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Biochemical Strategies of Cotton Defense: Osmolyte Accumulation and Stability Enhance Resistance to Cotton Leaf Curl Virus

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Abstract

Cotton leaf curl virus (CLCuV) is a major constraint to stable cotton yield, necessitating the screening of genotypes with higher levels of tolerance mechanisms. This study examined the accumulation of biochemical attributes and disease severity of 32 cotton genotypes under graft inoculation. Significant differences were revealed between uninoculated and inoculated genotypes in the concentration of glycine betaine (GB), proline (PRL), total soluble sugars (TSS), and total soluble proteins (TSP). Genotypes including FH-492, FH-Super Cotton and FH-938 exhibited the maximum folds of GB and PRL contents, among inoculated genotypes that indicate their important role in osmotic adjustment. Moderate accumulation of TSS was observed mostly in FH-938 and FH-494, while TSP demonstrated a varied trend, remaining comparatively stable in tolerant genotypes however low levels of accumulation in sensitive ones. This response of biochemical modification in genotypes is strongly linked with disease severity index. Genotypes FH-534, FH-933, FH-509, FH-5100, FH-415 were revealed maximum disease index with recorded elevated DSI values (>4.4), while FH-525, FH-930, FH-520, FH-Anmol, FH-Super Cotton were highlighted medium responses. Remarkably, FH-492 and FH-494 exhibited the lowest DSI (~1.6) with the highest accumulation of osmolytes and protein stability, recognizing them as promising donor parents for resistance breeding against disease. Overall, the findings of research figured that higher GB and PRL levels, moderate concentration of TSS, and constant TSP integrity establish a coordinated biochemical defense strategy against CLCuV disease. Genotypes exhibited the maximum tolerance levels in response to disease that are associated with maximum accumulation of osmolytes and stability in protein, such results suggest an applied approach in improvement of virus resistance and enhancing cotton production.

Keywords: Cotton leaf curl virus (CLCuV); osmolytes; glycine betaine; proline; resistant genotypes; biochemical markers
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Introduction

Cotton (*Gossypium hirsutum* L.) has significant importance as a fiber crop associated with the basic material for textiles, households and industry globally. Its cultivation and yield accommodate millions of farming communities that highlighted its wider adaptability and economic position worldwide (1-4). It is a distinct crop linked with oilseed and fiber outcomes, radically cultivated for its fundamental position in the oil and textile sector, positioning its importance definite. Pakistan is at 5th place in its yield for cotton production (5). However, climate change, abiotic and biotic factors contributing towards its lower produce (3,6,7). Cotton leaf curl disease (CLCuD) has the maximum influence in deteriorating its quality and quantity (8). This disease is caused by Begomovirus betasatellite complexes and the vector whitefly (Bemisia tabaci) spreads this disease resulting in maximum yield loss in affected crops (6,9,11,12). It has become a main menace to cotton, contributing up to 80-87% loss in the sub-continent (13,14). Maximum influence in yield decline was observed in Pakistan, India and China where widespread epidemic resulted in extreme economic (10,15,16,17). Therefore, this disease has major concern in parts of the Indian subcontinent and Africa (18-

22). Approximately growing G. hirsutum varieties in Pakistan are now mostly sensitive due to the development of cotton leaf curl Burewala virus (CLCuBuV) {(a recombinant of cotton leaf curl Multan virus (CLCuMuV) and cotton leaf curl Kokhran virus (CLCuKV)}, which has overwhelmed previously effective resistance (23-25). Even though Asiatic cottons (*Gossypium arboreum* and *Gossypium herbacium*) influence natural resistance, however their lower yield and quality limited the adaptability of these species (20,21). There is a dire need to create long-lasting resistance in upland cotton is highlighted by this incident.

It was examined that plants cope against pathogen attack through different mechanisms that relate to morphological barriers, biochemical directive, and molecular defense mechanisms (26,27). Biochemical osmolytes are the initial and major responses. Contribution of quaternary amine GB to photosystem II ion homeostasis, ROS detoxification, and prevention of protein aggregation is well documented (28,29). Another osmo-protectant proline has the major role in protein metabolism sequencing ROS regulating ROS balance and establishing plant enhancement under stress (30,47). Proteins have the remarkable function to enhance tolerance by protecting enzymatic activity and maintaining structural integrity during stress (3,31-33),

Response of plant against stresses to cope with the situation timely is mostly correlated with maximum accumulation of biochemical compounds (31,34). However, their precise role in virus-induced diseases is still vague, although the critical requirement to establish long-lasting resistance in upland cotton (36). Keeping in view the importance of biochemical attributes the information of their levels of accumulation and association with disease severity it is therefore obligatory to get information about insights into the biochemical mechanisms underlying resistance to CLCuV for advancing cotton breeding programs. In this research work, we assessed osmolyte accumulation (GB, PRL and TSS) and protein stability in cotton genotypes contrasting in their retort to graft inoculation. By linking biochemical attributes to DSI, the research focuses defense strategies allied with tolerance and suggests that osmolyte adjustment and proteome permanence can serve as potential biochemical indications for resistance breeding.

Materials and Methods

Seeds sterilization

Plant material and inoculation procedure

A set of thirty-two cotton (*Gossypium hirsutum* L.) genotypes were picked for screening under greenhouse conditions at Cotton Research Station, Ayub Agricultural Research Institute, Faisalabad, Pakistan. The controlled conditions at greenhouse were retained at a day/night temperature of 28 ± 2 °C / 22 ± 2 °C with a relative humidity of 60-70% during the experiment. The photoperiod of 14 hours light and 10 hours dark was set up utilizing natural daylight improved with artificial lighting when necessary to sustain uniform light environment. A sterilized growth medium was provided to grow the cotton seeds in earthen pots with a composition of medium loam soil, sand, and farmyard manure in a 2:1:1 ratio (v/v/v). Plants were watered frequently to maintain optimal soil moisture and balanced nutrient solutions were applied as needed. These balanced controlled conditions were kept minimizing variability and to make sure consistent CLCuV infection and symptom development across treatments.

Genotype FH-114 (the highly susceptible variety) was the source of inoculum to ensure uniform infection pressure, which was maintained as a constant reservoir of Cotton Leaf Curl Virus (CLCuV). Inoculation was transferred through grafting, according to the standard protocol described by Akhtar et al. (2013) (21). After the completion of six weeks, 5 plants per genotype were grafted with scions obtained from CLCuV-infected FH-114 donor plants. For each genotype, observations were recorded based on the DSI of grafts that successfully established, efficiency of disease transmission, the latent period, measured as the average number of days from grafting until the first visible symptom appeared, and symptom expression over a period of 90 days post-inoculation. To validate the symptom development a subsequent set of uninoculated plants was maintained for each genotype as negative controls. Calculations for disease severity index (DSI) were performed based on visual symptom scoring, according to the methodology defined by Akhtar et al. (2015) (22).

Fully expanded young leaves exhibiting visible symptoms were collected between 09:00 and 11:00 h to minimize daytime variation in metabolite folds. Instantly after collecting leaf samples these were wrapped in aluminum foil, placed on ice, and moved to the laboratory within 30 minutes. Samples were then rinsed quickly in distilled water, blotted dry, and partitioned for different assays. Prior to biochemical extraction and molecular analysis, leaf tissues were flash-frozen in liquid nitrogen and stored at -80 °C until analyses. During storage, all samples stayed frozen to keep RNA integrity and biochemical stability to prevent enzymatic degradation.

Biochemical assays

Analyses were performed on uppermost completely expanded stored from both inoculated and uninoculated plants. Bates et al (1973) method was employed to measure the proline content through ninhydrin-based quantification (37). Estimation of Total soluble sugars (TSS) were examined by the anthrone method according to Yemm and Willis (1954) (38). Lowry assay methodology was used to measure the total soluble protein (39), whereas quantification of glycine betaine (GB) was determined by using the spectrophotometric procedure of Grieve and Grattan,1983(40). Biochemical attributes on fresh weight basis were estimated with three replications per treatment for each genotype.

Statistical analysis

Completely randomized design (CRD) arrangement was performed and data collection for each trait was analyzed independently. Analysis of variance (ANOVA) was performed according to Lars et al. (1989) for which software package Statistix 8.1 was used (41). Calculations for descriptive statistics (mean values, standard deviation, and

range) were also performed to summarize the data set. Correlation statistics are conducted to recognize direction of relationships between variables and a heat map was generated to explore trait associations and visualize genotypic responses, using Python software (https://www.python.org/). Mean comparisons among genotypes were achieved using Tukey's Honest Significant Difference (HSD) test, and all observed values represent the mean performance of three replicates ± standard error (SE).

Results

Highly significant variations were found in the analysis of biochemical osmolytes evidenced from the differences between uninoculated control plants and those exposed to graft inoculation across the examined genotypes (Tables 1, 2: Figures 1-8).

Table 1 Mean squares of cotton traits under uninoculated and graft inoculated

SOV	Genotypes	Treatments	Treatment × Genotype	Error
DF	31	1	31**	128
GB	57.76**	5572.72**	52.07**	0.77
PRL	61.10**	4482.95**	52.95**	0.68
TSS	11.93**	343.82**	6.61**	0.22
TSP	13.06**	6.74**	0.34**	0.13

*p≤0.05, **p≤0.01, and ***p≤0.001. NS= Non–significant, GB= Glycine betaine, PRL= total soluble proline, TSS=total soluble sugars, TSP= total soluble protein

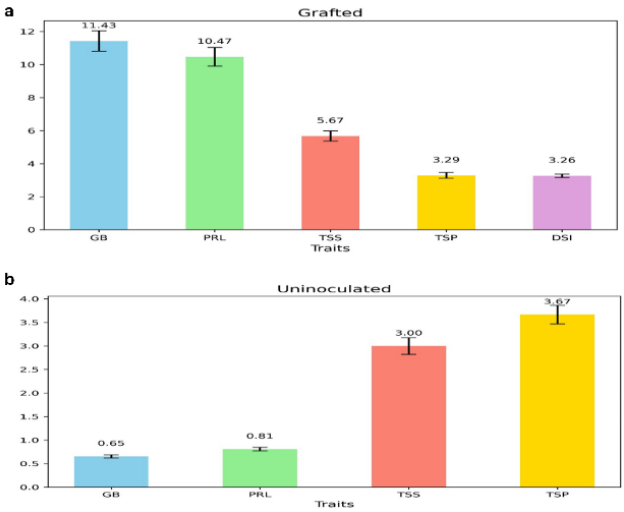


Figure 1: Mean performance of 32 cotton genotypes in uninoculated and Graft inoculated during 2023. *p≤0.05, **p≤0.01, and ***p≤0.001. NS= non-significant

Glycine betaine (GB)

It was revealed that very low accumulation of GB in uninoculated plants ranged from 0.37 to 1.46 µmol g^{-1} FW whereas this was increased in graft inoculation from 2.73 to 23.13 µmol g^{-1} FW. Genotype FH-494 accumulated the maximum fold for the trait with a value of 23.13 µmol g^{-1} FW followed by FH-938 (21.77), FH-492 (20.63), FH-super cotton (18.67), FH-Anmol (18.67) and FH-520 (17.56). In contrast, the minimum performance for accumulation of osmolyte in genotypes such as FH-534 (2.75) and FH-933 (2.73) was observed. Differences in osmolyte accumulation and the findings suggest that tolerant genotypes cope with stress by distinctly augmenting GB accumulation (Figures 2, 6,7).

Proline (PRL)

In graft inoculated treatment, it was revealed that during stress environment the PRL enhanced accumulation. In healthy (Uninoculated) plants, accumulation observed with a range from 0.18 to 1.17 µmol g^{-1} FW, while inoculated plants mean levels increased with a range from 3.45 to 21.07 µmol g^{-1} FW. The highest proline concentrations 21.07 µmol g^{-1} FW were observed in genotype FH-494 followed by FH-938 (21.06), FH-492 (21.05), FH-Super Cotton (21.04). While only minor increases were noticed in FH-534 (3.65) and FH-933 (3.45). These outcomes emphasize proline as an important osmolyte remarkably generated under treated plants (Figures 3, 6).

Table 2. Classification of cotton genotypes based on disease response and disease severity index (DSI).

Disease Response	Genotype(s)	DSI (Range)
Susceptible (S)	FH-534, FH-933, FH-509, FH-5100, FH-415	**4.4 – 4.7**
Moderately Susceptible (MS)	FH-903, FH-114, FH-416, FH-451, FH-453, FH-490, FH-529	**3.5 – 4.4**
Tolerant (T)	FH-554, FH-5099, FH-513, FH-5200, FH-522, FH-526, FH-889, FH-528, FH-5098, VH-327, FH-5300, FH-511	**2.6 – 3.4**
Moderately Resistant (MR)	FH-930, FH-525, FH-Anmol, FH-520, FH-Super Cotton, FH-938, FH-492, FH-494	**1.6 – 2.4**

Total soluble sugars (TSS)

Total soluble sugar (TSS) also exhibited significant variations in accumulation between inoculated and uninoculated treatments. In the uninoculated genotypes, TSS figured the mean value from 2.14 to 3.86 mg g^{-1} FW, whereas it was recorded that there was a moderate increase in inoculated plants, ranging from 3.78 to 10.74 mg g^{-1} FW. The maximum values were reflected in FH-938 (10.74), FH-494 (10.63), and FH-492 (10.27). Genotypes such as FH-534 (3.78) followed by with the mean value of 2.79 displayed the lowest increase, concluding restricted sugar-mediated protection responses in sensitive genotypes (Figures 4, 6,7).

Total soluble proteins (TSP)

Total soluble protein (TSP) content set out a varied inclination. Uninoculated healthy plants have been observed with the concentration of 1.96-7.15 mg g^{-1} FW, while graft

inoculated figured with a range between 1.92 and 6.82 mg g^{-1} FW. Susceptible genotypes demonstrated declines in protein folds with a range of 1.96 to 1.92 in FH-534 and 1.96 to 1.92 in FH-933. However, higher accumulation was figured in FH-494 (7.15 to 6.82) and FH-492 (7.14 to 5.20) in uninoculated and inoculated treatment. Genotypes that possessed relatively higher protein levels, reflect greater proteome permanence under stress conditions were found to be tolerant genotypes (Figures 5, 6, 7).

Overall findings revealed an increase in GB, PRL folds a moderate elevation in TSS levels, and marginally variable drops in TSP. Genotypes FH-494, FH-938, FH-Anmol, FH-520, and FH-Super Cotton exhibited the remarkable biochemical alterations and can therefore be recognized relatively tolerant. While, FH-534, FH-933 and FH-509 highlighted the minimum responses, indicative of susceptibility.

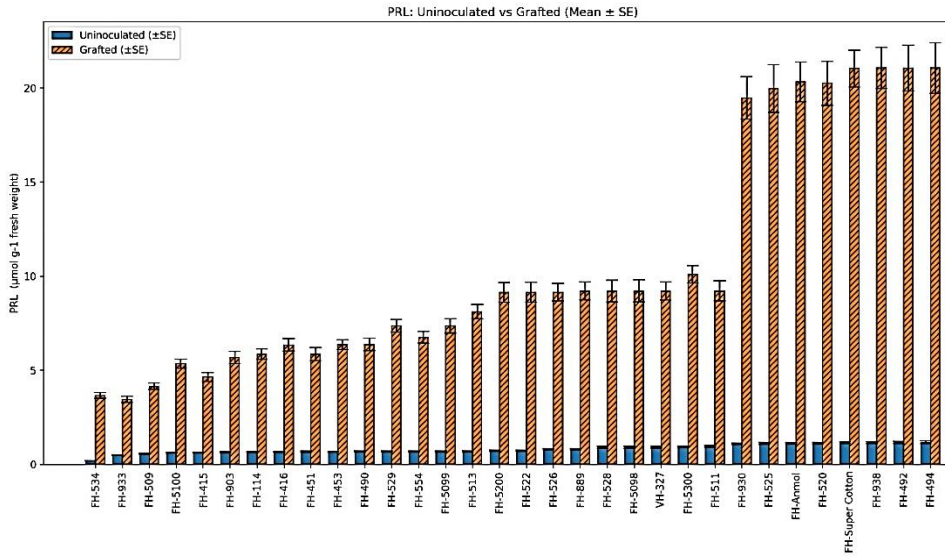


Figure 2 Mean value of glycine betaine in 32 cotton genotypes under uninoculated and graft inoculated during 2023.

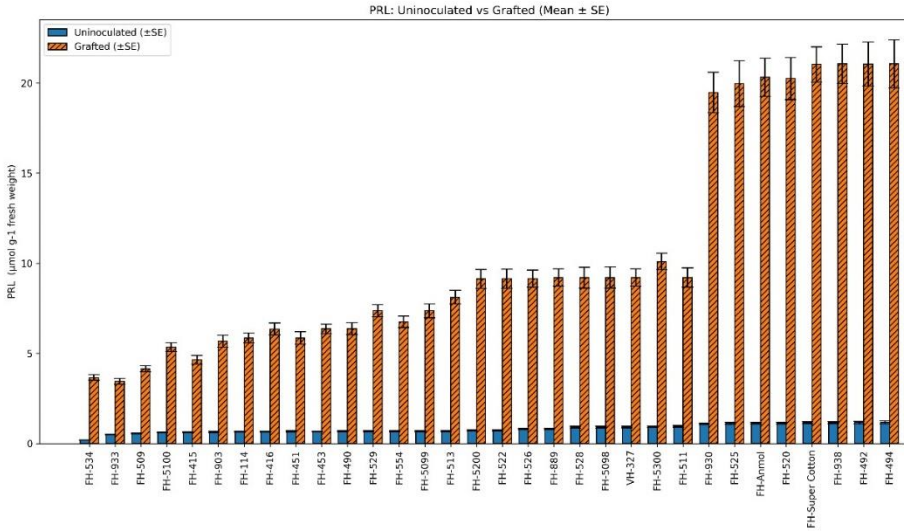


Figure 3 Mean value of proline in 32 cotton genotypes under uninoculated and graft inoculated during 2023.

Disease severity index (DSI)

The examined genotypic response to CLCuV further uncovered reasonable variation. Five genotypes (FH-534, FH-933, FH-509, FH-5100 and FH-415) displayed the maximum disease severity index (DSI) values form 4.4-4.7 that categorized them as susceptible. Whereas a set of 7 genotypes (FH-903, FH-114, FH-416, FH-451, FH-453, FH-490 and FH-529), figured relatively extreme DSI value form 3.5-4.4, grading them as moderately sensitive. Afterwards there was a set of 12 gentypes, including FH-554, FH-5099, FH-513, FH-5200, FH-522, FH-526, FH-889, FH-528, FH-5098, VH-327, FH-5300, and FH-511 figured with lower DSI values ranged from 2.6 to 3.4 and were recognized themselves in tolerant category. Remarkable results revealed that a set of eight gentypes (FH-930, FH-525, FH-Anmol, FH-520, FH-Super Cotton, FH-938, FH-492, and FH-494),

which documented the lowest DSI figures from 1.6 to 2.4 and were thus classified as moderately resistant. Two genotypes, FH-492 and FH-494 remarkably exhibited the best performance in graft inoculated treatment with minimum DSI (~1.6), emphasizing their better resistance potential (Table 2; Figures 6, 8).

Relative changes in osmolytes

Biochemical responses further explained with the percentage analysis Graft inoculation examination of GB and PRL demonstrated the maximum percentage increase ranging from +532% in FH-933 to +3157% in FH-Anmol. Similar findings were found for TSS, TSS increased in all genotypes, with percentage accumulation levels ranging from FH-451 (+37.7%) to FH-Anmol (+189.2%). Differing from these, TSP concentration usually decreased, excepting in a few tolerant genotypes. The highest percentage were documented in FH-492 (+27.15%), FH-525 (+18.85%), and FH-520 (+17.65%), while drops were monitored in FH-5100 (-16.78%) and FH-415(-16.19%). Relatively smaller increases in GB, PRL and TSS were witnessed in FH-933, FH-509, and FH-415, representing better osmotic adjustment. In comparison, FH-Anmol, FH-525, and FH-930 experienced substantial reductions, highlighting their extreme sensitivity to CLCuV disease (Figure 8).

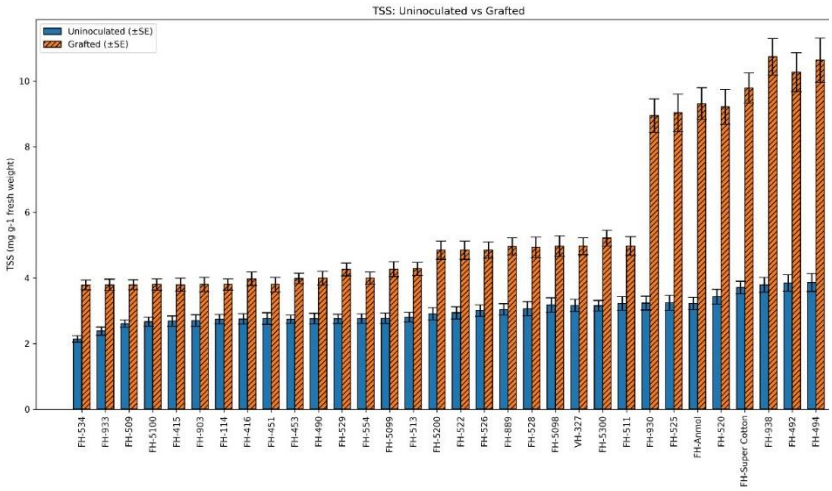


Figure 4 Mean value of total soluble sugars in 32 cotton genotypes under uninoculated and graft inoculated during 2023.

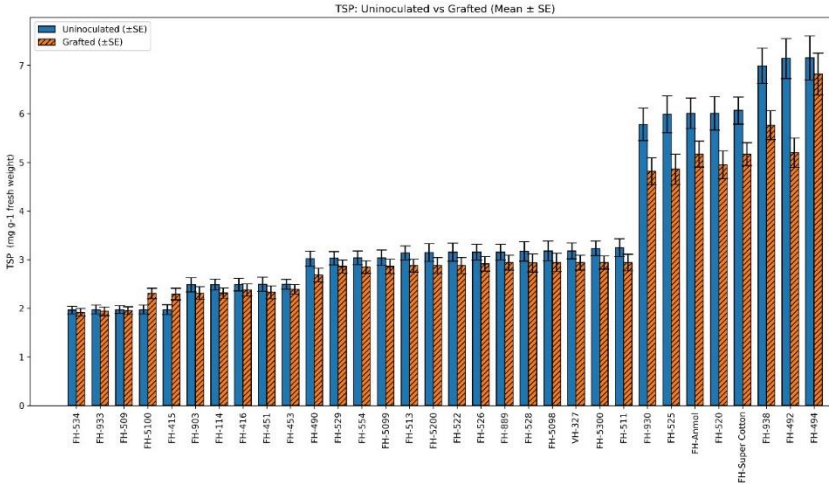


Figure 5 Mean value of total soluble protein in 32 cotton genotypes under uninoculated and graft inoculated during 2023.

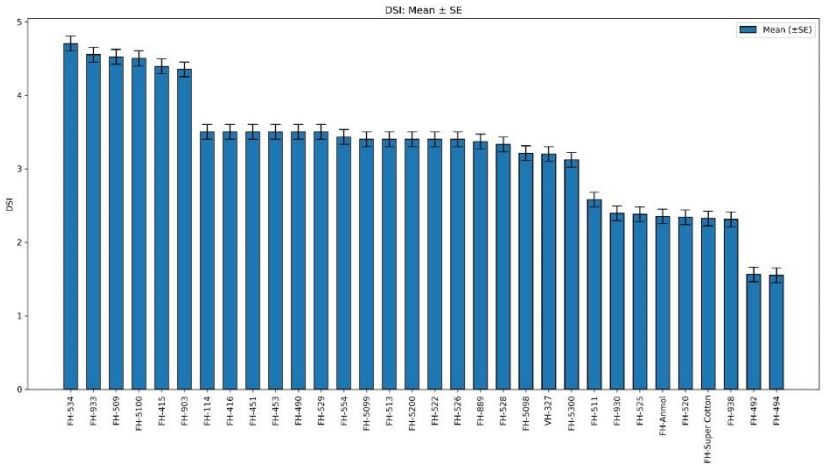


Figure 6. Classification of cotton genotypes based on disease severity index (DSI).

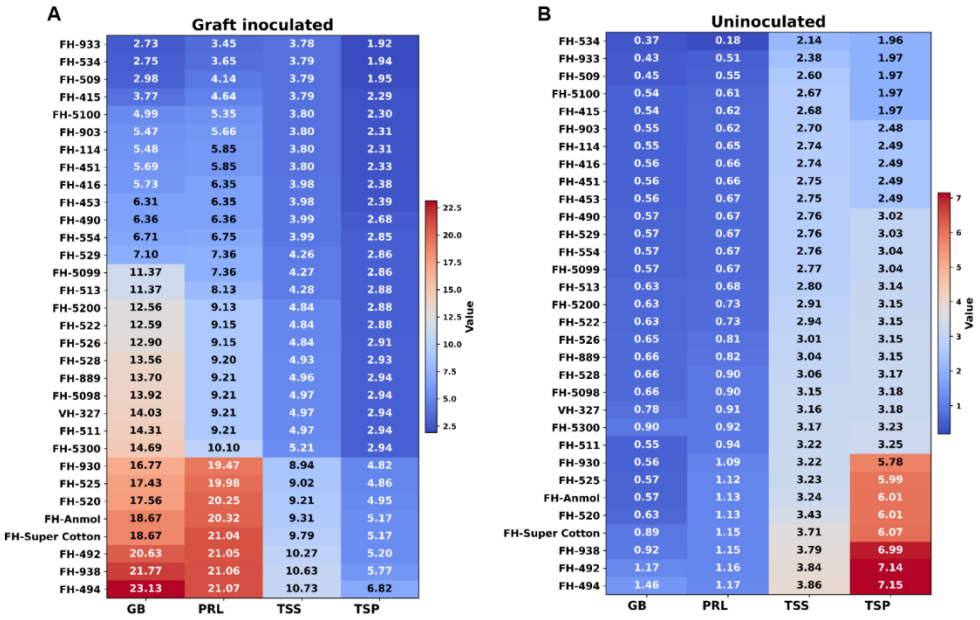


Figure 7 Heat map of mean performance of biochemical attributes in 32 genotypes under graft inoculated (A) and uninoculated (B) cotton genotypes during 2023.

Correlation Study between disease severity Index and biochemical Traits

The correlation analyses established significant negative relationship between DSI and biochemical attributes (GB (-0.92), PRL (-0.90), TSS (-0.87), and TSP (-0.90)}, representing that genotypes with maximum levels of these biochemical attributes experienced minimum disease severity index (Figure 9). This significantly negative association demonstrated that GB, PRL, TSS and TSP play protective roles in limiting disease development. However, GB, PRL, TSS, and TSP display remarkably positive correlations with each other (r = 0.88 to 0.99), indicating a organized metabolic and defense response during disease pressure. Such linkages indicate that these metabolites may act concertedly to improve cellular stability and disease tolerance. The maximum negative association between DSI and these attributes stresses their potential function as secondary biochemical indicators for disease resistance. Therefore, genotypes with improved osmolyte and protein accumulation can be pondered superior contestants' genotypes for breeding programs aiming enhanced disease tolerance.

Discussion

Genotypes exhibited biochemical accumulation and respond divergently to cotton leaf curl virus (CLCuV) disease, as signaled by differences in osmolyte concentrations and protein stability among tolerant and susceptible genotypes. A set of genotypes (FH-494, FH-492, FH-938, FH-Anmol, FH-520, and FH-Super Cotton), revealed moderately higher levels of GB and PRL, moderate rise in TSS levels, and subjective maintenance of TSP. These biochemical figures were steadily associated

with minimum DSI, suggesting that analogous to these traits are associated with increased stress adjustment under viral disease. However, non-existence of functional validation or molecular verification, these metabolic responses should be elucidated as associated indicators of tolerance rather than direct determinant factors of resistance. For example, FH-492 and FH-494 joined low severity scores (~1.6) with significant osmolyte accumulation and relative protein permanence, displaying their potential utility as tolerant genotypes without involving a definitive fundamental mechanism.

While extremely susceptible genotypes (FH-534, FH-933, FH-509, FH-5100 and FH-415) showed minimum osmolyte responses, noticeable reductions in TSP content, and substantially higher DSI (>4.4). The constant coincidence of raised DSI with reduced biochemical buffering capacity strengthens the interpretation that these attributes reflect the physiological position of plants under viral disease incidences, rather than functioning as separate resistance mechanisms. Such patterns affiliate with earlier explanations in CLCuV disease systems, where tolerant genotypes frequently exhibit more constant metabolic profiles than susceptible (7,42,43). Instead of this, decidedly susceptible genotypes (FH-534, FH-933, FH-509, FH-5100, and FH-415) revealed lower osmolyte responses, marked drops in TSP content and extensively higher DSI (>4.4). Osmolyte increase is extensively reported as a natural metabolic adjustment under varied stress conditions (3,44-46).

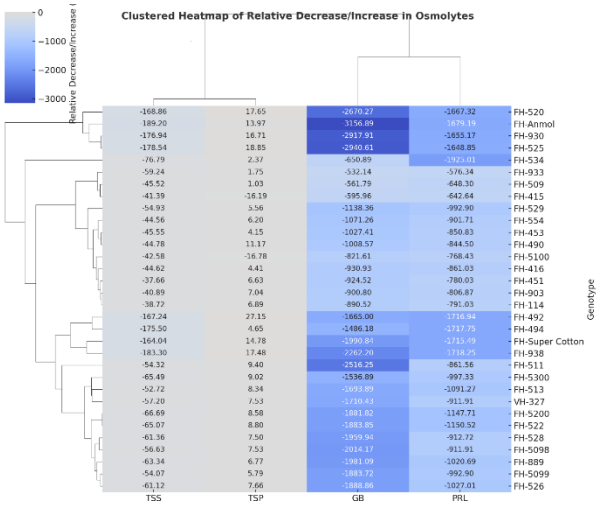
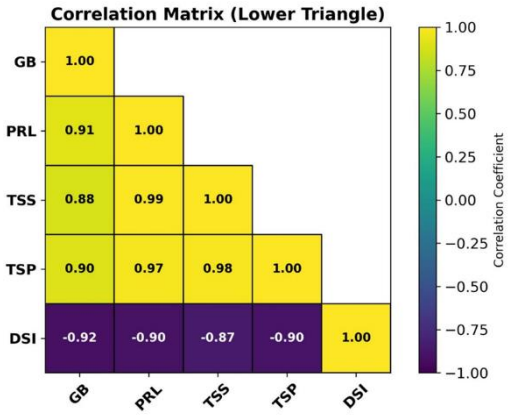


Figure 8 Heat map of relative decrease percentage (RD %) biochemical attributes in 32 genotypes under uninoculated and graft inoculated cotton genotypes during 2023.

In the present study, higher GB and PRL levels in genotypes with lower DSI levels were linked with lowered disease severity, indicating their contribution in maintaining cellular homeostasis during infection. Although reactive oxygen species (ROS) were not quantified here, previous studies propose that osmolytes may indirectly influence ROS homeostasis, membrane protection, and stress signaling pathways under adverse conditions (47,48). These reports provide a hypothetical framework for interpreting the observed osmolyte responses, rather than evidence of ROS-mediated mechanisms in the current dataset. The higher GB observed in FH-494, FH-938, and FH-Anmol is consistent with earlier findings describing its role in stabilizing photosystem II, preserving ionic balance, and reducing oxidative damage under stress (3,26,32,46,49). Similarly, proline accumulation, as observed in FH-492, FH-494 and FH-Super Cotton, may reflect heightened osmotic adjustment and metabolic flexibility, as suggested in prior studies (3,30,47).

Figure 9. Correlation plot of disease severity Index (DSI)



biochemical viz Glycine betaine (GB), Total Soluble protein (TSS), Proline (PRL), and Total Soluble sugars (TSS) of 32 cotton cultivars/genotypes. * Indicates significant (P≤0.05) and without sign (*) indicates non-significant (P ≥ 0.05).

Total soluble sugars exhibited reasonable boosts in tolerant genotypes, although their response was less marked than that of GB and PRL. This comparatively modest change may demonstrate genotypic variations in carbon allocation or stage-specific metabolic priorities during viral infection, as reported by Akbar et al. (2021) and Kaur et al. (2024) (50,51). Rather than acting as primary resistance factors, TSS may contribute to sustaining basic metabolic demands and signaling developments during stress, thereby establishing overall plant survival.

Protein stability also arose as an important biochemical trait associated with tolerance during abiotic and biotic stresses (3,7). Genotypes including FH-492 and FH-494 maintained comparatively higher TSP levels compared to susceptible genotypes, indicating lowered protein degradation during viral pressure. Previous studies suggest that protection of TSP can relieve maintain enzymatic activity, cellular structure, and stress alertness during pathogen attack (3,7,51,52,53). In contrast, the definite decline in TSP content observed in susceptible genotypes may outcome from virus-induced metabolic disturbance, enhanced proteolysis, or protein denaturation (42,54). These findings further support the interpretation of TSP as a biochemical marker of stress status, rather than a direct contributor to resistance.

The experiential relationships among DSI and biochemical attributes reveal that higher accumulation of GB, PRL, TSS, and TSP is inclined to accord with decreased disease expression. Such negative significant associations advocate coordinated metabolic adjustment in tolerant genotypes, where several biochemical pathways respond simultaneously to disease. The positive interrelations among studied metabolites indicate a degree of coregulation, potentially exhibiting integrated stress adaptation rather than independent defense mechanisms. Importantly, while these associations are useful, they do not establish connection and should be interpreted as reassuring indicators valuable for reasonable screening.

From a breeding goal to develop cultivars with disease resistance gentypes, FH-492 and FH-494 perform specifically promising, as they constantly combined minimal DSI with balanced biochemical profiles. Observing attributes including GB, PRL, and TSP may therefore express practical and profitable approach for initial screening for tolerant genotypes, balancing conventional phenotypic evaluations. Furthermore, exogenic function of osmolytes like proline or glycine betaine has been proposed as a short-term mitigation strategy. Association study highlighted that joining correlation-based authentication, metabolomics, transcriptomics, and targeted guidance of osmolyte biosynthetic pathways will be essential to elucidate the functional significance of these attributes and support their application in cotton cultivars development and improvement programs.

Conclusion

This study approves that resistance to CLCuV in cotton is significantly associated with heightened osmolyte accumulation and protein stability. Genotypes including FH-492 and FH-494, showed the maximum defense by maintaining low DSI, increase levels of GB and PRL, and reasonably stable TSP concentration. These attributes feature that mutually osmotic regulation and protection of protein integrity are indispensable for sustaining metabolic balance under disease occurrence. The different responses of susceptible genotypes additionally validate that osmolyte accumulation initiation is a functioning and operative defense mechanism. The recognition of important biochemical markers such as GB, PRL, and TSP delivers a applied basis for picking resistant cultivars and can be efficiently integrated into breeding programs. In addition, management practices involving exogenous osmolyte application may offer temporary relief, though long-term resistance will require the incorporation of tolerant germplasm and advanced molecular tools to enhance innate defense pathways.

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Authors' Contribution Statements: MKSS: Execution of experiments, data collection, Final revision and editing, supervised the experiment and provided the resources, MU: Statistical analysis, data collection, SM: data collection, MZ: data collection, MYA: Final revision and editing, Proofread the manuscript

References

1. Riello G, Parthasarathi P. The spinning world: a global history of cotton textiles, 1200-1850. Oxford University Press; 2011.
2. Voora V, Larrea C, Bermudez S. Global market report: cotton. Geneva, Switzerland: International Institute for Sustainable Development; 2020 Jun.
3. Sarwar MKS, Ghaffar A, Ahmad S, Rehman SU, Waheed U. Assessing genetic potential and development of crosses for breeding cotton (Gossypium hirsutum L.) against drought. Asian J. Agric. Biol. 2025; 2025:2024027.
4. Shani MY, Ashraf MY, Ramzan M, Khan Z, Batool N, Gul N, Bauerle WL. Unveiling drought tolerant cotton genotypes: insights from morpho-physiological and biochemical markers at flowering. Plants. 2025 Feb 18;14(4):616.
5. USDA. Global market analysis. Foreign Agricultural Service, United States Department of Agriculture; 2026.
6. Dilip M. Cotton leaf curl virus disease: an overview. Agric Res J. 2016;53(4):466–74.
7. Nadeem S, Riaz Khan MK, Hameed A. Screening and biochemical profiling of 'Mac-07'crosses for resistance to CLCuD under field and glasshouse conditions. BMC Plant Biology. 2025 Oct 6;25(1):1317.
8. Farooq J, Farooq A, Riaz M, Shahid MR, Saeed F, Iqbal MS, Hussain T, Batool A, Mahmood A. Cotton leaf curl virus disease a principle cause of decline in cotton productivity in Pakistan (a mini review). Can. J. Plant Prot. 2014; 2:9-16.
9. Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG.Geminivirus transmission and biological characterisation of Bemisia tabaci (Gennadius) biotypes from different geographic regions. Annals of applied biology. 1994 Oct;125(2):311-25.
10. Qadir R, Khan ZA, Monga D, Khan JA. Diversity and recombination analysis of Cotton leaf curl Multan virus: a highly emerging begomovirus in northern India. BMC genomics. 2019 Apr 6;20(1):274.
11. Kumar R, Tiwari RK, Jeevalatha A, Siddappa S, Shah MA, Sharma S, Sagar V, Kumar M, Chakrabarti SK. Potato apical leaf curl disease: current status and perspectives on a disease caused by tomato leaf curl New Delhi virusJ. Plant Dis. Prot. 2021 Aug;128(4):897-911.
12. Brown JK, Khan Z. Breeding cotton for cotton leaf curl disease resistance. InCotton breeding and biotechnology 2022 Mar 14 (pp. 171-197). CRC Press.
13. Mansoor S, Amin I, Iram S, Hussain M, Zafar Y, Malik KA, Briddon RW. Breakdown of resistance in cotton to cotton leaf curl disease in Pakistan. Plant Pathol. 2003 Dec 1;52(6):784.

14. Hussain M, Gao X, Qin D, Qin X, Wu G. Role of biotic and abiotic factors for sustainable cotton production. In: Best crop management and processing practices for sustainable cotton production. IntechOpen; 2023.
15. Briddon RW, Markham P. Cotton leaf curl virus disease. Virus Res. 2000;1;71(1-2):151-9.
16. Monga D. Cotton leaf curl virus disease. Technical bulletin, Central Institute for Cotton Research, Nagpur; 2014. p.1-34.
17. Sattar MN, Iqbal Z, Tahir MN, Ullah S. The prediction of a new CLCuD epidemic in the Old World. Front. Microbiol. 2017;8:631.
18. Azhar MT, Amin I, Anjum ZI, Arshad M, Briddon RW, Mansoor S. Both malvaceous and non-malvaceous betasatellites are associated with two wild cotton species grown under field conditions in Pakistan. Virus genes. 2010 Dec;41(3):417-24.
19. Azhar MT, Rehman MU, Aftab S, Zafar Y, Mansoor S. Utilization of natural and genetically-engineered sources in Gossypium hirsutum for the development of tolerance against cotton leaf curl disease and fiber characteristics. Int. J. Agric. Biol. 2010;12:744-48.
20. Akhtar KP, Haidar S, Khan MKR, Ahmad M, Sarwar N, Murtaza MA, Aslam M. Evaluation of Gossypium species for resistance to cotton leaf curl Burewala virus. Ann. Appl. Biol. 2010;157(1):135-47.
21. Akhtar KP, Ullah R, Khan IA, Saeed M, Sarwar N, Mansoor S. First symptomatic evidence of infection of Gossypium arboreum with cotton leaf curl Burewala virus through grafting. Int. J. Agric. Biol. 2013; 15:157-60.
22. Akhtar KP, Hussain M, Sarwar M, Sarwar N. Evaluation of Bt-cotton genotypes for resistance to cotton leaf curl disease under high inoculum pressure in the field and using graft inoculation in glasshouse. Plant Pathol. J. 2015;31(2):132.
23. Mansoor S, Briddon RW, Zafar Y, Stanley J. Geminivirus disease complexes: an emerging threat. Trends Plant Sci. 2003;8(3):128-34.
24. Iqbal N, Ashraf MY, Javed F, Ashraf M, Hameed S. Cotton leaf curl virus: Ionic status of leaves and symptom development. J. Integr. Plant Biol. 2006;48(5):558-62.
25. Tahir MN, Amin I, Briddon RW, Mansoor S. The merging of two dynasties identification of an African cotton leaf curl disease-associated begomovirus with cotton in Pakistan. PLoS One. 2011;6(5):e20366.
26. Jones JDG, Dangl JL. The plant immune system. Nature. 2006;444(7117):323-9.
27. Torres MA, Jones JD, Dangl JL. Reactive oxygen species signaling in response to pathogens. Plant Physiol. 2006;141(2):373-8.
28. Jarin A, Ghosh UK, Hossain MS, Mahmud A, Khan MAR. Glycine betaine in plant responses and tolerance to abiotic stresses. Discov. Agric. 2024;2(1):127.
29. Jarin AS, Islam MM, Rahat A, Ahmed S, Ghosh P, Murata Y. Drought stress tolerance in rice: physiological and biochemical insights. Int. J. Plant Biol. 2024 Jul 21;15(3):692-718.
30. Renzetti M, Funck D, Trovato M. Proline and ROS: A unified mechanism in plant development and stress response? Plants. 2024;14(1):2.
31. Ashraf MFM, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 2007;59(2):206-16.
32. Ashraf MHPJC, Harris PJ. Photosynthesis under stressful environments: An overview. Photosynthetica. 2013; 51(2): 163-90.
33. Abro AA, Qasim M, Younas MU, Ali I, Abbas M, Muhammad N, Khalid S, Ahmed J, Bibi U, Waqas M, Ercisli S. Impact of elevated temperatures on the genetic and morpho-physiological traits of cotton genotypes cultivation. Genet. Resour. Crop Evol. 2025;72(3):2533-60.
34. Ali A, Zafar S, Ashraf MY. Assessment of growth and biochemical indicators for drought tolerance in maize cultivars. Pak. J. Bot. 2025;57(1):125-34.
35. Sarwar MK, Ashraf MY, Rahman M and Zafar Y, 2012. Genetic variability in different biochemical traits and their relationship with yield and yield parameters of cotton cultivars grown under water stress conditions. Pak. J. Bot. 44:515-20.
36. Kumar R, Kaundal P, Tiwari RK, Siddappa S, Kumari H, Lal MK, et al. Establishment of a one-step reverse transcription recombinase polymerase amplification assay for the detection of potato virus S. J. Virol. Methods. 2022;307:114568.
37. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39(1):205-7.
38. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951;193(1):265-75.
39. Yemm EW, Willis AJ. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 1954;57(3):508.
40. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil. 1983;70(2):303-7.
41. St L, Wold S. Analysis of variance (ANOVA). Chemometrics and intelligent laboratory systems. 1989 Nov 1;6(4):259-72.
42. Montasser MS, Al-Own FD, Haneif AM, Afzal M. Effect of tomato yellow leaf curl bigeminivirus (TYLCV) infection on tomato cell ultrastructure and physiology. Can. J. Plant Pathol. 2012;34(1):114-25.
43. Chauhan P, Mehta N, Chauhan R, Kumar A, Singh H, Lal MK, et al. Utilization of primary and secondary biochemical compounds in cotton as diagnostic markers for measuring resistance to cotton leaf curl virus. Frontiers in Plant Science. 2023;14:1185337.
44. Hahn A, Bublak D, Schleiff E, Scharf KD. Crosstalk between Hsp90 and Hsp70 chaperones and heat stress transcription factors in tomato. Plant Cell. 2011;23(2):741-55.
45. Woodrow P, Ciarmiello LF, Annunziata MG, Pacifico S, Iannuzzi F, Mirto A, et al. Durum wheat seedling responses to simultaneous highlight and salinity involve

a fine reconfiguration of amino acids and carbohydrate metabolism. Physiol. Plant. 2017;159(3):290-312.
46. Singh CK, Rajkumar BK, Kumar V. Differential responses of antioxidants and osmolytes in upland cotton (Gossypium hirsutum) cultivars contrasting in drought tolerance. Plant Stress. 2021;2:100031.
47. Monteoliva MI, Rizzi YS, Cecchini NM, Hajirezaei M-R, Alvarez ME. Context of action of proline dehydrogenase (ProDH) in the hypersensitive response of Arabidopsis. BMC Plant Biology. 2014;14(1):21.
48. Rojas CM, Senthil-Kumar M, Tzin V, Mysore KS. Regulation of primary plant metabolism during plant–pathogen interactions and its contribution to plant defense. Front. Plant Sci. 2014;5:17.
49. Sarwar MKS, Ullah I, Ashraf M, Zafar Y. Glycinebetaine accumulation and its relation to yield and yield components in cotton genotypes grown under water deficit condition. Pak. J. Bot. 2006;38(5):1449-56.
50. Akbar S, Yao W, Yu K, Qin L, Ruan M, Powell CA, Chen B, Zhang M. Photosynthetic characterization and expression profiles of sugarcane infected by Sugarcane mosaic virus (SCMV). Photosynthesis Res. 2021 Dec;150(1):279-94.
51. Kaur T, Mandhania S, Singh V, Datten R, Banakar P, Malik K, Sankaranarayanan K, Jattan M, Kumar S, Saini AK, Jakhar A. Differential biochemical and physiological responses to cotton leaf curl virus infection in contrasting cotton genotypes. Acta Physiol. Plant. 2024;46(4):46.
52. Mahas JW, Roberts PM, Conner K, Graham S, Huseth AS, Steury TD, Mahas JB, Bag S, Bowen K, Jacobson AL. Cotton-Dominated Landscapes, Vector Abundance, and Temperature Influence Cotton Leafroll Dwarf Virus Incidence in Cotton. Phytopathology. 2026 Feb 13(ja).
53. Bawa G, Liu Z, Zhou Y, Fan S, Ma Q, Tissue DT, Sun X. Cotton proteomics: Dissecting the stress response mechanisms in cotton. Front. Plant Sci. 2022;13:1035801.
54. Chatterjee A, Ghosh SK. Alterations in biochemical components in mesta plants infected with yellow vein mosaic disease. Braz. J. Plant Physiol. 2008;20:267-75.