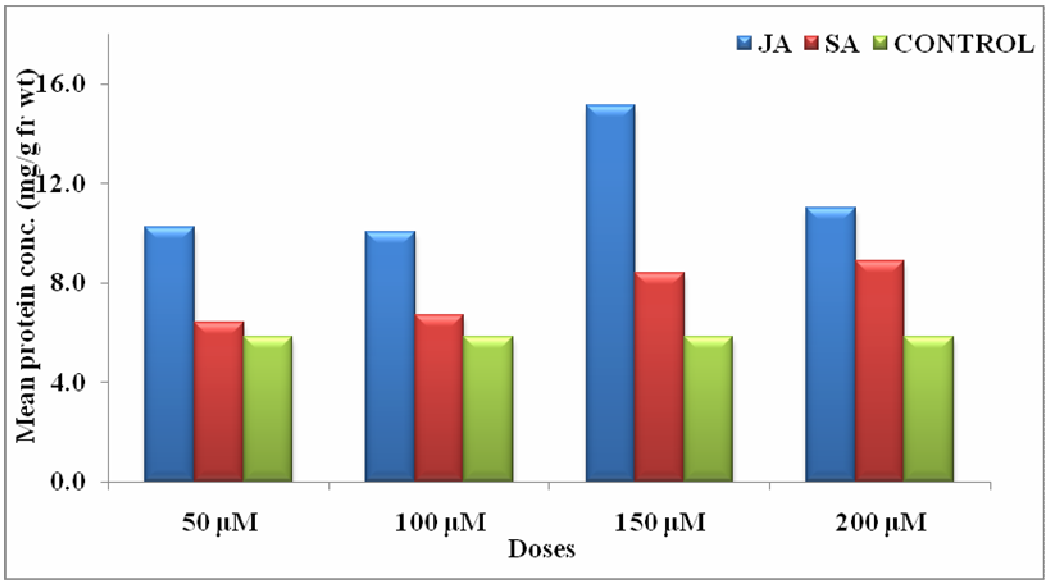
Statistically significant differences in protein concentration at different doses of each treatment were observed as indicated in Fig 4.1. JA resulted in mean maximum protein content (15.1 mg/g fr. wt.) at 150 µM whereas SA resulted in mean maximum protein value (9.0 mg/g fr. wt.) at 200 µM of SA when compared with control value of protein (5.8 mg/g fr. wt.).

The effect of different treatments (JA and SA) at various time intervals on protein concentration (Fig 4.2) showed statistically significant difference for JA and SA. JA was

found to induce mean maximum protein (9.9, 10.3, 11.4, 12.2 and 14.2 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) respectively followed by SA showing mean maximum protein (7.0, 7.2, 7.4, 7.9 and 8.6 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) whereas mean minimum protein (4.3, 5.3, 6.2, 6.4, and 7.0 mg/g fr. wt.) was observed for control at all the respective time intervals. At a week interval mean maximum protien (14.2 mg/g fr. wt.) was recorded for JA and (8.6 mg/g fr. wt.) for SA.

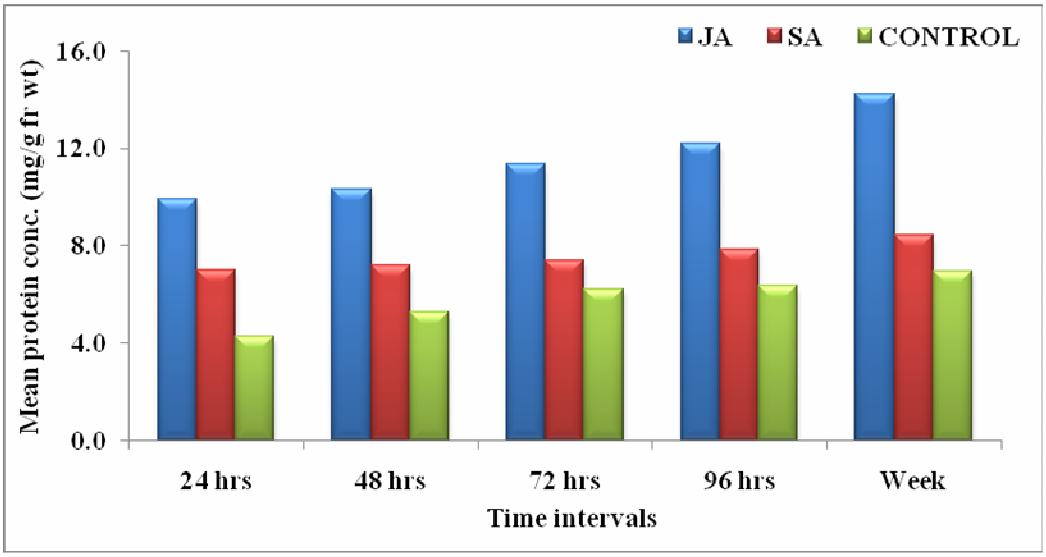
Table 4.1 Effect of different doses of JA and SA on leaf protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. RS 921 recorded at periodic time intervals

Dose	Treatment	Time interval					Treatment mean	Over all mean
		24 hrs	48 hrs	72 hrs	96 hrs	Week		
50 µM	JA	9.0	9.5	9.9	10.3	12.6	10.3	7.5
	SA	5.7	6.0	6.2	6.7	7.4	6.4	
	Water	4.3	5.3	6.2	6.4	7.0	5.8	
100 µM	JA	9.2	9.3	9.5	10.4	11.7	10.0	7.6
	SA	6.3	6.5	6.7	6.8	7.3	6.7	
	Water	4.3	5.3	6.2	6.4	7.0	5.8	
150 µM	JA	11.6	12.4	15.5	16.3	19.9	15.1	9.8
	SA	8.0	8.1	8.2	8.5	9.3	8.4	
	Water	4.3	5.3	6.2	6.4	7.0	5.8	
200 µM	JA	9.9	10.0	10.6	11.8	12.8	11.0	8.6
	SA	8.1	8.4	8.6	9.4	10.6	9.0	
	Water	4.3	5.3	6.2	6.4	7.0	5.8	
Overall mean		**JA** = 11.6, **SA** = 7.6, **Water** = 5.8						
LSD (0.05)		**Dose (A)** = 0.056, **Treatment (B)** = 0.048, **Time-interval (C)** = 0.062 **(A)(B)** = 0.097, **(A)(C)** = 0.12, **(B)(C)** = 0.10						



*Each value is mean of values of protein concentration at different doses of each treatment at respective time interval

Fig 4.1 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. RS 921



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

Fig 4.2 Effect of various treatments of JA ad SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. RS 921 at different time intervals

4.1.2 *Gossypium hirsutum* cv. LH 2076

The data pertaining to changes in protein concentration recorded at periodical intervals till a week in response to various doses of JA and SA i.e 50 µM, 100 µM, 150 µM and 200 µM revealed statistically significant difference amongst the various doses applied (Table 4.2). Mean maximum overall protein induction was observed at 150 µM i.e 8.9 mg/g fr. wt. followed by 8.5 mg/g fr. wt. at 200 µM and mean minimum protein 7.4 mg/g fr. wt. at 50 µM irrespective of treatment applied. Amongst the treatments (JA and SA) applied JA caused 1.6 fold increase in protein content in leaves of cv LH 2076 whereas SA resulted in 1.1 fold increase in protein concentration w.r.t to control indicating that JA is a better inducer of proteins.

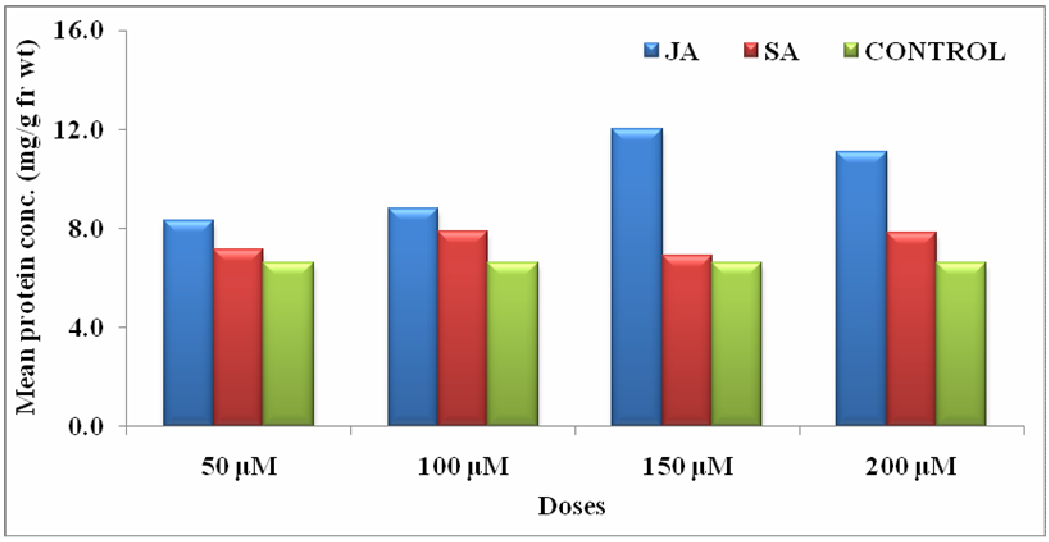
Statistically significant differences in protein concentration at different doses of each treatment were observed as indicated in Fig 4.3. JA resulted in mean maximum protein content (13.1 mg/g fr. wt.) at 150 µM whereas SA resulted in mean maximum protein value (8.0 mg/g fr. wt.) at 200 µM of SA when compared with control value of protein (6.6 mg/g fr. wt.).

The effect of different treatments (JA and SA) at various time intervals on protein concentration (Fig 4.4) showed statistically significant difference for JA and SA. JA was found to induce mean maximum protein (8.9, 9.3, 9.8, 11.0 and 12.7 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) respectively followed by SA showing mean maximum protein (6.6, 7.0, 7.5, 8.1 and 8.5 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) whereas mean minimum protein (6.0, 6.3, 6.5, 6.6 and 7.6 mg/g fr. wt.) was observed for control at all the respective time intervals. At a week interval mean maximum protien (12.7

mg/g fr. wt.) was recorded for JA and (8.6 mg/g fr. wt.) for SA.

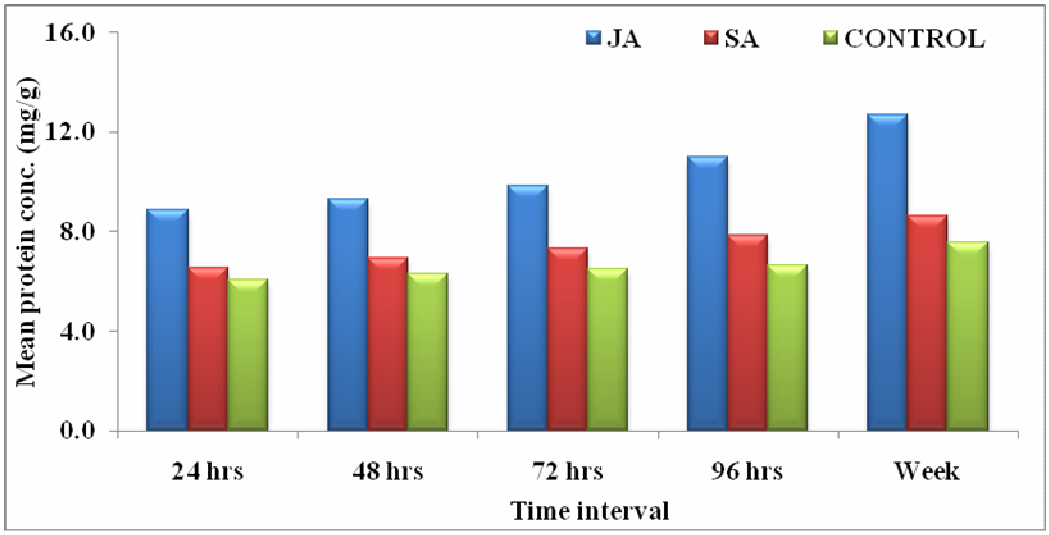
Table 4.2 Effect of different doses of JA and SA on leaf protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. LH 2076 recorded at periodic time intervals.

Dose	Treatment	Time interval					Treatment mean	Over all mean
		24 hrs	48 hrs	72 hrs	96 hrs	Week		
50 µM	JA	7.2	7.5	8.1	8.5	10.3	8.3	7.4
	SA	6.6	6.8	7.1	7.5	7.9	7.2	
	Water	6.0	6.3	6.5	6.6	7.6	6.6	
100 µM	JA	8.5	8.7	8.8	8.9	9.0	8.8	7.7
	SA	6.7	7.2	7.6	8.0	8.3	7.6	
	Water	6.0	6.3	6.5	6.6	7.6	6.6	
150 µM	JA	11.0	11.9	12.7	13.3	16.7	13.1	9.1
	SA	6.6	7.0	7.5	8.0	8.7	7.6	
	Water	6.0	6.3	6.5	6.6	7.6	6.6	
200 µM	JA	8.8	9.0	9.8	13.3	14.7	11.1	8.6
	SA	6.8	7.1	7.9	8.9	9.3	8.0	
	Water	6.0	6.3	6.5	6.6	7.6	6.6	
Overall mean		**JA** = 10.3, **SA** = 7.4, **Water** = 6.6						
LSD (0.05)		**Dose (A)** = 0.039, **Treatment (B) =** 0.034, **Time interval (C) = 0.04** **(A)(B)** = 0.069, **(A)(C)** = 0.089, **(B)(C)** = 0.077						



*Each value is mean of values of protein concentration at different doses of each treatment at respective time interval

Fig 4.3 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. LH 2076



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

Fig 4.4 Effect of various treatments of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. LH 2076 at different time interval

4.1.3 *Gossypium hirsutum* cv. PIL 8

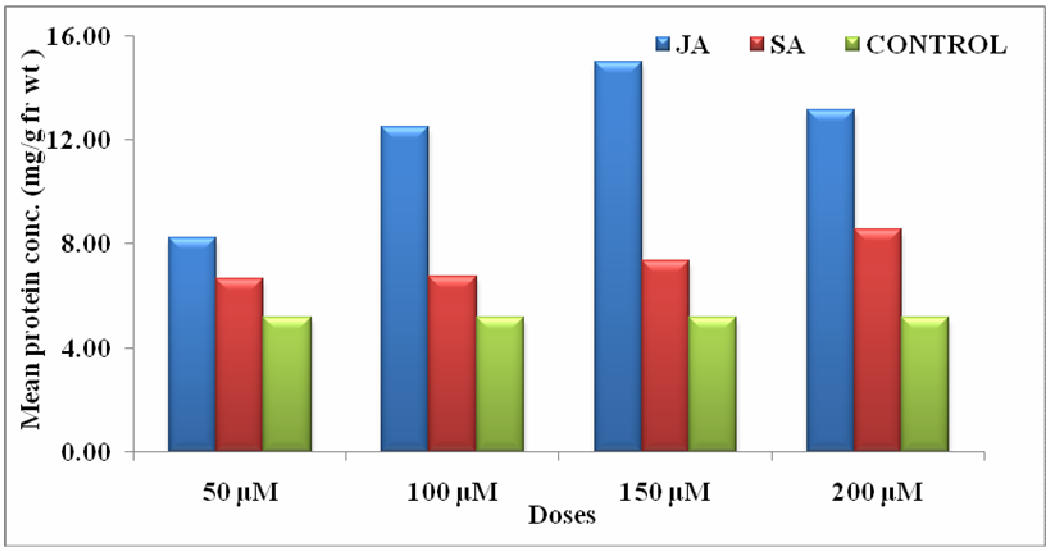
The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to various doses of JA and SA i.e 50 µM, 100 µM, 150 µM and 200 µM revealed statistically significant difference amongst the various doses applied (Table 4.3). Mean maximum overall protein induction was observed at 150 µM i.e 9.2 mg/g fr. wt. followed by 9.0 mg/g fr. wt. at 200 µM and mean minimum protein 6.7 mg/g fr. wt. at 50 µM irrespective of treatment applied. Amongst the treatments applied JA caused 2 fold increase in protein content in leaves of cv PIL 8 whereas SA resulted in 1.2 fold increase in protein concentration w.r.t to control indicating that JA is a better inducer of proteins.

Statistically significant differences in protein concentration at different doses of each treatment were observed as indicated in Fig 4.5. JA resulted in mean maximum protein content (15.0 mg/g fr. wt.) at 150 µM whereas SA resulted in mean maximum protein value (8.6 mg/g fr. wt.) at 200 µM of SA when compared with control value of protein (5.1 mg/g fr. wt.).

The effect of different treatments (JA and SA) at various time intervals on protein concentration (Fig 4.6) showed statistically significant difference for JA and SA. JA was found to induce mean maximum protein (10.5, 11.3, 11.8, 12.7 and 14.7 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) respectively followed by SA showing mean maximum protein (6.7, 6.9, 7.2, 7.5 and 8.2 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) whereas mean minimum protein (3.3, 4.3, 5.8, 6.2 and 6.3 mg/g fr. wt.) was observed for control at all the respective time intervals. At a week interval mean maximum protien (14.7 mg/g fr. wt.) was recorded for JA and (8.2 mg/g fr. wt.) for SA.

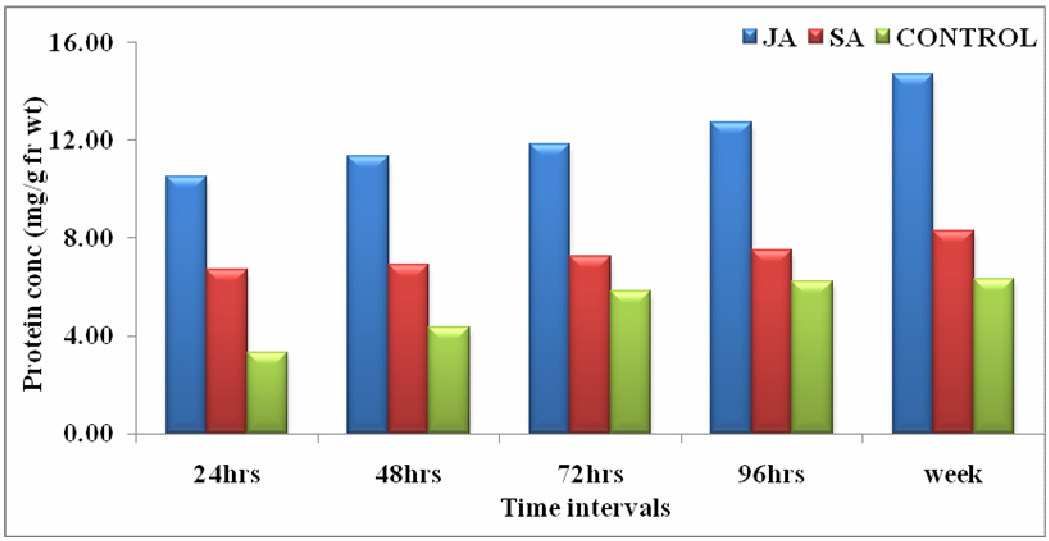
Table 4.3 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. PIL 8 recorded at periodic time intervals.

Dose	Treatment	Time interval					Treatment mean	Over all mean
		24 hrs	48 hrs	72 hrs	96 hrs	Week		
50 µM	JA	6.9	7.6	8.2	8.7	9.9	8.3	6.7
	SA	6.3	6.4	6.7	6.7	7.3	6.7	
	Water	3.3	4.3	5.8	6.2	7.0	5.2	
100 µM	JA	11.7	12.0	12.3	12.7	13.9	12.5	8.1
	SA	6.5	6.6	6.7	6.9	7.3	6.7	
	Water	3.3	4.3	5.8	6.2	7.0	5.2	
150 µM	JA	11.9	13.3	14.1	16.1	19.6	15.0	9.2
	SA	6.7	6.9	7.2	7.7	8.2	7.3	
	Water	3.3	4.3	5.8	6.2	7.0	5.2	
200 µM	JA	11.6	12.4	12.9	13.4	15.4	13.1	9.0
	SA	7.3	7.6	8.4	8.8	9.9	8.6	
	Water	3.3	4.3	5.8	6.2	7.0	5.2	
Overall mean		**JA** = 12.2, **SA** = 7.3, **Water** = 5.9						
LSD (0.05)		**Dose (A)** = 0.04, **Treatment (B)** = 0.03, **Time interval (C)** = 0.05 **(A)(B)** = 0.07, **(A)(C)** = 0.1, **(B)(C)** = 0.08						



*Each value is mean of values of protein concentration at different doses of each treatment at respective time interval

Fig 4.5 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. PIL 8



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

Fig 4.6 Effect of various treatments of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. PIL 8 at different time intervals

4.1.4 *Gossypium hirsutum* cv. Ankur 3028 BGII

The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to various doses of JA and SA i.e 50 µM, 100 µM, 150 µM and 200 µM revealed statistically significant difference amongst the various doses applied (Table 4.4). Mean maximum overall protein induction was observed at 150 µM i.e 8.8 mg/g fr. wt. followed by 8.4 mg/g fr. wt. at 200 µM and mean minimum protein 7.4 mg/g fr. wt. at 50 µM irrespective of treatment applied. Amongst the treatments applied JA caused 1.6 fold increase in protein content in leaves of cv Ankur 3028 BGII whereas SA resulted in 1.2 fold increase in protein concentration w.r.t to control indicating that JA is a better inducer of proteins.

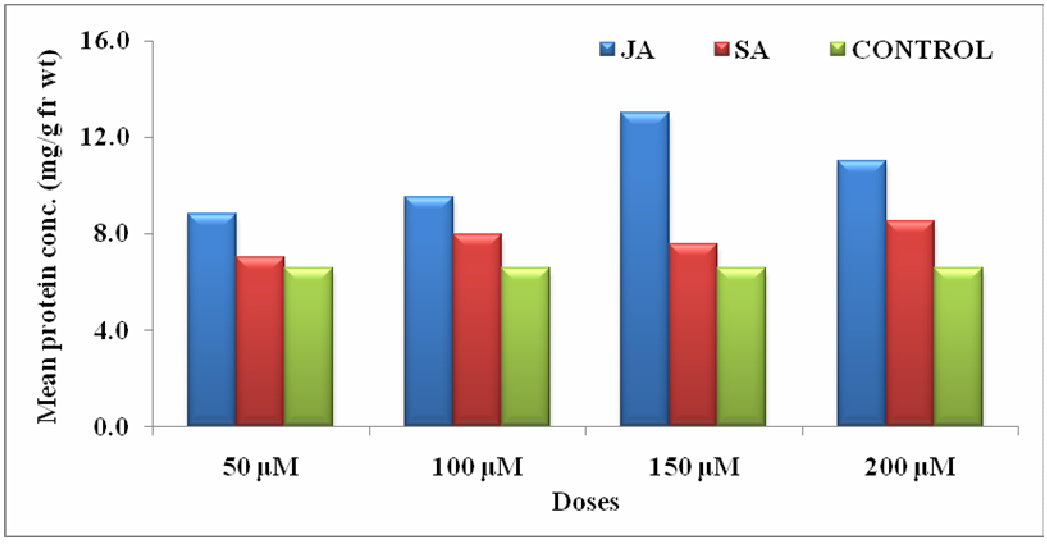
Statistically significant differences in protein concentration at different doses of each treatment were observed as indicated in Fig 4.5. JA resulted in mean maximum protein content (12.0 mg/g fr. wt.) at 150 µM whereas SA resulted in mean maximum protein value (8.5 mg/g fr. wt.) at 200 µM of SA when compared with control value of protein (6.5 mg/g fr. wt.).

The effect of different treatments (JA and SA) at various time intervals on protein concentration (Fig 4.6) showed statistically significant difference for JA and SA. JA was found to induce mean maximum protein (8.5, 9.1, 9.4, 10.7 and 12.8 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) respectively followed by SA showing mean maximum protein (6.9, 7.2, 7.6, 8.2 and 8.9 mg/g fr. wt.) at (24, 48, 72, 96 hrs and at a week interval) whereas mean minimum protein (5.9, 6.0, 6.3, 7.0 and 7.5 mg/g fr. wt.) was observed for control at all the respective time intervals. At a week interval mean maximum protien (12.8

mg/g fr. wt.) was recorded for JA and (8.9 mg/g fr. wt.) for SA.

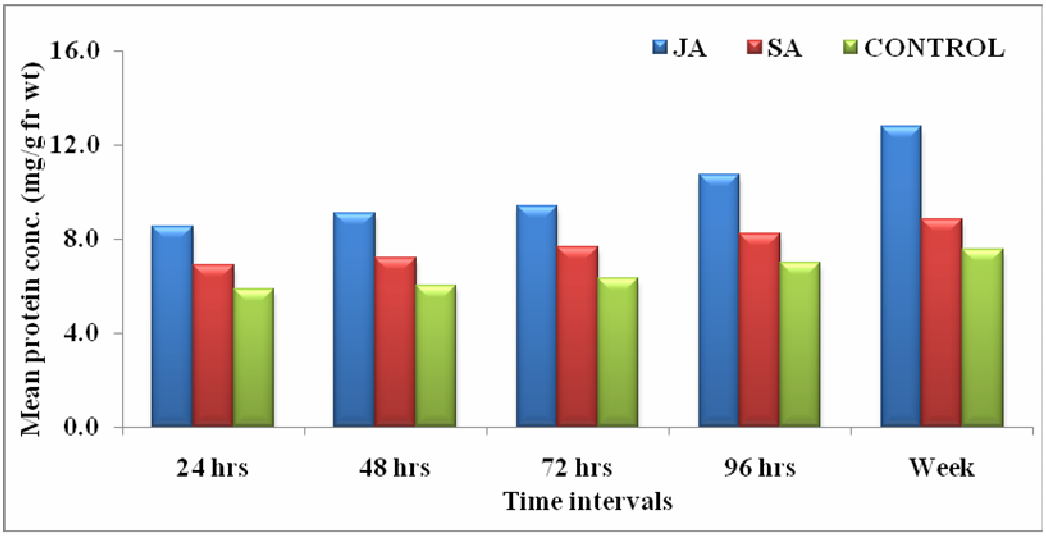
Table 4.4 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. Ankur 3028 BGII recorded at periodic intervals

Dose	Treatment	Time interval					Treatment mean	Over all mean
		24 hrs	48 hrs	72 hrs	96 hrs	Week		
50 µM	JA	7.1	7.9	8.0	9.0	12.0	8.8	7.4
	SA	6.0	6.2	6.5	7.7	8.6	7.0	
	Water	5.9	6.0	6.3	7.0	7.5	6.5	
100 µM	JA	9.0	9.2	9.5	9.9	10.0	9.5	8.0
	SA	6.6	6.9	7.6	7.9	8.8	7.6	
	Water	5.9	6.0	6.3	7.0	7.5	6.5	
150 µM	JA	9.6	10.8	11.1	12.6	15.0	12.0	8.8
	SA	7.0	7.5	7.9	8.3	9.0	7.9	
	Water	5.9	6.0	6.3	7.0	7.5	6.5	
200 µM	JA	8.4	8.6	8.8	11.4	14.0	10.2	8.4
	SA	8.0	8.1	8.5	8.9	9.0	8.5	
	Water	5.9	6.0	6.3	7.0	7.5	6.5	
Overall mean		**JA** = 10.0, **SA** = 7.8, **Water** = 6.5						
LSD (0.05)		**Dose (A)** = 0.033, **Treatment (B)** = 0.029, **Time interval (C)** = 0.037 **(A)(B)** = 0.058, **(A)(C)** = 0.075, **(B)(C)** = 0.065						



*Each value is mean of values of protein concentration at different doses of each treatment at respective time interval

Fig 4.7 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. Ankur 3028 BGII



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

Fig 4.8 Effect of various treatments of JA and SA on protein concentration (mg/g fr. wt.) of *G. hirsutum* cv. Ankur 3028 BGII at different time intervals

4.1.5 *Gossypium arboreum* cv. LD 694

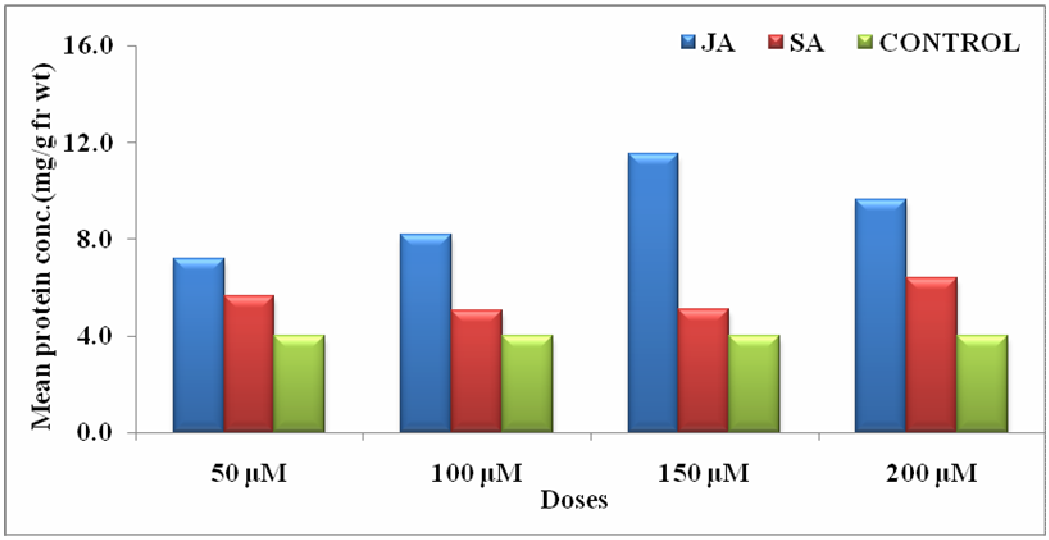
The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to various doses of JA and SA i.e 50 µM, 100 µM, 150 µM and 200 µM revealed statistically significant difference amongst the various doses applied (Table 4.5). Mean maximum overall protein induction was observed at 150 µM i.e 6.9 mg/g fr. wt. followed by 6.7 mg/g fr. wt. at 200 µM and mean minimum protein 5.6 mg/g fr. wt. at 50 µM irrespective of treatment applied. Amongst the treatments applied JA caused 2.3 fold increase in protein content in leaves of cv LD 694 whereas SA resulted in 1.4 fold increase in protein concentration w.r.t to control indicating that JA is a better inducer of proteins.

Statistically significant differences in protein concentration at different doses of each treatment were observed as indicated in Fig 4.9. JA resulted in mean maximum protein content (11.5 mg/g fr. wt.) at 150 µM whereas SA resulted in mean maximum protein value (6.4 mg/g fr. wt.) at 200 µM of SA when compared with control value of protein (4.0 mg/g fr. wt.).

The effect of different treatments (JA and SA) at various time intervals on protein concentration (Fig 4.10) showed statistically significant difference for JA and SA. JA was found to induce mean maximum protein (7.6, 8.4, 9.2, 9.7 and 10.7 mg/g fr. wt. at 24, 48, 72, 96 hrs and at a week interval respectively followed by SA showing mean maximum protein (4.7, 5.0, 5.3, 5.9 and 6.8 mg/g fr. wt.) at (24, 48, 72, 96hrs and at a week interval) whereas mean minimum protein (3.6, 3.7, 3.8, 3.9 and 5.0 mg/g fr. wt.) was observed for control at all the respective time intervals. At a week interval mean maximum protien (10.7 mg/g fr. wt.) was recorded for JA and (6.8 mg/g fr. wt.) for SA.

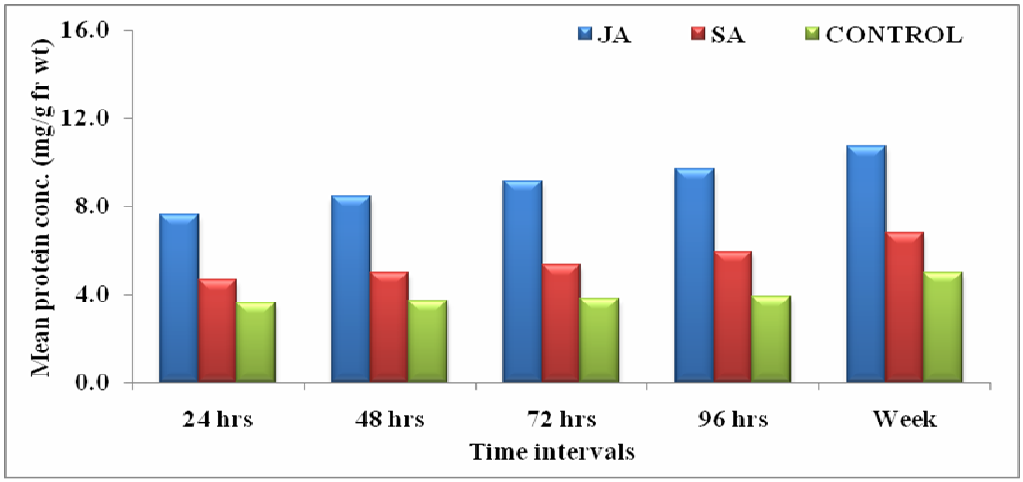
Table 4.5 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. arboreum* cv. LD 694 recorded at periodic intervals

Dose	Time interval						Treatment mean	Over all mean
	Treatment	24 hrs	48 hrs	72 hrs	96 hrs	Week		
50 µM	JA	6.3	6.9	7.1	7.5	8.0	7.2	5.6
	SA	5.0	5.3	5.6	6.0	6.3	5.6	
	Water	3.6	3.7	3.8	3.9	5.0	4.0	
100 µM	JA	7.2	7.7	8.3	8.8	9.0	8.2	5.8
	SA	3.9	4.3	4.6	5.6	6.9	5.1	
	Water	3.6	3.7	3.8	3.9	5.0	4.0	
150 µM	JA	9.7	10.3	11.2	12.0	14.4	11.5	6.9
	SA	4.4	4.6	5.1	5.5	5.7	5.1	
	Water	3.6	3.7	3.8	3.9	5.0	4.0	
200 µM	JA	7.3	8.8	10.0	10.4	11.5	9.6	6.7
	SA	5.4	5.7	6.0	6.6	8.1	6.4	
	Water	3.6	3.7	3.8	3.9	5.0	4.0	
Overall mean		**JA** = 9.1, **SA** = 5.5, **Water** = 4.0						
LSD (0.05)								
Dose (A) =0.041, **Treatment (B)** = 0.036, **Time interval** (C) = 0.046								
(A)(B) = 0.072, **(A)(C)** = 0.093, **(B)(C)** = 0.08								



*Each value is mean of values of protein concentration at different doses of each treatment at respective time interval

Fig 4.9 Effect of different doses of JA and SA on protein concentration (mg/g fr. wt.) of *G. arboreum* cv. LD 694



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

Fig 4.10 Effect of various treatments of JA and SA on protein concentration (mg/g fr. wt.) of *G. arboreum* cv. LD 694 at different time intervals

4.1.6 Comparative analysis of induction of proteins in different cultivars w.r.t to treatment with JA (150µM) and SA (200µM)

At 150 µM of JA and 200 µM of SA highest protein induction was observed in all the treated cultivars as compared to their controls. Comparative analysis of mean protein induction in different cotton cultivars treated with 150 µM of JA and 200 µM SA after a week interval as shown in Fig 4.11 revealed highest protein induction in cv RS 921 and PIL 8 followed by Ankur 3028 BGII and LH 2076. Similar results were obtained when cotton cultivars were treated with 200 µM of SA. Treatment with 150 µM of JA resulted in 15.1, 15.0, 13.1, 12.0 and 11.5 mg/g fr. wt. protein in RS 921, PIL 8, LH 2076, Ankur 3028 BGII and LD 694 whereas 200µM of SA resulted in 9.0, 8.6, 8.0, 8.5 and 6.4 mg/g fr. wt. protein in RS 921, PIL 8, LH 2076, Ankur 3028 BGII and LD 694 respectively as compared to protein values in control which were 5.8, 5.2, 6.6, 6.5 and 4.0 mg/g fr. wt..

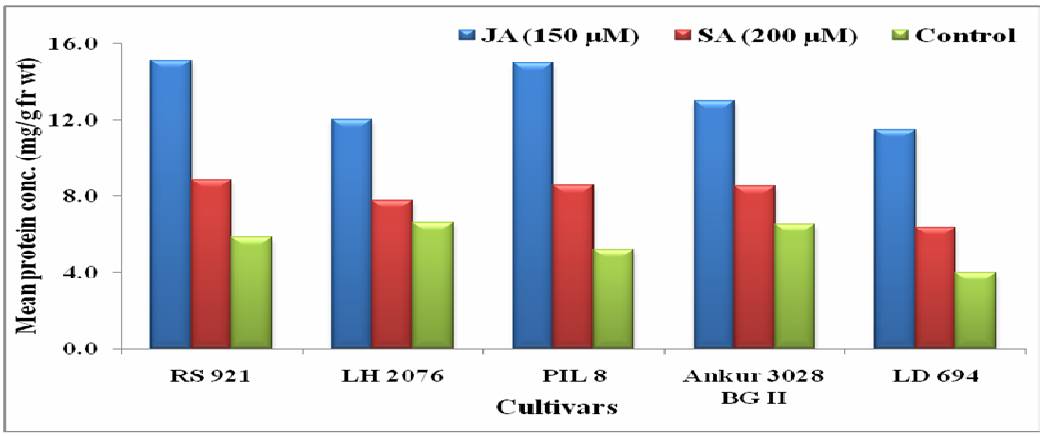


Fig 4.11 Comparative protein content in different cotton cultivars w.r.t 150 µM of JA and 200 µM of SA after a week interval

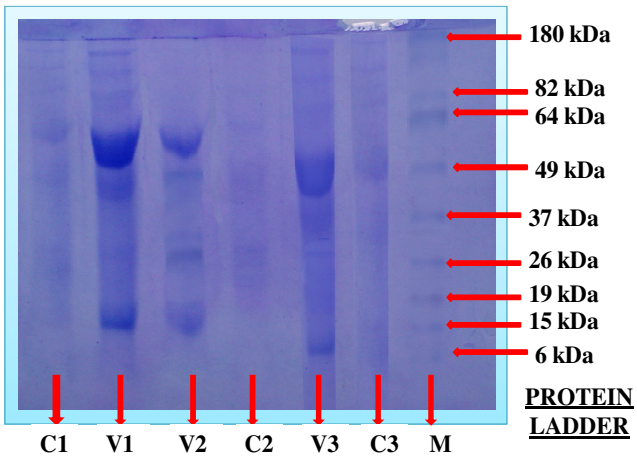
Work done by different research workers fully support the induction of proteins in different crops other than cotton. Formation of JA induced proteins (JIP) and Pathogenesis

related proteins (PR) was reported by Schweizer *et al* (1993) in barley *Hordeum vulgare* L. with the application of Jasmonic acid. With the application of MeJA in melon seedlings induction of various PR proteins was reported by Buji *et al* (2004). Yuan Jiao *et al* (2007) also indicated the induction of PR proteins by the application of JA in Bt Corn (*Zea mays*) and also found increase in Bt proteins concentration in the treated plants. It was found by Jing shi *et al* (2010) that exogenous application of JA and SA resulted in the induction of proteins in transgenic *Nicotiana benthamiana* with the expression of GhMPK7 gene. Haggag *et al* (2010) in his work reported that MeJA application on to the Beet mosaic virus (BtMV) infected sugarbeet plants resulted in the accumulation of total soluble proteins, chitinases etc which belong to various PR families. Yamada *et al* (2012) also reported that treatment of rice plants with jasmonates results in the induction of OsJAZ8 protein.

Exogenous application of SA also showed induction of various proteins in different crops. For the first time White (1979) reported the accumulation of PR proteins in tobacco plants which were treated with SA. SA and its structural analogue BTH are successfully used in inducing protection against various plant viruses like Tobacco mosaic virus (TMV) in tobacco, Turnip crinkle virus (TCV) in *Arabidopsis* (Lawton 1996), and Cucumber mosaic virus (CMV) in tomato (Anfoka 2000). Similar, results were observed in case of SA as reported by Guleria *et al* (2001) in peas (*Pisum sativum*) accumulation of PR proteins was reported by the application of SA.

4.5 Electrophoretic study of different cotton cultivars treated with JA and SA

The cotton leaf proteins extracted by the procedure of 3.1.5 in materials and methods were subjected to SDS-PAGE electrophoresis. Leaf samples of different cultivars namely RS 921, LH 2076, PIL 8, Ankur 3028 BGII and desi cotton variety LD 694 showed maximum protein induction at 150 µM of JA and 200 µM of SA for which SDS-PAGE was carried out to study the protein profile of different cotton cultivars. Total leaf proteins were resolved with molecular weights ranging from 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 (PLATE 2 and PLATE 3). It is known that PR proteins fall under the range of 15.8 kDa to 45 kDa (Van Loon *et al* 1982).Work presented by different workers fully support the finding of PR protein induction by the application of SA and JA in different crops. Van Loon *et al* (1982) and Bol *et al* (1990) reported the induction of various PR proteins of molecular size ranging from 15.8 - 45 kDa with the application of SA in Tobacco plants. Ding *et al* (2002) reported that pre-treatment of tomato fruit with MeJA induces the synthesis of PR proteins such as PR-2b, PR-2a, PR-3b etc. Jishan *et al* (2011) also revealed the induced expression of defense related genes like PR 2, PR 3, PR 5, PR 10 and Ta-JA2 which encode β,1-3 glucanase, chitinase, thaumatin-like protein, peroxidase etc. in different wheat cv. Chinese Spring, Pumai 9 and Zhoumai 18 with MeJA treatment. Thus, it shows that



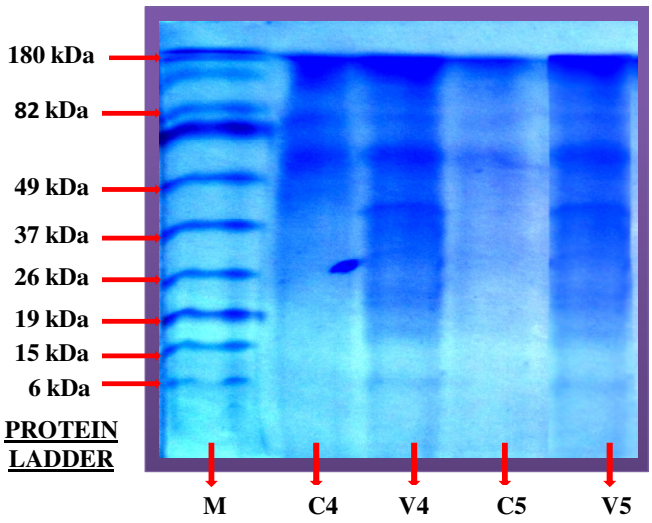


PLATE 2: SDS-PAGE of leaf proteins of different cotton cultivars at 150 µM of JA

M- Protein Ladder (6 kDa- 180 kDa), V1- Ankur 3028 BGII, V2- LH 2076, V3- LD 694, V4- RS 921, V5- PIL 8;
C1, C2, C3, C4, C5 represent control of Ankur 3028 BGII, LH 2076, LD 694, RS 921 and PIL 8 cultivars

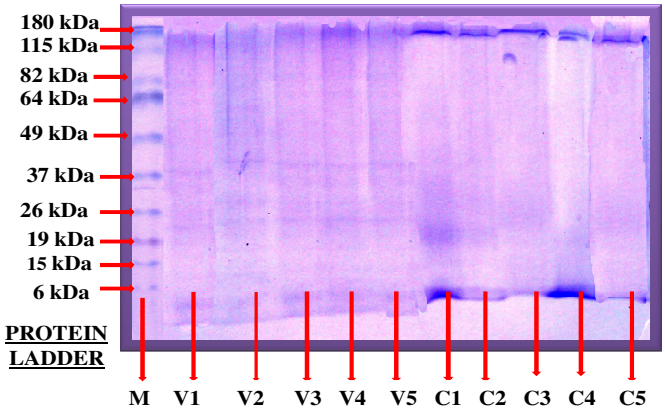


PLATE 3: SDS-PAGE of leaf proteins of different cotton cultivars at 200 µM of SA

M- Protein Ladder (6 kDa- 180 kDa), V1- PIL 8, V2- RS 921, V3- LH 2076, V4- Ankur 3028 BGII, V5- LD 694;

C1, C2, C3, C4, C5 – represent control of PIL 8, RS 921, LH 2076, Ankur 3028 BGII and LD 694 cultivars

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 all the cotton cultivars under treatment.

4.6 Effect of different doses of JA and SA on CLCuD incidence, severity and phytotoxicity

Different doses (50, 100, 150 and 200 µM) of JA and SA were found to affect the CLCuD incidence and severity. At 50 µM concentration of JA and SA no disease incidence was observed in cultivar PIL 8 and LD 694 at 14, 21, 28 days after spray (DAS) whereas LH 2076 and Ankur 3028 BGII showed disease incidence 21, 28 DAS which was found to be higher as compared to control and in case of highly susceptible cv RS 921 disease incidence and severity was found to be highest at all the time intervals (14, 21 and 28 DAS) Similar, results were obtained with moderate doses (100 µM) of JA and SA Table 4.6 and 4.7 clearly indicates higher disease incidence and severity with the application of SA followed by JA as compared to check. But comparatively, at 100 µM of JA and SA disease incidence and severity was lower as compared to the incidence and severity values recorded at 50 µM of JA and SA. This higher incidence and disease severity with the application of lower doses of JA and SA is due to the fact that as these chemicals are known to affect various physiological and biochemical activities of plants and may play a key role in regulating growth and development (Arberg 1981). Khan *et al* (2003) reported that SA and its analogues enhanced leaf area and dry mass production in corn and soybean. Fariduddin *et al* (2005) reported increase in dry matter accumulation in *Brassica juncea* with lower doses of SA and JA. In an other study Khodary *et al* (2004) observed a significant increase in growth characteristics, carbohydrate content, pigment content, photosynthetic rate in maize plant sprayed with lower doses of SA. Increase in mass of plantelets was reported by Shakirova (2007). This increase in leaf area, dry matter, carbohydrate content, photosynthetic rate affected the infestation rate of viruliferous whiteflies resulting in more disease incidence and disease severity as compared to plants in check.

Table 4.6 Effect of JA and SA at a dose of 50 µM on disease incidence and severity of CLCuD

Cultivar	Disease Incidence (%) Dose @ 50 µM									Disease Index (%)		
	14 *DAS			21 DAS			28 DAS					
	Control	JA	SA	Control	JA	SA	Control	JA	SA	Control	JA	SA
RS 921	20	0	33	40	37	83	50	62	100	78	75	100
LH 2076	0	0	0	20	35	40	46	50	62	65	63	75
PIL 8	0	0	0	0	0	0	0	0	0	0	0	0
Ankur 3028 BGII	0	0	0	28	15	42	45	45	50	63	68	85
LD 694	0	0	0	0	0	0	0	0	0	0	0	0

*DAS - Days after spray

Table 4.7 Effect of JA and SA at a dose of 100 µM on disease incidence and severity of CLCuD

Cultivar	Disease Incidence (%)									Disease Index (%)		
	Dose @ 100µM											
	14 *DAS			21 DAS			28 DAS					
	Control	JA	SA	Control	JA	SA	Control	JA	SA	Control	JA	SA
RS 921	20	12	37	40	35	62	50	50	75	78	63	70
LH 2076	0	0	0	20	16	37	46	45	62	65	60	67
PIL 8	0	0	0	0	0	0	0	0	0	0	0	0
Ankur 3028 BGII	0	0	0	28	25	38	45	45	56	63	60	65
LD 694	0	0	0	0	0	0	0	0	0	0	0	0

*DAS - Days after spray

Table 4.8 shows the effect of JA and SA applied at a concentration of 150 µM on the incidence and severity of CLCuD. At 150 µM of JA and SA disease incidence and severity values were found to be lower as compared to check in cultivars RS 921, LH2076 and Ankur 3028 BGII. Disease incidence was 37%, 30%, 30% and disease index was 48%, 40%, 40% in RS 921, LH 2076, Ankur 3028 BGII at 150 µM concentration of JA which was 50%, 46%, 45% and 78%, 65%, 63% when compared with disease incidence and disease index values at control. No disease was observed in PIL 8 and LD 694 cotton cultivars.

Table 4.9 shows the effect of 200 µM of JA and SA which also indicated lower disease incidence and severity as compared to check. At 200 µM SA disease incidence was 48%, 36%, 34% and disease index was 57%, 50%, 50% in RS 921, LH 2076, Ankur 3028 BGII which was 50%, 46%, 45% and 78%, 65%, 63% when compared with disease incidence and disease index values at control.

Table 4.8 Effect of JA and SA at a dose of 150 µM on disease incidence and severity of CLCuD.

Cultivar	Disease Incidence (%)									Disease Index (%)		
	Dose @ 150 µM											
	14 *DAS			21 DAS			28 DAS					
	Control	JA	SA	Control	JA	SA	Control	JA	SA	Control	JA	SA
RS 921	20	0	37	40	27	50	50	37	75	78	48	60
LH 2076	0	0	0	20	25	37	46	30	62	65	40	53
PIL 8	0	0	0	0	0	0	0	0	0	0	0	0
Ankur 3028 BGII	0	0	0	28	20	33	45	30	55	63	40	50
LD 694	0	0	0	0	0	0	0	0	0	0	0	0

*DAS - Days after spray

Table 4.9 Effect of JA and SA at a dose of 200 µM on disease incidence and severity of CLCuD.

Cultivar	Disease Incidence (%)									Disease Index (%)		
	Dose @ 200 µM											
	14 *DAS			21 DAS			28 DAS					
	Control	JA	SA	Control	JA	SA	Control	JA	SA	Control	JA	SA
RS 921	20	0	12	40	35	32	50	45	48	78	50	57
LH 2076	0	0	0	20	16	15	46	33	36	65	48	50
PIL 8	0	0	0	0	0	0	0	0	0	0	0	0
Ankur 3028 BGII	0	0	0	28	25	28	45	32	34	63	45	50
LD 694	0	0	0	0	0	0	0	0	0	0	0	0

*DAS - Days after spray

It was observed that 150 µM concentration of JA and 200 µM concentration of SA was most effective in lowering CLCuD incidence and severity which is found to be positively correlated with the amount of protein induced at these concentrations as at above mentioned concentration maximum protein induction was reported which was responsible in lowering the disease incidence. Thomma *et al* (1998) showed that application of JA resulted in the expression of PR-2a, PR-1 genes which induced PR proteins in the treated plants and imparted protection against *Alternaria brassicicola* in *Arabidopsis.* In an important work Van Loon (2006) reported that most PR proteins and related proteins gets induced through the action of signaling compounds like SA , JA, ethylene etc which play an important role in activating plants defense against various pathogens. Cohen *et al* (1993) found that foliar spray of JA and MeJA protected tomato and potato plants against *Phytophthora infestans* by enhancing the production of proteinase inhibitors (PI) and certain acid soluble proteins. In cowpea Chandra *et al* (2001) showed that foliar spray of 0.02 per cent of SA twice at a week interval caused reduction in root rot disease caused by *Rhizoctonia solani* by enhancing the production of proteins. Application of SA was also found to induce the synthesis of PR proteins in bean plants and thus protected it against various pathogens. Spraying Samsun NN tobacco with SA induced the production of PR-1 proteins which played role in inhibiting the multiplication of alfalfa mosaic virus (A1MV) (Huijsduijnen *et al* 1986).

Phytotoxicity

Observations were recorded for different parameters to detect phytotoxicity in different cotton cultivars when different doses of JA and SA (50, 100, 150 and 200 µM) were applied. Different parameters namely epinasty, hyponasty, vein clearing, yellowing, necrosis and wilting were recorded 21, 28, 35 days after treatment with JA and SA. No phytotoxicity was reported on different cotton cultivars at various concentrations of JA and SA application.

4.7 Detection of latent carry over of CLCuD

Leaf samples from symptomless plants of different cotton cultivars which were earlier treated with 150 µM of JA and 200 µM of SA at which maximum protein induction was

reported were collected to detect the presence/absence of β satellite DNA through PCR amplification using β primer. A major band at 600-700 bases was observed in case of all the cotton cultivars except LD 694 when sprayed with JA and SA which indicated the presence of virus in all the cotton cultivars except LD 694 which signifies that PR proteins does not eliminate virus as their presence was confirmed through PCR (Plate 4 and Plate 5)

The results are consistent with the findings of Briddon *et al* (2002), where a major band at 600-700 bases was observed. Further, amplification of genomic DNA extracted from healthy plants did not produce any amplicon. Sekhon and Chopra (2002) in their findings reported latent carry over of CLCuV in *G. hirsutum*. Similarly, these findings are also supported by Sabhiki (2004) confirmed the presence of latent infection of CLCuV in apparently disease free plants and healthy plants of resistant genotypes.

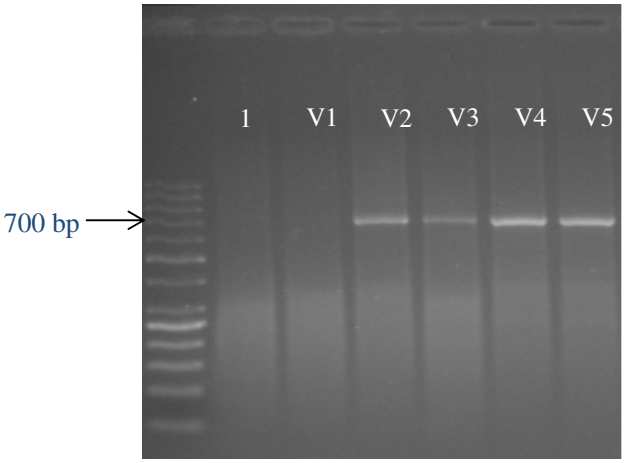


PLATE 4: Detection of latent carry over of CLCuD in symptomless plants of different cotton cultivars treated with 150 µM of JA through PCR based amplification using primer β

1- Negative control, V1- LD 694, V2- RS 921, V3- LH 2076, V4- PIL 8, V5- Ankur 3028 BGII

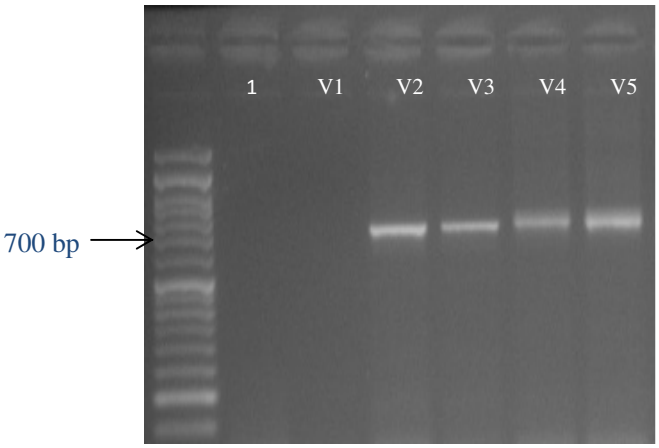


PLATE 5: Detection of latent carry over of CLCuD in symptomless plants of different cotton cultivars treated with 200 µM of SA through PCR based amplification using primer β

1- Negative control, V1- LD 694, V2- RS 921, V3- LH 2076, V4- PIL 8, V5- Ankur 3028 BGII

Chapter V

SUMMARY

American cotton (*Gossypium hirsutum* L.) is the predominantly cultivated cotton species in the world. India has the distinction of growing all the four cultivated cotton species and their interspecific hybrids. Cotton is grown in three distinct agro-climatic zones in the country. Punjab, Haryana and Rajasthan are the major North Indian cotton growing states which contribute substantially to the total cotton production and productivity of the country. However, the American cotton cultivation in these states is seriously threatened by the cotton leaf curl disease. CLCuD was first reported in 1912 from Nigeria then in Sudan in 1924 and subsequently from Tanzania in 1926. In India, the disease was first reported on *G. hirsutum* near Sriganganagar in Rajasthan in 1993. While in Punjab, it was observed at a few locations in Hindumal Kot area of Abohar, during 1994. In 1997, it assumed serious proportion and was observed in almost all the cotton growing areas of North India with a disease intensity touching as high as 98 per cent at some locations.

CLCuD is known to be caused by begomovirus belonging to family *Geminiviridae* and is transmitted by whitefly (*Bemicia tabaci*). The virus complex consists of DNA A and DNA β. DNA A is ~2.75 kb long circular single-stranded DNA molecule which actually causes CLCuD. DNA β is required by the virus for induction of typical symptoms of the CLCuD. Breakdown of resistance to CLCuD has necessitated the need to look for new approaches of disease control. So present study was planned with the objective of studying the role of Jasmonic acid (JA) and Salicylic acid (SA) (signaling molecules) in the induction of resistance to CLCuD in cotton, as these molecules are found to activate SAR in plants by resulting in induction of pathogenesis related (PR) proteins of various types.

Different American cotton cultivars namely RS 921, LH 2076, PIL 8, Ankur 3028 BGII and *G. arboreum* cv LD 694 were grown in pots under screen cages at Cotton Research Area, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during the year 2012-13. At four to six leaf stage potted plants of different cotton cultivars were treated with 50 µM, 100 µM, 150 µM, 200 µM of JA and SA whereas water sprayed plants of corresponding genotypes served as control. Quantification of proteins was done using Lowry method from the leaf samples collected 24, 48, 72, 96 hrs and a week after treatment with JA and SA. Application of JA and SA resulted in the induction of proteins 24 hrs after spray. After a week interval at 150 µM dose of JA mean maximum protein induction (19.9, 16.7, 19.6, 15.0 and 14.4 mg/g fr. wt.) as compared to protein values in control (7.0, 7.6, 7.0, 7.5 and 5.0 mg/g fr. wt.) in RS 921, LH 2076, PIL 8, Ankur 3028 BGII and LD 694 respectively. In case of SA, 200 µM dose showed maximum protein induction (9.8, 9.3, 9.9, 9.0 and 8.1 mg/g fr. wt.) as compared to protein concentration for contol (7.0, 7.6, 7.0, 7.5

and 5.0 mg/g fr. wt.) and in RS 921, LH 2076, PIL 8, Ankur 3028 BGII and LD 694 respectively. JA was found to be more effective than SA in the induction of proteins.

Electrophoretic study of protein pattern of cotton cultivars treated with 150 µM of JA and 200 µM of SA was carried out. Total leaf proteins were resolved in the molecular weights ranging from 6-180 kDa with respect to standard protein markers. Specific bands falling in the range of 6-49 kDa were reported in treated samples as compared to their respective control. It is known that PR proteins fall under the range of 15.5 kDa to 45 kDa which, signified that application of JA and SA in cotton resulted in PR protein induction along with some other molecular wt proteins.

Effect of different concentration of JA and SA was studied against CLCuD. Values for disease incidence was 37%, 30%, 30% and disease index was 48%, 40%, 40% in RS 921, LH 2076, Ankur 3028 BGII at 150 µM concentration of JA which was 50%, 46%, 45% and 78%, 65%, 63% when compared with disease incidence and disease index values at control. Whereas, at 200 µM SA disease incidence was 48%, 36%, 34% and disease index was 57%, 50%, 50% in RS 921, LH 2076, Ankur 3028 BGII at 200 µM which was 50%, 46%, 45% and 78%, 65%, 63% when compared with disease incidence and disease index values at control. It was observed that 150 µM dose of JA and 200 µM dose of SA was most effective in lowering CLCuD incidence and severity and which was found to be positively correlated with the amount of protein induced at these doses as at above mentioned concentration maximum protein induction was reported which was found to be responsible in lowering the disease incidence. JA was found to be more effective in lowering disease progress as compared to SA. For the latent carry over detection of symptomless plants treated with 150 µM of JA and 200 µM of SA *in-vitro* amplification in the thermal cycler was carried out using beta 1 (Forward 5´ ACCGTGGGCGAGCGGTGCCCGAT3´ and Reverse 5`CACG TGTAATACGTCTCCATCGTC 3`) which confirmed the presence of virus in all the tested cotton cultivars except LD 694 which signifies that PR proteins does not eliminate virus but it could be rightly said that application of JA and SA resulted in imparting tolerance with the induction of PR proteins but does not lead to complete resistance against the disease.

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