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PR-proteins Mediated Disease Resistance in Cotton Genotypes against Cotton Leaf Curl Disease Upon Pretreatment with Salicylic Acid and β-aminobutyric Acid

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Abstract: Cotton leaf curl disease, caused by the *Geminivirus* and transmitted by whitefly, is a major concern for cotton growers. This study observed the effect of resistance-inducing chemicals (RICs); salicylic acid (SA) and β-aminobutyric acid (BABA), on pathogen-related proteins, total soluble protein, disease incidence, and disease index. Both elicitors were applied @ 250 µM using two methods: seed priming (once) and foliar spray (thrice at 5, 19, and 33 days after germination) on three cotton cultivars showing differential responses: F1378 (susceptible), LH2076 (moderately resistant), and FDK124 (resistant). The activities of proteins such as chitinase (PR-3), β-1,3-glucanase (PR-2), peroxidase (PR-9), and total soluble protein were estimated at 7, 21, and 35 days after germination. These resistance-inducing chemicals work via systemic acquired resistance, an eco-safe technique providing long-term protection. Our results revealed that SA and BABA significantly increased PR-proteins' activities viz. chitinase, β-1,3-glucanase, peroxidase and total soluble protein content compared to the controls. Furthermore, treated plants showed a remarkable reduction in cotton leaf curl disease incidence and index compared to untreated plants. The higher activity of PR-proteins might be responsible for the decreased disease incidence and index in cotton cultivars. In addition, β-1,3-glucanase and chitinase negatively correlated with disease incidence and index, strongly indicating PR-proteins' role in the plant defense mechanism. A comparison between modes of elicitor treatment indicates seed priming to be the most effective as seed treatment given once sustained its effect up to 35 DAG whereas foliar spray in general gave better results. Although both elicitors seemed at par in their effectiveness, economy-wise SA will be the preferred elicitor. Therefore, SA treatment is suggested as an effective and eco-safe method to induce resistance against cotton leaf curl disease.

Keywords: β-aminobutyric acid, Cotton leaf curl disease, Salicylic acid, Systemic acquire resistance, Pathogen-related protein

Cotton is one of the most important cash crops and is also known as white gold. China, India, the United States, Pakistan, Türkiye, Brazil and Uzbekistan are major cotton-growing countries that contribute more than 84% of the total cotton production around the globe (Akın 2024). According to ICAC Journal 'Cotton This Month' in June 2023 the worldwide production of cotton was approximately 24.51 million tonnes (144.1 million bales) from 32.41 million hectares with productivity of 756 Kgs/hectare (Anonymous 2023). India holds 1st position in the world in cotton cultivation area of 12.7 million hectares with a production of 5.84 million tonnes and an average productivity of 447 Kgs/hectare (Anonymous 2023 and Keelery 2024). Cotton leaf curl disease (CLCuD), caused by a Geminivirus belonging to the family *Geminiviridae*, genus *Begomovirus*, is a severe and constant threat to cotton growers worldwide (Sattar et al., 2013). The DNA-1/DNA-A/DNA-β complex of Begomovirus is responsible for CLCuD. The whitefly's spreading vector is difficult to control due to the prevalence of multiple virulent viral strains or related species (Akhtar et al., 2010, Ullah et al., 2014, Zubair et al., 2017).

Several short-term (mostly management practices) and long-term approaches (developing resistant cotton varieties) were formulated to control this disease. The use of resistant varieties is the safest, most economical and most effective option to manage, but unfortunately, introgressed host plant resistance was rapidly overcome by the resistant breeding strain of CLCuBuV during 2005 and all the available cultivated genotypes from *G. hirsutum* were susceptible (Akhtar et al., 2010). This situation highlights the urgent need to explore new approaches to manage this devastating disease effectively.

Currently, several new strategies are being investigated to control various diseases for example use of various synthetic and biological compounds like 2,6-dichloroisonicotinic acid (INA), benzothiadiazole (BTH), salicylic acid (SA), jasmonic acid (JA) and β-aminobutyric acid (BABA) called resistance inducing chemicals (RICs). RICs can regulate numerous plant diseases by activating systemic acquired resistance (SAR) (Kumari et al., 2020 a,b). This mechanism involves activating the plant's resistance through chemical or biological agents, offering a non-conventional and eco-friendly approach to plant protection (Stockwell 2004, Raikhel and Pirrung 2005). SAR induces various PR-proteins such as PR-1, β-1,3-glucanase (PR-2), acidic and basic class III chitinases, hevein-like protein (PR-4), thaumatin-like or osmotin-like proteins (PR-5), among others, which function as antiviral, antifungal, antibacterial, insecticidal, and nematocidal agents (Ward et al., 1991). For instance, in TMV-infected tobacco plants, SAR genes code for various pathogenesis-related proteins, with β-1,3-glucanase and chitinase hydrolyzing the microorganism's cell wall made of glucan and chitin (Gozzo 2003). Peroxidase activation is crucial for cell wall reinforcement and serves as a marker of the induced state (Kumari et al., 2022). Numerous studies have demonstrated the effective use of RICs to trigger SAR and control viral diseases in plants (War et al., 2012, Gordy et al., 2015, Thakur et al., 2016, Raj et al., 2016, Kumari et al., 2020b). PR-proteins like β-1,3-glucanase and chitinase reduce TMV infection in tobacco plants (Sindelarova and Sindelar 2005, Kumari et al., 2020a).

Thus, keeping cognizance of the above, the current study, was undertaken to investigate the effects of different SA and BABA for induction of pathogenesis-related proteins against cotton leaf curl disease

MATERIAL AND METHODS

Experimental Design

The experiment was conducted on three cotton cultivars *viz*. susceptible (F1378) and moderately resistant (LH2076) and resistant (FDK124). The cotton seeds were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Before sowing seeds were treated with 250μM concentration of the different resistance-inducing chemicals *viz*, SA and BABA for seed priming. Followed by foliar spray of both SA and BABA with the same concentration was sprayed on three different periods 5, 9 and 21 days after germination (DAG). For control, seeds were imbibed in distilled H_2O for 6 hrs. Samples were collected at 7, 21 and 35 DAG for biochemical analysis. After 35 DAG plants were transferred to cages and viruliferous whiteflies were released on them @ six whiteflies/ plant and plants were disturbed two times a day for uniform and overall manifestation of CLCuD. The data on disease incidence and disease index was collected at 20, 30, and 40 days after infestation (DAI).

Extraction and Assay of Chitinase and □-1, 3-Glucanase

100mg leaf sample was crushed in a pre-chilled pestle and mortar using glass capillaries, in ice-cold 0.1 M sodium acetate buffer (pH 5.0 containing 2-mercaptoethanol). The sample extract was centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant obtained after centrifugation was used as an enzyme extract for the estimation of chitinase and □-1, 3-Glucanase activities. The standard procedure by Boller and Mauch (1988) and Kauffmann et al., (1987) was followed for the assay of Chitinase and □-1, 3-Glucanase.

Extraction and Assay of Peroxidase (POD)

Plant samples (100mg) were crushed in ice-cold potassium phosphate buffer (0.1 M, pH 7.5 containing 1 mM EDTA, 1% PVP and 10 mM of 2-mercaptoethanol) and centrifuged at 10,000 rpm for 30 min. The supernatant obtained was used for enzyme assay of peroxidase. All operations were done at 4 °C and assayed at 25 °C ± 1. Standard procedure of Claiborne and Fridovic (1979) was used for estimation of peroxidase.

Extraction and Assay of total soluble protein content

Standard procedure of Lowry et al., (1951) is used for estimation of total soluble protein.

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

Disease index (%)

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

Where,

N1= Number of plants in control

N2= Number of plants in test entry ,

S1= Sum of all infection grades in control

S2= Sum of all infection grades in test entry

Rating scale	Symptoms
0	Plants free from CLCuD
1	Thickening of small veins, only a 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 the underside of leaves
4	Thickening of veins, cupping, enations, stunting of plants and few bolls

The plants were graded according to the revised CLCuD scale described by AICCIP (Anonymous 2008) as given below.

Statistical Analysis

All the experiments were conducted in triplicates and presented as mean±SD. Means were compared by using Duncan's Multiple Range test at p≤ 0.05. All analysis was done by using two ways of analysis variance (ANOVA) by SPSS software version 23.

RESULTS AND DISCUSSION

The plant's defense mechanism is typically not based on only single biochemical components but depends upon the whole spectrum of biochemical components (Tripathi et al., 2019). Systemic acquired resistance (SAR) is a distinctive and vital mechanism because of its possible implication in terms of eco-friendly techniques providing long-duration protection to the plant (Shine et al., 2019). In the SAR mechanism plant's resistance mechanism is activated by prior treatment of chemical or biological agents known as resistance-inducing chemicals (RICs) (Akram and Anjum 2011, Gaikwad and Balgude 2016). Exogenous application of RICs for instance SA and BABA leads to induction of SAR against the cotton leaf curl disease. In the present investigation, treatment with BABA through seed priming resulted in a significant increase in PR-protein compared to control. The highest average increase of β-1, 3 glucanase activity through seed priming was observed in susceptible genotypes F1378 (3.0-fold) followed by resistant genotypes FDK 124 (1.7-fold) as compared with control plants. However, via foliar spray of BABA enzymes activity either increased or remained the same till 35 DAG. In contrast, maximum induction in β-1, 3 glucanase activity was recorded at 35 DAG in moderately resistant genotypes LH2076 (1.2-fold) than the control (Table 1). RICs such as SA, BABA, JA and other organic compounds were reported for induced resistance through the SAR mechanism when applied as seed priming, foliar spray and soil drench (Gordy et al., 2015, Thakur et al., 2016, Kumari et al., 2020b). BABA is also registered to persuade resistance against several environmental stresses and plant pathogens including viruses, bacteria and fungi when applied as seed imbibition and foliar spray (Jakab et al., 2001, Cohen 2010, Walters et al., 2013). The possible mechanism of BABA is explained by Wang et al. (2019) in grape barries against *Botrytis cinerea*, BABA caused early H_2O_2 burst which leads to the activation of various PR-proteins genes and accumulation of phytoalexins.

Treatment with SA via both modes that is seed treatment and foliar treatment exhibited a significant increase of β-1, 3 glucanase activity. The highest increase was observed in F1378 and FDK124 (1.6-fold) via seed priming. LH2076 and FDK124 (1.6-fold) showed the highest increment in enzyme activity with foliar spray as compared to control at all stages of plant development (Table 1). A previous study reported that exogenous application of SA and BABA via seed imbibition increases β-1,3-glucanase and chitinase activity in tomato plants providing defense against *Alternaria* leaf blight (Raut and Borkar 2014).

In current study application of RICs (BABA and SA) significantly increased the activity peroxidase in treated cotton plant compared to the untreated plants with both mode of application (Table 3). Seed priming with BABA exhibited highest significant average increase of peroxidase activity was in F1378 (1.6-fold) as compared to untreated plants. Similarly, a comparable result was recorded with foliar spray which exhibited maximum significant increase was recorded in F1378 (1.7-fold) whereas lowest increases were LH2076 (1.4-fold) with BABA treatment at all stage of stages. However, seed priming with SA showed a significant increase of peroxidase activity in FDK124 (2.0-fold) followed by F1378 (1.5-fold) via seed priming. However, FDK124 (1.5-fold) and LH2076 (1.4-fold) showed the highest average increase in POX activity via foliar spray compared to control plants at all stages of sampling (Table 3). Similar results were also obtained by Thakur et al., (2016), that is foliar application of SA resulted in induction of peroxidase, polyphenol oxidase, protease inhibitor and amylase inhibitor with JA and SA in brassica genotypesmight be help in strengthening of plant cell wall. Which is further negatively correlated with peroxidase and polyphenol oxidase leads reduction in aphid population. Peroxidases are involved in varied range of physiological processes such as strengthening of host plant cell walls by oxidation of phenolic compounds, lignification, suberization, auxins metabolism, wound healing associated with H_2O_2 decomposition against pathogens and insect pest (Pandey et al., 2017). Peroxidase is also registered to be a significant controller of various disease regulatory responses which are accompanying with SAR or HR by using RICs *viz*. INA, SA, and BTH through seed imbibition, foliar spray and soil drench (Baccelli and Mauch-Mani 2016, Kumari et al., 2020b).

Chitinase enzyme was induced either local or systemic after pathogen attack or with elicitors treatment which might be responsible for defence mechanism in plants against various pathogens. Chitinase activity was significantly upregulated with SA and BABA when seed priming and foliar spray were applied at different sampling stages (Table 2). Application of BABA through seed priming resulted in the highest average increase of chitinase activity in moderately resistant genotype LH2076 (2.9-fold) and resistant genotype FDK124 (1.8-fold) in comparison to untreated plants. Induction of these PR-protein by seed imbibition with SA and acetylsalicylic acid (ASA) was also observed in different studies (Abd-El-Kareem et al., 2004, El-Mougy 2004, Gaikwad and Balgude 2016, Kumari et al., 2020a). Likewise, SA and BABA also reported an increase in chitinase activity in brinjal and harvested peaches (Mahesh et al., 2017, Wang et al., 2018). In the case of foliar spray with BABA, the maximum average increase was in FDK124 (2.5-fold) and LH2076 (2.3-fold) as compared to untreated plants. While seed priming with SA, the highest increase was in LH2076 (3.4-fold) and FDK124 (2.3-fold) whereas foliar spray caused a maximum

Table 1. Effect of 250 µM of BABA and SA on β-1, 3-gluanase activity (mg Glucose released /min/g FW)through foliar spray and seed priming in cotton genotypes

Genotypes	Treatments	β-1,3 Glucanase (mg glucose released /min/g FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	1.45^{c}	1.57^{e}	1.67^{g}	1.45^{cd}	1.57^{defg}	1.67^{def}
	BABA	1.36^{b}	1.49^{cd}	1.66^{fg}	4.38^{n}	4.85^{o}	4.81^{o}
	SA	1.26^{a}	1.90^{i}	2.02^{j}	2.28^{j}	2.42^{jk}	2.57^{k}
LH2076	Control	1.35^{b}	1.51^{d}	1.64^{fg}	1.35^{l}	1.51^{bc}	1.64^{def}
	BABA	1.22^{a}	1.48^{cd}	1.92^{m}	1.51^{fg}	1.66^{def}	1.94^{g}
	SA	2.23^{k}	2.34^{l}	2.51^{fg}	1.01^{hi}	1.59^{efg}	1.84^{h}
FDK124	Control	1.32^{b}	1.46^{bc}	1.63^{cd}	1.32^{b}	1.46^{cde}	1.63^{fg}
	BABA	1.48^{cd}	1.62^{f}	1.83^{h}	2.01^{i}	2.44^{k}	2.85^{m}
	SA	2.24^{k}	2.36^{l}	2.49^{m}	2.32^{jk}	2.42^{k}	2.59^{l}

Significant difference in peroxidase activity is indicated by Star in comparision to their respective control $P \leq 0.05$ analyzed by Tukey 's post hoc test

Table 2. Effect of 250 µM of BABA and SA on chitinase activity (µM NAG released/ min/g FW)through foliar spray and seed priming ine cotton genotypes

Genotypes	Treatments	Chitinase activity (µM NAG released/ min/g FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	0.53^{ab}	0.73^{cd}	1.11^{fg}	0.53^{ab}	0.73^{cd}	1.11^{h}
	BABA	0.80^{d}	1.2^{gh}	1.83^{kl}	0.74^{d}	1.04^{gh}	1.49^{ij}
	SA	1.24^{h}	1.61^{j}	2.11^{m}	1.45^{i}	1.57^{jk}	1.61^{k}
LH2076	Control	0.45^{a}	0.61^{b}	0.93^{e}	0.45^{a}	0.61^{b}	0.93^{ef}
	BABA	0.93^{e}	1.42^{i}	2.27^{no}	1.11^{h}	2.03^{l}	2.69^{n}
	SA	1.07^{f}	1.91^{l}	2.36^{o}	0.99^{fg}	2.59^{n}	3.27^{o}
FDK124	Control	0.49^{a}	0.63^{bc}	0.93^{e}	0.49^{a}	0.63^{bc}	0.93^{ef}
	BABA	1.15^{fgh}	1.78^{k}	2.19^{mn}	0.62^{b}	1.04^{gh}	2.28^{m}
	SA	0.96^{e}	1.82^{kl}	2.7^{p}	0.87^{e}	1.64^{k}	2.38^{m}

Significant difference in peroxidase activity is indicated by Star in comparision to their respective control $P \leq 0.05$ analyzed by Tukey 's post hoc test

Table 3. Effect of 250 µM of BABA and SA on peroxidase activity (∆A/min/g FW) through foliar spray and seed priming in three cotton genotypes

Genotypes	Treatments	Peroxidase activity (∆A/mint/g FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	1.42^{a}	2.52^{cd}	3.23^{f}	1.42^{a}	2.52^{bc}	3.23^{de}
	BABA	2.53^{cd}	3.79^{ijk}	6.04^{n}	4.14^{l}	3.64^{hij}	3.8^{ijk}
	SA	1.72^{b}	2.79^{e}	4.18^{l}	3.33^{defg}	3.81^{ijk}	4.10^{l}
LH2076	Control	2.75^{de}	3.28^{fg}	3.65^{hij}	2.75 c	3.28^{def}	3.65^{hij}
	BABA	3.49^{gh}	4.03^{kl}	6.26^{n}	3.23 de	3.62^{ghij}	4.02^{kl}
	SA	3.52^{gh}	4.13^{kl}	5.99^{n}	3.4^{efgh}	3.53^{fghij}	3.9^{jkl}
FDK124	Control	2.31^{bcd}	3.05^{efg}	3.53^{ghi}	2.31^{b}	3.05^{d}	3.53^{fghi}
	BABA	3.68^{hijk}	3.85^{kl}	5.66^{m}	2.56^{bcd}	3.25^{def}	3.72^{ij}
	SA	3.22^{f}	4.05^{kl}	5.67^{m}	5.33^{m}	5.97^{n}	6.53^{o}

Significant difference in peroxidase activity is indicated by Star in comparision to their respective control $P \leq 0.05$ analyzed by Tukey 's post hoc test average increment in FDK124 (2.7fold) and LH2076 (2.6-fold) as compared to untreated plants. In addition, a significant and positive correlation was also observed between β-1, 3 glucanase and chitinase suggesting their defensive role in in cotton against CLCuD (Table 6).

SA is a small phenolic molecule that induces SAR by activating different defence processes at basal and molecular levels and should be considered as a possible chemical control for the pest/pathogen (Lu et al., 2016) (Fig. 1). Tripathi et al. (2019) documented that phenyl ammonia lyase pathways mainly synthesize the SA which acts as a systemic signal in SAR and is the main reason for plant defence mechanisms under adverse conditions. Various plant protein serves as viral suppress and mediate several numbers of defence mechanisms. Total soluble protein was increased in all three cultivars after being treated with BABA and SA by seed priming and foliar spray (Table 4). The highest average protein content was in FDK124 (1.8-fold) with BABA when applied through seed priming. A similar trend was observed with SA which caused a 1.8-fold increase in total soluble protein content. In foliar spray, maximum increase was recorded with F1378 (1.7-fold) in comparison to untreated plants. SA treatment resulted in a maximum average increase in total soluble protein in FDK124 (1.8-fold) via seed priming whereas LH2076 (1.6-fold) showed maximum increment via foliar spray compared to untreated plants at all three stages of sampling. Application of SA and BABA with seed priming and foliar spray increased total soluble protein at all development stages is might be due to the increased concentration of PR-protein in treated cotton plants. Proteins play a vital role in plant defense mechanisms in the form of many defense enzymes and other protein-based nonenzymatic compounds (War et al., 2011, Kumari et al., 2020a). Earlier studies reported upregulation of total soluble protein due to SA and JA might be due to an increase in PR-protein in brassica and cotton genotypes (Thakur et al., 2016, Raj et al., 2016). It is also documented that external application SA induced the total soluble proteins compared to untreated plants which provide resistance against tobacco necrosis virus (TNV) (Faheed and Mahmoud 2006). El-Khallal (2007) also recognized an increase in total soluble protein in tomato plants when treated with SA and jasmonic acid. When *B. napus* and *B. Juncea* were sprayed with SA and BTH increased total soluble protein. It was reported that

Table 4. Effect of 250 µM of BABA and SA on chitinase activity (µM NAG released/ min/g FW) through foliar spray and seed priming in cotton genotypes

Genotypes	Treatments	Total soluble protein activity (µmol min^{-1} mg^{-1} FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	7.48^{c}	8.80^{e}	10.28^{h}	7.48^{b}	8.80^{c}	10.28^{e}
	BABA	9.51^{j}	11.23^{n}	15.38^{o}	9.51^{d}	11.23^{f}	15.38^{m}
	SA	9.56^{f}	12.36^{i}	13.44^{l}	9.56^{d}	12.36^{h}	13.44^{j}
LH2076	Control	8.51^{e}	8.68^{e}	9.43^{fg}	8.51^{c}	8.68^{c}	9.43^{d}
	BABA	10.11^{h}	11.93^{j}	14.35^{l}	10.11^{e}	11.93^{g}	14.35^{l}
	SA	11.78^{k}	12.85^{l}	16.21^{n}	11.78^{g}	12.8^{i}	16.21^{n}
FDK124	Control	6.42^{b}	7.53^{c}	9.61^{g}	6.42^{a}	7.53^{b}	9.61^{d}
	BABA	12.76^{i}	14.46^{l}	15.23^{m}	12.76^{hi}	14.46^{l}	15.23^{m}
	SA	12.46^{a}	13.88^{b}	15.13^{d}	12.46^{hi}	13.88^{k}	15.13^{m}

Significant difference in peroxidase activity is indicated by Star in comparision to their respective control *P* ≤ 0.05 analyzed by Tukey 's post hoc test

Table 5. Effect of seed priming and foliar spray of SA and BABA@ 250 µM on disease incidence and disease index

Cultivar	Disease Incidence (%)					Disease Incidence (%)					Disease Index (%)				
	30 DAI Seed priming			30 DAI Foliar spray		40 DAI Seed priming			40DAI Foliar spray		Seed priming			Foliar spray	
	Con	SA	BABA	SA	BABA	Con	SA	BABA	SA	BABA	Con	SA	BABA	SA	BABA
F1378	34	17	0	20	14	67	28	28	34	29	75	40	50	53	60
LH2076	17	0	0	10	17	34	17	20	14	25	62	45	44	52	57
FDK124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Con= Control, SA=salicylic acid, BABA= β-aminobutyric acid, DAI= day after infestation the combined effect of SA + BTH exhibited higher total soluble protein content in both genotypes after each spray (Thakur and Sohal 2014).

Disease incidence and disease index: Both SA and BABA showed decreases in disease incidence and index (Table 1). No disease symptoms were observed upto 20 DAI on the cultivars. In cucumber seedlings, *Fusarium* blight disease was reduced by up to 66% when seeds were imbibed with SA (Wisniewska and Chelkowski 199). In cacao, SA was applied as seed imbibition, and foliar spray resulted in the reduction of *Phytophthora palmivora* infection. The resistant cultivar *i.e* FDK124 showed zero disease incidence and disease index.

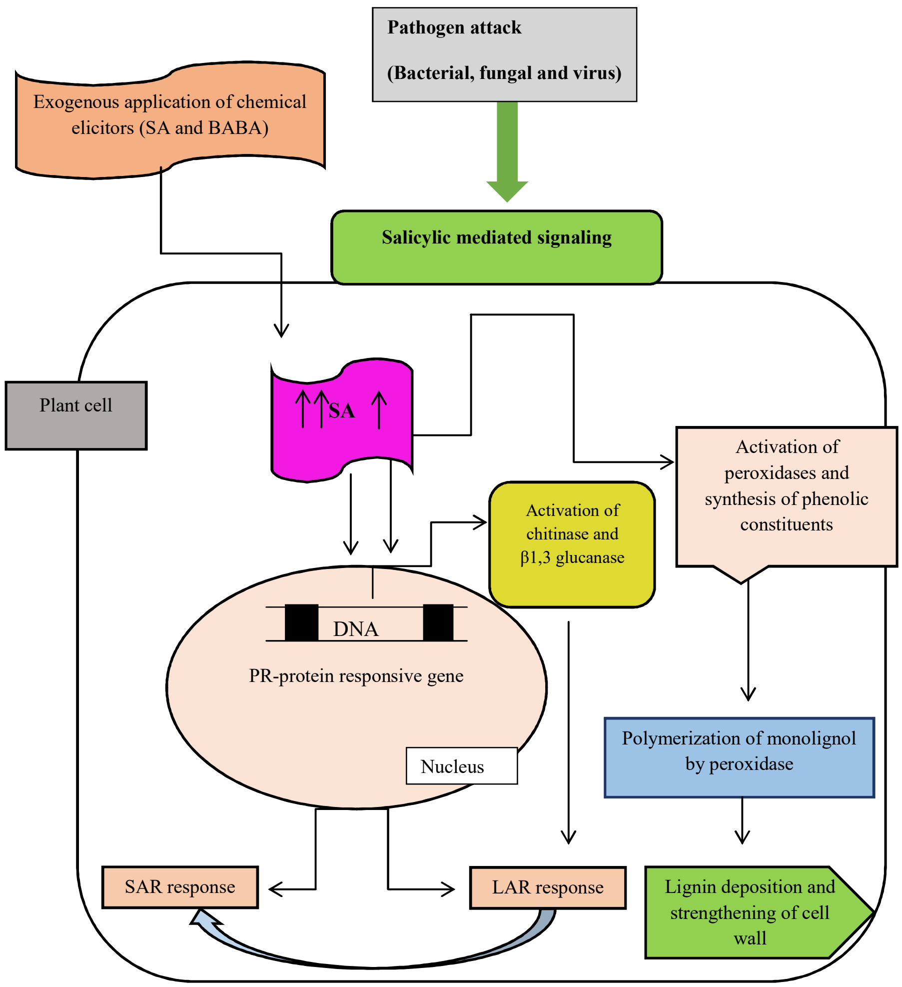

Fig. 1. SAR by activating different defense process at basal and molecular level considered as a possible chemical control for the pest/pathogen

Table 6. Correlation between BABA treated biochemical parameters disease incidence and disease index

	β-1,3 glucanase	Chitinase	Peroxidase	Total soluble protein	Disease incidence	Disease Index
β-1,3 glucanase	1					
Chitinase	0.73792*	1				
Peroxidase	-0.24870	0.56281*	1			
Total soluble protein	-0.24156	0.43767	0.21614	1		
Disease incidence	-0.57225*	0.09012	-0.12024	-0.42414*	1	
Disease Index	-0.02049	-0.54673*	0.34113	0.58509	-0.61895*	1

*Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Susceptible genotypes were recorded with more severe disease symptoms compared to moderately resistant cultivars. In treated plants, susceptible genotypes F1378 showed 28% increases in disease incidence with both modes of application. s moderately resistant genotype recorded with 20 and 25% disease incidence via seed priming and foliar spray respectively at 40 DAI. Disease index for susceptible cultivars was 40 - 53% whereas it was 50-60% for moderately resistant cultivars it ranges from 45-52 and 44-57% with SA and BABA via seed priming and foliar spray, at 30 DAI to 40 DAI respectively. Our results also recorded with lower disease incidence and index with 250μM concentration of both RICs via seed priming and foliar spray as compared to untreated plants (Table 5). This lower disease incidence and index could be explained based on the upregulation of PR-protein after RIC treatment (Raj et al., 2016). These scientists reported that implementation of MeJA and JA led to the upregulation of different PR-proteins which induced the resistance against *Moniliniafructicola*and CLCuD in peach and cotton genotypes. Similarly, Kumari et al. (2020a) also documented the induction of different PR-protein and reduction of CLCuD with INA via three modes of application that is seed priming, foliar spray, and soil drench.

CONCLUSION

Seed priming and foliar pray of SA and BABA induced the activities of PR-proteins, β-1,3 glucanase, peroxidase, chitinase as well as total soluble protein content in all tested cotton cultivars during the different stages of sampling. Increase in PR-proteins activity caused induction of SAR which might be responsible for lower disease incidence and disease index in cotton. Among the two methods, seed priming was more effective in term of higher PR-protein activities and total protein as it was done once in the plant's life sustained its impact upto 35 DAG. However, among the resistance inducing chemical SA was more eco-safe elicitor can be tried as protective regime against CLCuD. This study has scope for future in term of authentication of the result and application on large scale in crop field.

REFERENCES

Abd-El-Kareem F, El-Moungy NS, El-Gamal NG and Fotouh YO 2004. Induction of resistance in squash Plants against Powdery mildew and *Alternaria* leaf spot disease using chemical inducers as protective or therapeutic treatments. *Egypt Journal Phytopathology* **32**(1-2): 65-76.

Akhtar KP, Haidar S, Khan MKR, Ahmad M, Sarwar N, Murtaza MA and Aslam 2010. Evaluation of *Gossypium* species for resistance to *Cotton leaf curl Burewala virus* (CLCuBuV). *Annals of Applied Biology* **157:** 135-147.

Akın S 2024. *The Strategic Importance of Cotton Production for the World and Türkiye*. Agricultural Sciences. IntechOpen. Available at: http://dx.doi.org/10.5772/intechopen.114084.

Akram W and Anjum T 2011. Use of bioagents and synthetic chemicals for induction of systemic resistance in tomato against diseases. *International Research Journal of Agricultural Science and Soil Science* **1**(8): 286-292.

Anonymous 2023. *Annexure-VII: Note on Cotton Sector, 2023*. Annexure-VII-Note on Cotton Sector.pdf

Anonymous 2008. *All India Coordinated Cotton Improvement Project.* Annual Report, CICR, Coimbatore

Baccelli I and Mauch-Mani B 2016. Beta-aminobutyric acid priming of plant defense: The role of ABA and other hormones. *Plant Molecular Biology* **91**: 703-711.

Boller T and Mauch F 1988. Colorimetric assay for chitinase, *Methods in Enzymology* **161**: 430-435.

Claiborne A and Fridovic I 1979. Purification of the o-dianisidine peroxidise from *E. coli*. *Journal of Biological Chemistry* **254**: 4245-4252.

Cohen Y, Rubin AE and Kilfin G 2010. Mechanisms of induced resistance in lettuce against *Bremia lactucae* by DL-β-amino-butyric acid (BABA). *European Journal of Plant Pathology* **126**: 553-573.

El- Khallal SM 2007. Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (*Arbuscular mycorrhiza*) and/or hormonal elicitors (Jasmonic acid & Salicylic acid): 2-Changes in the antioxidant enzymes, phenolic compounds and pathogen related- proteins. *Australian Journal of Basic and Applied Sciences* **1**(4): 717-732.

El-Mougy NS, Nehal SF, Abd-El-Kareem, El-Gamal NG and Fotouh YO 2004. Application of fungicides alternatives for controlling cowpea root rot diseases under greenhouse and field conditions. *Egypt Journal of Phytopathology* **32**(1-2): 23-35.

Faheed FA and Mahmoud S Y M 2006. Induction of resistance in Phaseolus vulgaris against TNV by salicylic acid and kinetin. *International Journal of Agriculture and Biosciences* **8**(1): 47-51.

Gaikwad AP and Balgude YS 2016. Induction of systemic resistance in rice against blast disease by bioagents and chemicals. *Journal of Rice* **9**: 2.

Gordy JW, Leonard BR, Blouin D, Davis JA and Stout MJ 2015. Comparative Effectiveness of Potential Elicitors of Plant Resistance against *Spodoptera frugiperda* (J. E. Smith) (*Lepidoptera: Noctuidae*) in Four Crop Plants. *PLoS ONE* **10**(9): e0136689.

Gozzo F 2003. Systemic resistance in crop protection: from nature to a chemical approach. *Journal of Agricultural and Food Chemistry* **51**: 4487-503.

Gupta SK, Gupta PP, Yadava TP and Kaushik CD 1990. Metabolic change in mustard due to Alternaria leaf blight. *Journal of Phytological Research* **43**(1): 64-69.

Jakab G, Cottier V, Toquin V, Rigoli G, Zimmerli L, Metraux JP and Mauch-Mani B 2001. β-Aminobutyric acid-induced resistance in plants. *European Journal of Plant Pathology* **107**(1): 29-37.

Johnson J, MacDonald S, Meyer L and Stone L 2018. *The world and United States cotton outlook Agricultural Outlook Forum*, In Cotton Outlook.

Kauffmann S, Legrand M, Geoffroy P and Fritig B 1987. Biological function of 'pathogenesis-related' proteins: four PR proteins of tobacco have 1,3-β-glucanase activity. *EMBO Journal* **6**: 3209-3212.

Kumari A, Kaur SM, Prinka G and Dharminder P 2020b. Effectiveness of elicitors in cotton against cotton leaf curl virus (CLCuV) in relation to parthenogenesis-related proteins. *Research Journal of Biotechnology* **15**(10): 19-27.

Kumari A, Kaur SM, Pashupat V, Javed A and Dharmender P 2020a. Role of 2, 6 Dichloroisonicotinic acid inducing resistance in cotton against cotton leaf curl disease. *Research Journal of Biotechnology* **15**(5): 67-74.

Kumari A, Goyal M, Mittal A and Kumar R 2022. Defensive capabilities of contrasting sorghum genotypes against *Atherigona soccata* (Rondani) infestation. *Protoplasma* **259**: 809-822.

Lowry OH, Rosebrough NJ, Farr AL and Randal RJ 1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **198**: 265-275.

Lu H, Greenberg JT and Holuigue L 2016. Editorial: Salicylic acid signaling networks. *Frontiers in Plant Science* **7**: 238.

Mahesh HM, Murali M, Chandra Pal MAC, Melvin P and Sharada MS 2017. Salicylic acid seed priming instigates defense mechanism by inducing PR-Proteins in *Solanum melongena* L. upon infection with *Verticillium dahliae*leb. *Plant Physiology and Biochemistry* **117**: 12-23.

Pandey VP, Awasthi M, Singh S, Tiwari S and Dwivedi UN 2017. A Comprehensive Review on Function and Application of Plant Peroxidases.*Biochemistry & Analytical Biochemistry* **6**: 1.

Raikhel N and Pirrung M 2005. Adding precision tools to the plant biologists' toolbox with chemical genomics. *Plant Physiology* **138**: 563-564.

Raj R, Sekhon PS and Sangha MK 2016. Protection against cotton leaf curl disease by jasmonic acid induced proteins. *Journal of Cotton Research and Development* **30**(1): 84-89.

Raut SA and Borkar SG 2014. PR-proteins accumulation in tomato plant due to application of resistance inducing chemicals during period of induced resistance against alternaria leaf blight. *Scientific International* **2**(3): 72-75.

Sattar MN, Kvarnheden A, Saeed M and Briddon RW 2013. Cotton leaf curl disease: An emerging threat to cotton production worldwide. *Journal of General Virology* **94**: 695-710.

Shine MB, Xiao X, Kachroo P and Aardra Kachroo A 2019. Signaling mechanisms underlying systemic acquired resistance to microbial pathogens. *Plant Science* **279**: 81-86.

Sindelarova M and Sindelar L 2005. Isolation of pathogenesis-related protein from TMV-infected tobacco and their influence on Infectivity of TMV. *Plant Protection Science* **41**(2): 52-57.

Stockwell BR 2004 Exploring biology with small organic molecules. *Nature* **432**: 846-854.

Thakur M and Sohal BS 2014. Effect of elicitors on physio morphological and biochemical parameters of Indian mustard (*Brassica juncea*) and rapeseed (*B. napus*). *Journal of Applied and Natural Science* **6**(1): 41-46.

Thakur T, Sangha MK, Arora R and Javed M 2016. Effect of foliar spray of elicitors on status of defense proteins in relation to mustard aphid infestation in crop *Brassica* cultivars. *Journal of Applied and Natural Science* **8**(4): 2242-2248.

Tripathi D, Raikhy G and Kumar D 2019. Chemical elicitors of systemic acquired resistance-Salicylic acid and its funtional analogs. *Current Plant Biology* **17**: 48-59.

Ullah R, Akhtar KP, Moffett P, Mansoor S, Briddon RW and Saeed M 2014. An analysis of the resistance of *Gossypium arboreum*to cotton leaf curl disease by grafting. *European Journal of Plant Pathology* **139:** 837-847.

Walters DR, Ratsep J and Havis ND 2013. Controlling crop diseases using induced resistance: Challenges for the future. *Journal of Experimental Botany* **64**(5): 1263-1280.

Wang J, Cao S, Wang L, Wang X, Jin P and Zheng Y 2018. Effect of b-Aminobutyric acid on disease resistance against rhizopus rot in harvested peaches. *The Frontiers in Microbiology* **9**: 1505.

Wang KT, Wu DZ, Guo DQ and Du MY 2019. Aminobutyric acid induces disease resistance against *Botrytis cinerea* in grape berries by a cellular priming mechanism. *Acta Alimentaria* **48**(2): 177-186.

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S and Sharma HC 2012. Mechanisms of Plant Defense against Insect Herbivores. *Plant Signaling & Behavior* **7(**10): 1306-1220.

War AR, Paulraj MG, War MY and Ignacimuthu S 2011. Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). *Plant Signaling & Behavior* **6**(11): 1787-1792.

Ward ER, Uknes SJ, Williams SC, Dincher S, Wiederhold DL, Alexander DC, Ahl Goy P, Métraux JP and Ryals JA 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* **3**: 1085-1094.

Wisniewska H and Chelkowski J 1999. Influence of exogenic salicylic acid on Fusarium seedling blight reduction in barley. *Acta Physiologiae Plantarum* **21**: 63-66.

Zubair M, Shan-e-Ali Zaidi S, Shakir S, Farooq M, Amin I, Jodi A, Scheffler, Scheffler BE and Mansoor S 2017. Multiple begomoviruses found associated with cotton leaf curl disease in Pakistan in early 1990 are back in cultivated cotton. *Scientific Reports* **7**: 680.

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