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Dissection of phenylpropanoid pathway during salt stress in cotton (*Gossypium hirsutum* L.)

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Abstract

Salt stress is an abiotic stress in cotton (*G. hirsutum* L.) that affects evenly yield. The present investigation carried out to study the significance of integrative biochemical and molecular approaches against salt stress in cotton. Four different varieties, two salt tolerant (G.Cot.-16, GISV.218) and two susceptible (G. Cot.10, G.Cot.-100) were used screened at different salinity levels viz., 0.8 dS/m, 3 dS/m, 7 dS/m and 10 dS/m. Bio-chemical and molecular parameters analysed at seedling (15 DAS) and squaring stage (45 DAS). Biochemical traits like Protein content, total phenol content and Proline content was found to be increased with increasing salinity levels. Enzyme activity related to phenylpropanoid pathway (PAL, C4H and C3H) was found to be also increased with the advancement in salt stress, hence it implies possible role of these enzymes in imparting tolerance to salt stress in cotton. Reactive oxygen species (ROS) scavenging enzymes such as SOD, POD, CAT and PPO recorded increase in their activity at both seedling and squaring stages with increasing salinity levels. Expression analysis of key genes of phenylpropanoid pathway viz. *PAL*, *C4H* (*C4H1* and *C4H2*) revealed maximum fold expression of *PAL* and *C4H1* in the leaves, whereas least fold expression was observed for *C4H2*. Thus, it may be concluded that phenylpropanoid pathway play significant role in imparting tolerance against salt stress in cotton.

Keywords: Cotton, phenylpropanoid pathway, PAL, C4H

Introduction

Cotton, the white gold, is the world's leading fibre crop and second most important oilseed crop. Man has utilized cotton for his benefits since ancient times (Fryxell, 1992). Salinity is prior threat to world agriculture and it imposes a major setback in increasing the yield of cotton. Salt stress suppresses the growth in general across the plants but the rate of reduction in growth only depends on the tolerance level of plants species. Generally, salt stress reduces the water potential in the soil and causes disturbances in ion-homeostasis and ion toxicity in plant cells that repress enzymatic activity (Zhang, *et al.*, 2001) [36]. Plant growth suppression is directly related to the total concentration of soluble salts or the osmotic potential of soil water (Greenway & Munns, 1980) [12]. Salt damage of leaf tissues is usually the result of Na⁺ accumulation in leaf cells that shortens the life span of individual leaves, thus reducing their net photosynthetic productivity and crop yield. Increased NaCl levels result in a significant decrease in the root, shoot, leaf biomass, increase in the root/shoot ratio, and cottonseed abortion leading to both yield loss and lower fibre quality (Davidonis, *et al.*, 2005) [5]. Generally salt tolerance in cotton has been associated with Na⁺ exclusion. High salinity reduces N and P uptake in cotton, whereas low salinity does not have a significant effect on the absorption of either of the ions. (Yaser *et al.*, 2018) [35] Phenylpropanoid compounds are precursors to a wide range of phenolic compounds that contributes plant responses towards biotic and abiotic stimuli. They are not only indicators of plant stress responses but are also key mediators of the plant's resistance/tolerance towards pests, mechanical or environmental damage, like salinity, drought, or wounding (Camera, *et al.*, 2004) [20]. With the increase of salinity stress, most plants become unable to extract water from the soil and face osmotic stress. Soil salinity also disturbs nutrient balance in soil as well as in plants and creates specific ion toxicity in plant. (Muhammad *et al.*, 2018) [27] Therefore, the present work aims study the effect of salinity levels on biochemical parameters and molecular responses especially enzymes/genes of phenylpropanoid pathway.

Materials and Methods

The experiment was conducted at, Main Cotton Research Station, Navsari Agricultural University, Surat, Gujarat. The seed of four cotton varieties of G. Cot-16 (Tolerant), GISV 218 (Tolerant) G. Cot 10 (Susceptible) and G. Cot 100 (Susceptible) were used for experiment. In pre-constructed channels, different levels of salinity viz., 0.8 dS/m, 3 dS/m and 10 dS/m. were maintained with four plants per treatment per replication in complete randomised design with factorial concept (FCRD).

Biochemical parameters

Fresh second upper leaf samples were collected and washed twice with tap water followed by deionized water. All biochemical parameters were analyzed at 15 and 45 day after sowing (DAS).

Proline was estimated by using acid ninhydrin method as described by Bates, *et al.*, (1973) [3]. Phenol extraction and estimation were elucidated by the method as described by Malick & Singh, (1980) [24]. Peroxidase (POD) activity was estimated as described by Reddy & Gasber, (1971) [32]. Superoxide dismutase (SOD) activity was measured by the method as described by Beyer & Fridovich, (1987) [4]. Catalase (CAT) and polyphenol oxidase (PPO) activity was measured by method as described by Thimmaiah, (1999) [33]. Na⁺ and K⁺ ratio from root and shoot was estimated by flame photometer as described by AOAC, (1990) [1]. Enzyme activity related to phenylpropanoid pathway viz. Phenylalanine ammonia lyase (PAL) activity was measured by the method as described by Mahatma, *et al.*, (2011) [23]. Cinnamic acid-4-hydroxylase (C4H) and p-Coumaric acid-3-hydroxylase (C3H) activity was measured by the method as described by Padhiar & Sharma, (2008) [30].

Molecular analysis

RNA was extracted from Fresh leaves (0.1 g) collected at 15 and 45 days after sowing (DAS) from treated and non-treated plants of each genotype and were powdered in liquid nitrogen using autoclaved pestle and mortar. The resulting powder was transferred to a 1.5 ml tight capped eppendorf tube and RNA was extracted using Spectrum™ Plant Total RNA Kit (Sigma).

Synthesis of cDNA

The cDNA was synthesized by using high capacity cDNA

reverse transcription kit (thermo) following manufacturer's instructions. For cDNA synthesis, 2µl of template RNA (100 ng), 1µl of Oligo (dT) primer (0.5g) and 8µl of nuclease free water were mixed by brief centrifugation and incubated at 65°C for 5 min followed by immediate cooling on ice. After that 4 µl of 5x reaction buffer, 1 µl of RibolockRNase Inhibitor (20 u/µl), 2 µl of 10 mM dNTP mix and 2µl of M-MuLV Reverse Transcriptase (20u/µl) were mixed by brief centrifugation and incubated for 60 min at 37°C. The reaction was terminated by heating at 70 °C for 5 min. The reverse transcription reaction products (cDNA) were stored at -20 °C until used.

Gene specific primers were used to amplify a set of cotton pphenylpropanoid pathway key genes and housekeeping genes in quantitative real time-PCR (qRT-PCR) assays and details are given in Table 1. Gene specific primers *PAL*, *C4H*, *C4H2* & *Ubiquitin* were selected according to the Xu *et al.* (2011).

Table 1: Sequences of primers used for expression analysis

Primer	Sequence	Gene ID
<i>PAL F</i>	CAATGGACAATGCTCGTTTGGCTAT	CL1Contig4241
<i>PAL R</i>	CACGGTGTTCCTCACTGTGCTCCTC	
<i>C4H1 F</i>	ATCCAGAGATCCAGCAGAACTCCG	CL23Contig4
<i>C4H1 R</i>	GACAGCTCCTCCTTCCAACACCAAA	
<i>C4H2 F</i>	GGGTTGTGTGGCTGTATTGTGTGG	CL23Contig5
<i>C4H2 R</i>	GGCTAAGTCTGTGAGGTTGCGGTGG	
<i>Ubiquitin F</i>	ACCAAAATCCAAGATAAGGAAGGCAT	DQ116441
<i>Ubiquitin R</i>	ACGCAAACGAAGGACAAGTGGAG	

Results and Discussion

Biochemical parameters

Biochemical analysis was done from fresh sample that was collected from cotton plant at seedling (15 DAS) and squaring stage (45 DAS). Sample collected from normal plot was served as control.

Significant varietal differences in response to salinity had been observed. Maximum proline content at 15 DAS was observed at highest salinity level i.e.10 dS/m in G.Cot.-16 (0.86 mg/g tissue) followed by GISV-218 (0.77mg/g tissue), G. Cot-10 (0.45 mg/g tissue) while, it was found minimum in susceptible variety G. Cot 100 (0.33mg/g tissue) as compared with control. Further, at 45 DAS it was observed that at highest salt concentration proline content was significantly increased in tolerant genotype *i.e.* GISV-218 (1.35 mg/g tissue) followed by G.Cot-16 (1.30 mg/g tissue) while, it was found minimum in susceptible genotype G.Cot-100 (0.74mg/g tissue) followed by G. Cot 10 (0.97 mg/g tissue). However, proline content was increased with increase of salinity level in the genotype studied. (Figure 1)

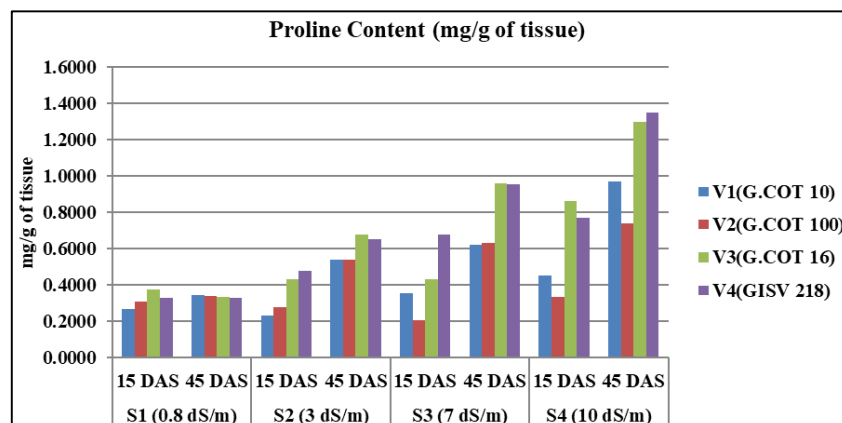


Fig 1: Change in Proline content in leaves of cotton during different level of salt stress

At highest salinity (10 dS/m) there was significant difference recorded in total phenol content among the varieties. At the seedling stage total phenol content was found on par in resistant variety GISV-218 (18.86 mg/g tissue) and G. Cot.-16 (18 mg/g tissue) followed by G.Cot.100 (12.40 mg/g tissue). While, least phenol content was found in G.Cot.-10 (11.60 mg/g tissue), Similarly at squaring stage also it was found at

par in resistant variety GISV-218 (21.26 mg/g tissue) and G.Cot-16 (20.49 mg/g tissue) followed by G.Cot.100 (14.55 mg/g tissue) While, least phenol content was found in G.Cot.-10 (14.11 mg/g tissue). From these results it can be concluded that salinity significantly affects total phenol content at both stages. (Figure 2)

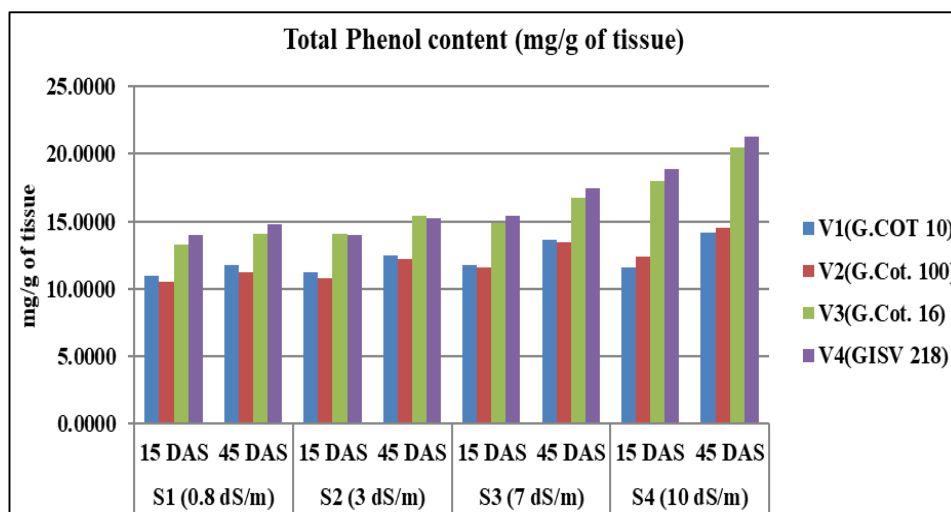


Fig 2: Change in Total Phenol content in leaves of cotton during different level of salt stress

At 15 DAS peroxidase (POD) was highest in G.Cot.-16 (7.81 unit/mg protein) followed by GISV-218 (8.61 unit/mg protein) while it was lowest in G.Cot.-100 (4.89 unit/mg protein) followed by G.Cot.-10 (4.91 unit/mg protein) under most salty condition (10 dS/m). As compare to control it was 4.3, 4.2, 5.7 and 6.8 fold higher in G.Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively. At 45 DAS POD

activity was maximum in GISV-218 (8.82 unit/mg protein) followed by G.Cot.-16 (8.10 unit/mg protein) while it was lowest in G.Cot.-100 (5.35 unit/mg protein) followed by G.Cot.-10 (5.49 unit/mg protein) under high salinity condition (10 dS/m). As compare to control it was 4.42, 4.10, 5.53 and 6.61 fold increase in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively.(Figure 3)

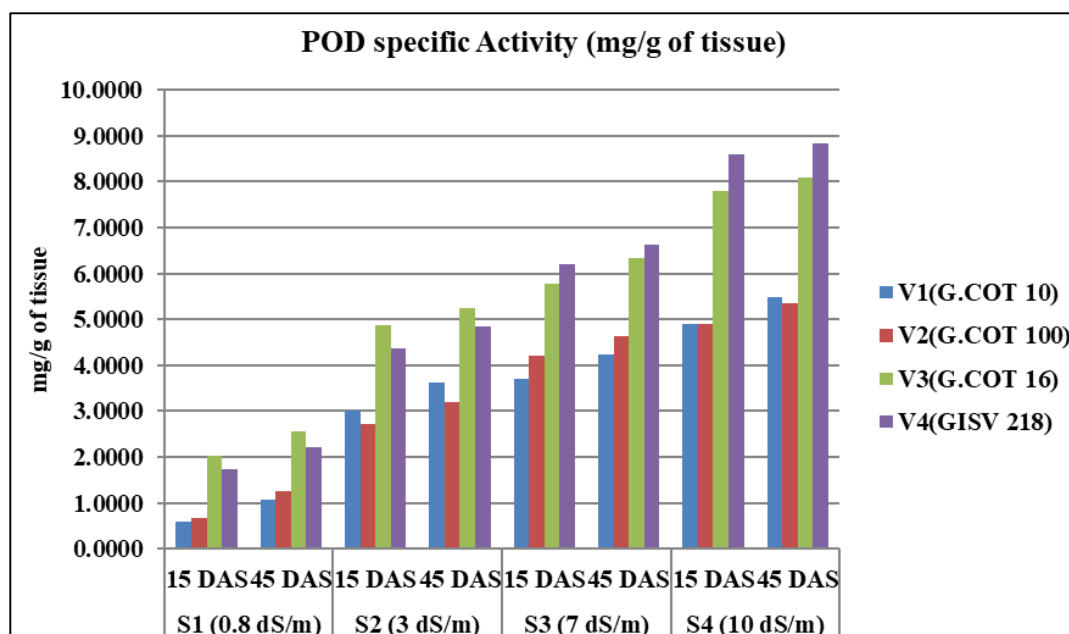


Fig 3: Change in peroxidase (POD) specific activity in cotton leaves during different levels of salt stress

At both the stages SOD enzyme activity was observed higher in tolerant genotype then in susceptible genotype. At 15 DAS it was highest in GISV 218 (0.33 unit/mg protein) followed by G.Cot-16 (0.30 unit/mg protein) while it was lowest in G.Cot.-100 (0.19 unit/mg protein) followed by G.Cot.-10 (0.21 unit/mg protein) under highest saline condition (10

dS/m). Similar trend was found at 45 DAS, the SOD activity was maximum in GISV-218 (0.35 unit/mg protein) followed by G.Cot.-16 (0.34 unit/mg protein) while it was lowest in G.Cot.-100 (0.23 unit/mg protein) followed by G.Cot.-10 (0.25 unit/mg protein). SOD activity was enhanced at both stages with increasing salinity levels. (Figure 4)

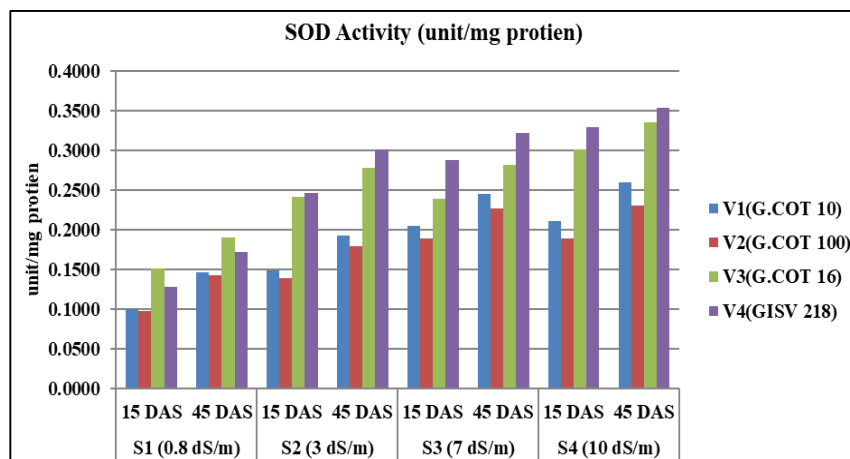


Fig 4: Change in Superoxide Dismutase (SOD) specific activity in cotton leaves during different levels of salt stress

At 15 DAS Catalase enzyme activity was highest in GISV-218 (0.123 unit/mg protein) followed by G.Cot.-16 (0.119 unit/mg protein) while it was lowest in G.Cot.-100 (0.091 unit/mg protein) followed by G.Cot.-10 (0.102 unit/mg protein) under most salty condition (10 dS/m). As compare to control it was 0.087, 0.082, 0.097 and 0.096 fold higher in G.

Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively. Similarly at 45 DAS SOD activity was maximum in GISV-218 (0.138 unit/mg protein) followed by G. Cot.-16 (0.134 unit/mg protein) while it was lowest in G.Cot.-100 (0.104 unit/mg protein) followed by G.Cot.-10 (0.116 unit/mg protein). (Figure 5)

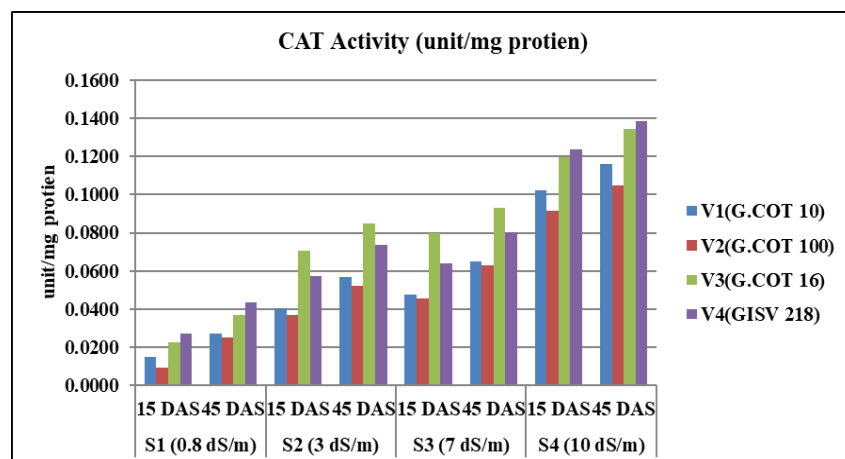


Fig 5: Change in Catalase (CAT) specific activity in cotton leaves during different levels of salt stress

At both 15 and 45 DAS, highest percent increase Na^+/K^+ ratio in leaf was recorded in susceptible variety G.Cot-10 (23%, 45%) and G.Cot-100 (33%, 37%) at the highest salt

concentration as compared to their respective normal soil and least Na^+/K^+ ratio in G.Cot-16 (57%, 42%). (Figure 6)

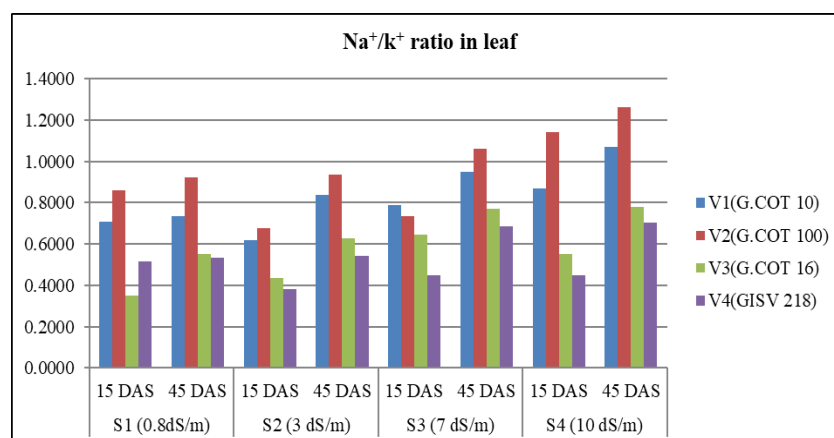


Fig 6: Change in Na^+/K^+ ratio in cotton leaf during different level of salt stress

The results of proline, phenol, POD, SOD, CAT and Na^+/K^+ ratio were in agreement with Ramani *et al.* (2018) [31] who screened eleven cotton genotypes that are grown up to

squaring stage in different soil ratio of normal soil and saline soil. Proline content raised from 0.23 to 0.56 mg/g of tissue in normal soil condition and it was 0.75 to 1.35 mg/g of tissue in

saline soil condition similarly highest total phenol content was found in GISV-218 and G. Cot-16 and Lowest total phenol content was found in G.Cot-10 and G.Cot-100. Further highest peroxidase activity was recorded in GISV-218 and G. Cot-16 while highest catalase activity was found in GISV-218 and Surat Dwarf in ratio of 1:2 (Normal soil: Saline soil). Highest Superoxide dismutase activity was found in GISV-218 and in ratio of 1:2 (Normal soil: Saline soil). Lowest Na/K ratio was found in GISV-218 and G. Cot-16 in root while lowest Na/K ratio was found in GISV-218 and G. Cot-16 in shoot.

At both the stages PPO enzyme activity was higher in tolerant genotype then in susceptible genotype. At 15 DAS it was highest in G.Cot.-16(4.16 unit/mg protein) followed by GISV-218 (3.63 unit/mg protein) while it was lowest in G.Cot.-10 (1.84 unit/mg protein) followed by G.Cot.-100 (2.50 unit/mg

protein) under most saline condition (10 dS/m). As compare to control it was 39.40, 130.86, 246.27 and 195.39 percent increase in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively. Similar trend was observed at 45 DAS also. At 45 DAS PPO activity was highest in G.Cot.-16 (5.16 unit/mg protein) followed by GISV-218 (4.20 unit/mg protein) while it was lowest in G.Cot.-10 (2.54 unit/mg protein) followed by G.Cot.-100 (3.62 unit/mg protein) under higher saline condition. As compare to control it was 64.58, 180.16, 281.78 and 199.29 percent increase in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively (Figure 7). Earlier study by Dhimir & Kocacaliskan, (2001) [6] also showed significant correlation between NaCl concentration and PPO activity. Niknam, *et al.*, (2006) [29] opined that PPO together with other enzyme effectively remove the ROS formed under salt stress.

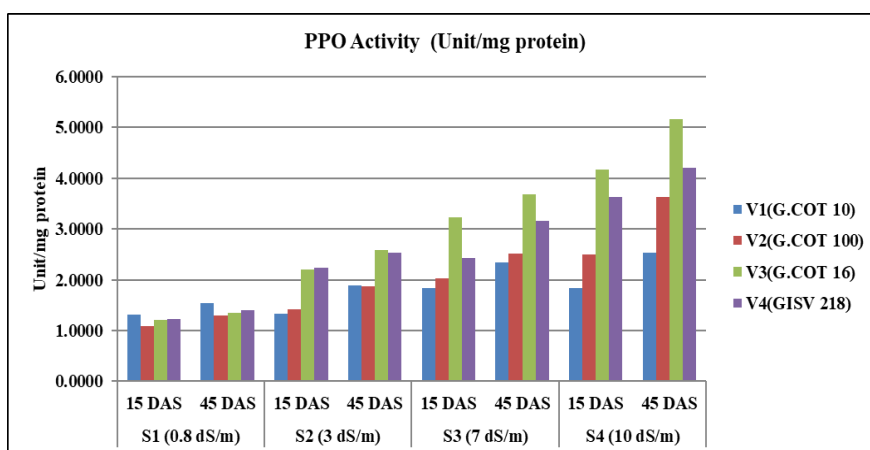


Fig 7: Change in Enzyme activity of Polyphenol Oxidase (PPO) in cotton leaves during salt stress

PAL enzyme activity at seedling and squaring was higher in tolerant genotype then in susceptible genotype. At 15 DAS it was highest in G.Cot.-16(50.25 unit/mg protein) followed by GISV-218 (5.00 unit/mg protein) while it was lowest in G.Cot.-10 (2.48 unit/mg protein) followed by G.Cot.-100 (2.86 unit/mg protein) under most saline condition (10 dS/m). As compare to control it was 80.59, 112.91, 230.61 and 233.78 percent higher in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively. Similar trend of PAL activity was maximum in G.Cot.-16 (6.22 unit/mg protein) followed by GISV-218 (6.15 unit/mg protein) while it was lowest in G.Cot.-10 (2.70 unit/mg protein) followed by G.Cot.-100 (3.04 unit/mg protein) under high salinity condition (10 dS/m) at 45 DAS. As compare to control it was 64.50, 89.24, 248.50 and 260.54 percent increase in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively (Figure 8). Miladinova

et al. (2012) also correlated PAL activity with the salt tolerant capacity. Gholizadeh and Kohnehrouz (2010) [11] found that PAL increased quickly when exposed to high salt concentrations (150 mM NaCl) at 24h salt stress in both maize inbreds. Gao *et al.* (2008) [11], observed that PAL activity in the cotyledons of *Jatropha curcas* was linearly and positively correlated with increasing NaCl concentrations, but the peak activity in the cotyledons was observed at NaCl concentration of 150 mM and the highest activity increased by 117.2% compared to the control. Further they concluded that increased PAL activity could be a response to the cellular damage provoked by higher NaCl concentrations. by 117.2% compared to the control. Further they concluded that increased PAL activity could be a response to the cellular damage provoked by higher NaCl concentrations.

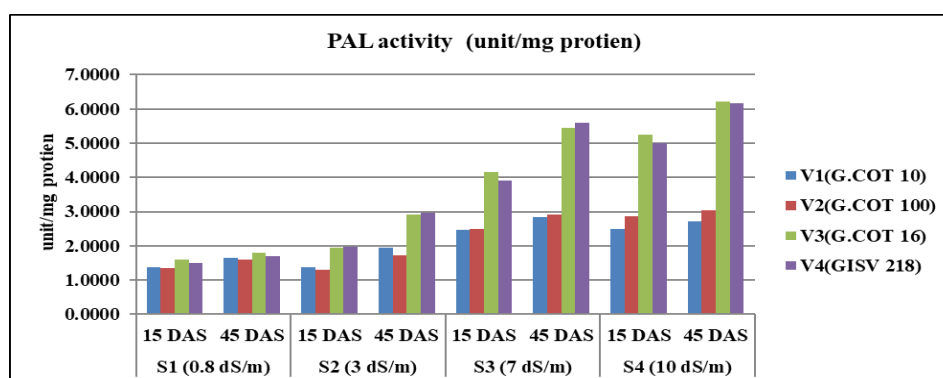


Fig 8: Change in Enzyme activity of Phenylalanine Ammonia Lyase (PAL) in cotton leaves during different levels of salt stress

The C4H enzyme activity measured in leaves of cotton at different salinity levels showed a significant increasing trend with the increase of salt concentration at both 15 and 45 DAS (Figure 9). At both the stages C4H enzyme activity was observed higher in tolerant genotype then in susceptible genotype. At 15, 45 DAS it was highest in G.Cot.-16 (3.91, 5.09 unit/mg protein) followed by GISV-218 (2.83, 3.58 unit/mg protein) while it was lowest in G.Cot.-10 (0.98, 1.36

unit/mg protein) followed by G.Cot.-100 (1.66, 2.42 unit/mg protein) under most saline condition (10 dS/m). As compare to control it was 110.69, 227.61, 574.70, and 411.77 percent amplified in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively. Our results are in correlation with Mizutani *et al.* (1997) [25], who proved C4H expression was clearly higher in roots than in leaves in *Arabidopsis* plant.

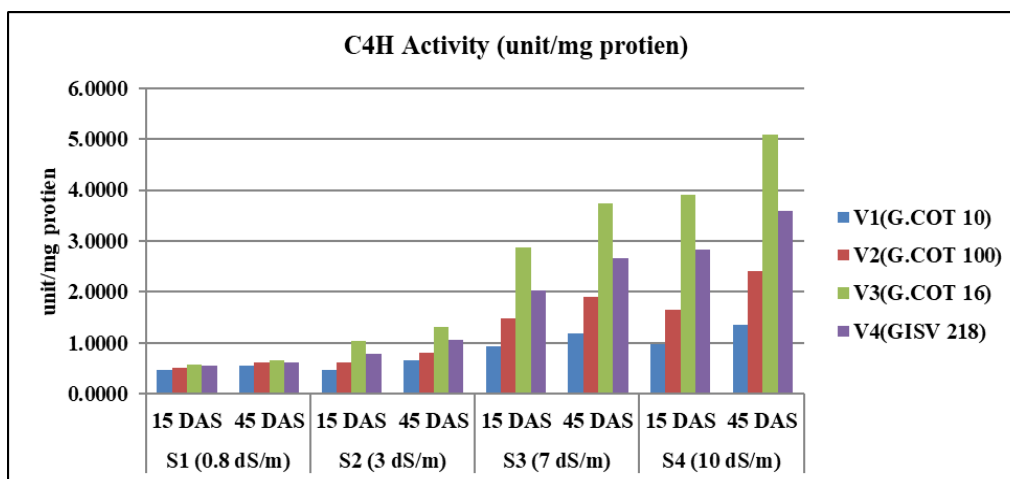


Fig 9: Change in Enzyme activity of Cinnamate-4-hydroxylase (C4H) in cotton leaves during different level of salt stress

At both the stages C3H enzyme activity was observed higher in tolerant genotype then in susceptible genotype. At 15 DAS it was highest in G.Cot.-16 (3.53 unit/mg protein) followed by GISV-218 (2.26 unit/mg protein) while it was lowest in G.Cot.-10 (0.88 unit/mg protein) followed by G.Cot.-100 (1.57 unit/mg protein) under most saline condition (10 dS/m). As compare to control it was 83.33, 247.07, 612.03 and 358.13 percent amplified in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively. At 45 DAS C3H activity was maximum in G.Cot.-16 (4.50 unit/mg protein) followed by

GISV-218 (2.84 unit/mg protein) while it was lowest in G.Cot.-10 (1.21 unit/mg protein) followed by G.Cot.-100 (2.27 unit/mg protein) under high salinity condition (10 dS/m). As compare to control C3H activity was 121.21, 328.30, 728.88 and 429.16 percent increase in G. Cot.-10, G.Cot.-100, G.Cot.-16 and GISV-218 respectively (Figure 10). The increase C3H activity changes the composition of lignin inturn alters the stiffness of cellwall and then, provides tolerance to salinity (Moura, *et al.*, 2010) [26].

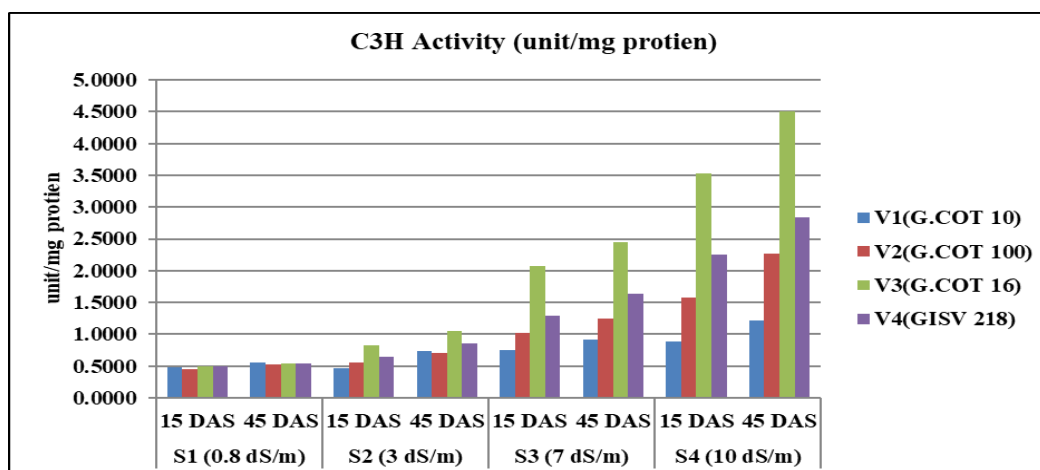


Fig 10: Change in enzyme activity of P-Coumaric acid-3-hydroxylase (C3H) in cotton leaves during different levels of salt stress

Molecular Analysis

Expression analysis of key genes of phenylpropanoid pathway was carried out at 15 DAS and 45 DAS after treatment with different concentrations of salt in both treated and non treated plants. The intact RNA isolated is converted to cDNA and is subjected RT-PCR analysis with PAL, C4H₁ and C4H₂ gene activity.

PAL gene expression of different cotton genotypes is presented in Figure 11. It was observed that PAL gene expression increased with increasing salt concentration. At 15

DAS, maximum increase in PAL gene expression was observed in G.Cot.-16 (6.11 fold) followed by GISV-218 (5.74 fold); while, least increase was found in susceptible varieties i.e. G.Cot.-10 (2.93 fold) and G.Cot.-100 (2.45 fold) at highest salinity level (10 dS/m) as compared to normal soil (0.8 dS/m). Similar kind of trend was observed at 45 DAS also, maximum increase in PAL transcripts were observed in G.Cot.-16 (7.36 fold) followed by GISV-218 (6.77 fold), while, least increase was found in susceptible varieties i.e. G.Cot.-100 (3.14 fold) and G.Cot.-10 (2.85 fold) at highest

salinity level as compared to normal soil. Similar kind of increase in PAL activity was reported by Ibrahim, *et al.* (2019) [17, 18] who observed the effect of drought and salinity,

alone or in combination, on secondary metabolism-related enzyme activities using two cotton.

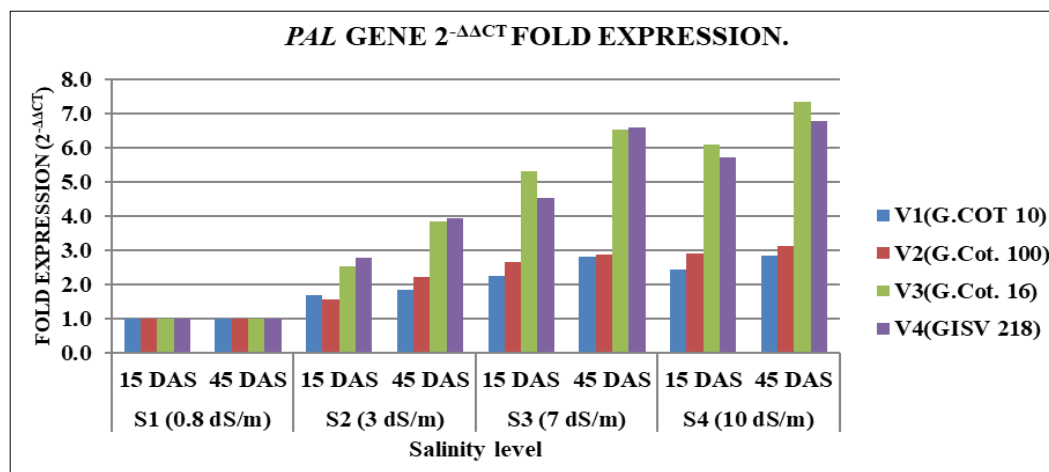


Fig 11: Comparative real time PCR results in fold expression ($2^{-\Delta\Delta\text{CT}}$) of PAL gene in leaves of cotton during salt stress

genotypes Zhongmian 23 (salt tolerant) and Zhongmian 41 (salt sensitive). They found that PAL expression was increased in Zhongmian 23 by five fold, eight fold and six fold under drought, salinity and D+S respectively as compared to control. Similarly, Jeong, *et al.* (2012) observed that PAL transcript was significantly induced by 200 mM NaCl treatment was maximum after 24 h in Kenaf seedlings. Induction of PAL gene by NaCl was also observed by Guo and Wang (2009) [13] in the tomato.

Expression analysis of another important gene, *C4H* was performed using *C4H* (*C4H1* & *C4H2*) genes specific primers. The gene *C4H1* & *C4H2* was found to be up regulated in salinity treated cotton plants as compared to non-treated plants. It was observed that *C4H1* gene expression increased with increasing salt stress. At 15 DAS, maximum increase in *C4H1* gene expression were observed in G.Cot.-16 (4.96 fold) followed by GISV-218 (4.47 fold) while, least

increase was found in susceptible variety *i.e.* G.Cot.-100 (1.66 fold) and G.Cot.-10 (1.38 fold) at highest salinity level as compared to control (Figure 12). Similar kind of trend was observed at 45 DAS also, maximum increase in *C4H1* gene expression were observed in G.Cot.-16 (6.92 fold) followed by GISV-218 (6.32 fold) while, least increase was found in susceptible variety *i.e.* G.Cot.-100 (2.19 fold) and G.Cot.-10 (1.38 fold) at highest salinity level as compared to control (Figure 13). It was observed that *C4H2* gene expression increased with increasing salt stress. At 15 DAS, maximum increase in *C4H2* gene expression were observed in G.Cot.-16 (4.47 fold) followed by GISV-218 (3.56 fold) while, least increase was found in susceptible variety *i.e.* G.Cot.-100 (1.20 fold) and G.Cot.-10 (1.01) at highest salinity level as compared to control. Similar kind of trend was observed at 45 DAS also, maximum increase

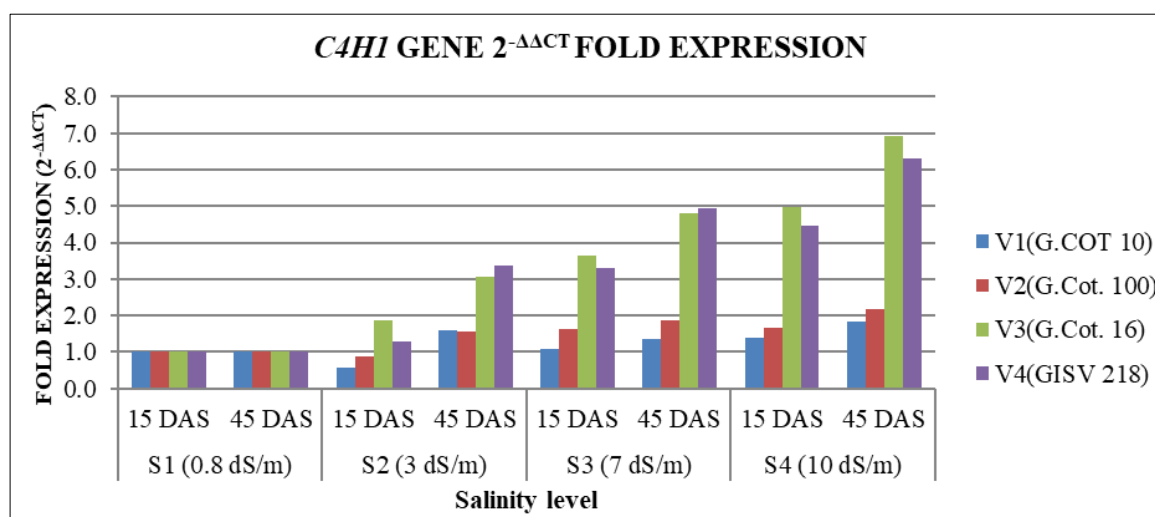


Fig 12: Comparative real time PCR results in fold expression ($2^{-\Delta\Delta\text{CT}}$) of *C4H1* gene in leaves of cotton during salt stress

compared to control. Similar kind of trend was observed at 45 DAS also, maximum increase in *C4H1* gene expression were observed in G.Cot.-16 (5.13 fold) followed by GISV-218

(4.72 fold) while, least increase was found in susceptible variety *i.e.* G.Cot.-100 (1.82 fold) and G.Cot.-10 (1.64 fold) at highest salinity level as compared to control.

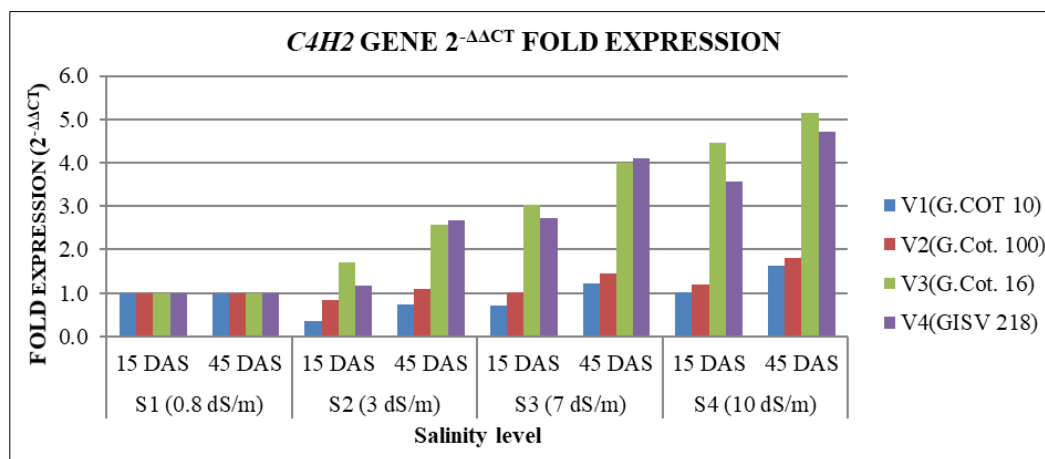


Fig 13: Comparative real time PCR results in fold expression ($2^{-\Delta\Delta CT}$) of C4H1 gene in leaves of cotton during salt stress

The *C4H1* and *C4H2* are differentially expressed isoforms of *C4H* gene. The existence of *C4H* isoforms was previously reported in maize and alfalfa and this differential expression of *C4H* isoforms might reflect the specific need for certain compounds at a certain time or compartment. *C4H2* expressed constitutively and seems to play the role of a 'housekeeping' gene in the phenylpropanoid pathway. Transcripts of *C4H1* detected in wound induced response while *C4H2* transcripts detected without wound also. Similar trend increase in fold expression of *C4H1* and *C4H2* isoforms is observed in our study. The obtained results are in comparison with Kim *et al.* (2013) they observed that *C4H* induction was increased for up to 12 h after treatment, followed by a decreased at 24 h after treatment and a subsequent increased at 48 h after treatment at 150-200 mM NaCl /treatment in kenaf seedlings. The co-ordinated increase in *C4H* and *PAL* may participate in the reinforcement of cell walls by increased deposition of the lignin building units (Monolignols) and coumarates in the stressed plants.

Conclusion

The plants grown under saline condition have the ability to biosynthesize more biochemical metabolites in comparison to plants growing under normal conditions; this might be happening as a defence mechanism in plants. Looking to the tolerant genotypes, which performed better in stress environment have more actively produced phenols, proline, ROS enzymes and the key enzymes of phenylpropanoid pathway *viz.* *PAL*, *C4H* and *C3H* in cotton as compared to salt sensitive genotypes. Further, the molecular study of genes of phenylpropanoid pathway also revealed that the pathway was more active during stress condition from this it can be concluded that under saline stress the secondary metabolite pathway especially phenylpropanoid pathway is active in cotton.

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