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RESEARCH ARTICLE

Comparative analysis of salt tolerance in cotton cultivars under saline water stress

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Abstract

Soil salinity imposes multiple stresses on cotton (*Gossypium* spp.) and the use of saline water for irrigation is common in coastal regions. However, the effects of borewell saline water (NaCl) on cotton cultivars remains underexplored. A pot experiment conducted during 2017-2018 aimed to evaluate the morphological, biochemical and physiological responses of six cotton cultivars- *G. herbaceum* (G-Cot 25, Jayadhar), *G. arboreum* (Phule dhanwantry, Roja) and *G. hirsutum* (Suraj, LRA-5166) subjected to NaCl treatments (100, 150 and 200 mM) and a control. All cultivars exhibited a progressive decline in growth parameters with increasing salinity. Among them, Jayadhar and Suraj demonstrated the highest tolerance, maintaining superior growth and physiological performance under NaCl stress. Relative water content decreased significantly across all cultivars; however, G-Cot 25 and LRA-5166 retained higher water content under saline conditions. Biochemically, NaCl stress resulted in reduced levels of total chlorophyll and carotenoids, alongside an increase in protein content, suggesting adaptive responses to salinity. Jayadhar, Suraj and Phule Dhanwantry exhibited greater chlorophyll retention, indicating enhanced biochemical stability. Malondialdehyde (MDA) levels increased under salinity, signifying lipid peroxidation; however, Jayadhar and Suraj accumulated lower MDA levels, suggesting reduced oxidative damage. Proline content increased in all cultivars, with Jayadhar and Suraj showing the highest accumulation, reflecting improved osmotic adjustment. Activities of antioxidant enzyme including superoxide dismutase, catalase and peroxidase were significantly enhanced, particularly in G-Cot 25, Jayadhar and Phule dhanwantry respectively. These findings indicate that antioxidant defense mechanisms play a crucial role in mitigating oxidative damage by scavenging reactive oxygen species under saline conditions. Based on an integrative assessment, the *G. herbaceum* cultivar Jayadhar demonstrated the highest tolerance to salinity.

Keywords: antioxidant; biochemical; cotton; morphology; NaCl stress; physiological; salinity

Introduction

Soil salinity is a major constraint to agricultural productivity, affecting approximately 6.73 million ha in India; equivalent to 5 - 8 % of cultivated land, depending on regional variability. Salinity reduces soil fertility, impairs water and nutrient uptake and causes physiological drought, ultimately lowering crop yields. Annual crop production losses due to salinity in India are estimated at 5.66 million t, translating to economic losses of around ₹80 billion (1).

Cotton (*Gossypium* spp.) is considered moderately tolerant to salinity, though responses vary significantly among species and cultivars (2). Salt stress adversely affects germination, seedling growth and early vegetative development, primarily through osmotic stress, ion toxicity and nutrient imbalance (3). These stresses disrupt key physiological processes such as photosynthesis, stomatal regulation and cellular homeostasis, often leading to excessive generation of reactive oxygen species (ROS) and subsequent oxidative damage. Additionally, salinity negatively affects root development, shoot growth and overall biomass accumulation.

In saline-affected areas, high concentrations of soluble salts, such as NaCl, $NaHCO_3$, $MgSO_4$ and $CaCl_2$, dissociate into anions (chloride [Cl^-], sulfate [SO_4^-], carbonate [CO_3^-] and bicarbonate [HCO_3^-]) and cations (calcium [Ca], magnesium [Mg] and sodium [Na]). This imbalance in ionic composition reduces soil fertility, disrupts water and nutrient uptake and hampers transpiration. Consequently, physiological drought occurs and the availability of essential nutrients such as phosphorus and micronutrients is diminished.

Salt stress also reduces the photosynthetic rate, limiting biomass production. High salinity alters several physiological, morphological and biochemical factors in plants, including modifications to the water potential of cells, reduced water availability and impaired CO_2 assimilation. These adversely affects stomatal function (4). Salt stress causes ionic imbalance at the cellular ions, resulting in ion toxicity, osmotic stress and the production of ROS. These factors collectively impair plant growth, morphology and survival (5). Ion toxicity can damage roots and shoot membranes, alter hormonal levels, disrupt enzyme activities and interfere with metabolic processes, potentially leading to plant death and crop failure (6). Furthermore, both drought and salt stress contribute to oxidative stress in plants (7).

The root cell membrane is typically the first structure affected by salinity, as roots are essential for water and nutrient absorption. Salinity often results in stunted root and shoot growth and varying salt concentrations in the root zone significantly impact root growth and development (8). Cotton's response to salinity is genotype-dependent, with different cultivars exhibiting variable tolerances across a salinity range of 1-4 dS m^{-1} (9). Most cotton cultivars display substantial variation under saline conditions, especially during critical stages such as germination and vegetative growth. It is essential to examine changes in root and shoot morphology, physiological processes, antioxidant activity and biochemical parameters to better understand cultivar specific responses to salinity (10).

Salinity is a major factor limiting factor in global crop production, accounting for up to 40 % of total yield losses (11). Crops are categorized based on yield loss under saline conditions: sensitive (soybean, maize and rice); moderately sensitive (tomato); moderately tolerant (cotton, wheat); tolerant (sorghum and barley). On average, cotton yield reductions due to salt stress are reported to be in the range of 20 % to 50 %, depending on the salinity level. Central Institute for Cotton Research (CICR) project focused on breeding cotton cultivars suitable for saline and sodic soils in regions like Gujarat, Punjab and Maharashtra. Similarly, project under National Agricultural Technology Project (NATP) aimed to improve abiotic stress tolerance, including salinity, in key crops including cotton.

Similarly, among the three cotton species cultivated, the salt tolerance mechanism is partially understood. Previous studies have explored salinity tolerance in cotton using transgenic approaches or by evaluating seedling stage under controlled conditions. However, limited attention has been paid to field-relevant or pot culture studies using regionally adapted cultivars exposed to realistic levels of salinity (1-4 dS m^{-1}). Moreover, interspecific variation in morphological, physiological and biochemical responses, particularly in relation to antioxidant defense mechanisms and osmotic adjustment, remains under characterized in Indian cotton cultivars.

Therefore, improving the development of salt-tolerant cotton cultivars is vital for the sustainable utilization of saline soils and for addressing challenges posed by climate change and increasing global food and fibre demand. Enhancing cotton productivity, ensuring ecological sustainability in coastal regions and improving resilience for resource-poor farmers are key goals in this context.

The aim of this study is to evaluate the impact of exogenous NaCl application on cotton morphology, biochemical and physiology. Specifically, the study focuses on integrative parameters across three different species [*G. herbaceum*, *G. arboreum* and *G. hirsutum*] cotton plants cultivated in India under varying salt stress conditions. The goal is to identify cultivars with enhanced salt tolerance for potential use in saline prone agricultural systems.

Materials and Methods

Location and experimental setup

The experiment was conducted during the off season (summer 2017-18) at the Indian Council for Agricultural Research (ICAR) - Central Institute for Cotton Research (CICR), Nagpur, located at 79°04′40″ E longitude and 21°04′71″ N latitude. Meteorological data recorded during the salinity experiment are provided in Fig. 1 and the physico-chemical properties of the experimental soil are detailed in Table 1. Soil for the pot experiment was collected from Plot (E-44) of the ICAR-CICR, Panjari farm. Cotton stubbles, weed roots, pebbles and trash were cleaned by primary sieving. The clayey soil, which had low organic matter, was further sieved using a 2 mm mesh and each pot received 10 kg soil. In each pot compost (20 g) and vermicompost (20 g) were applied

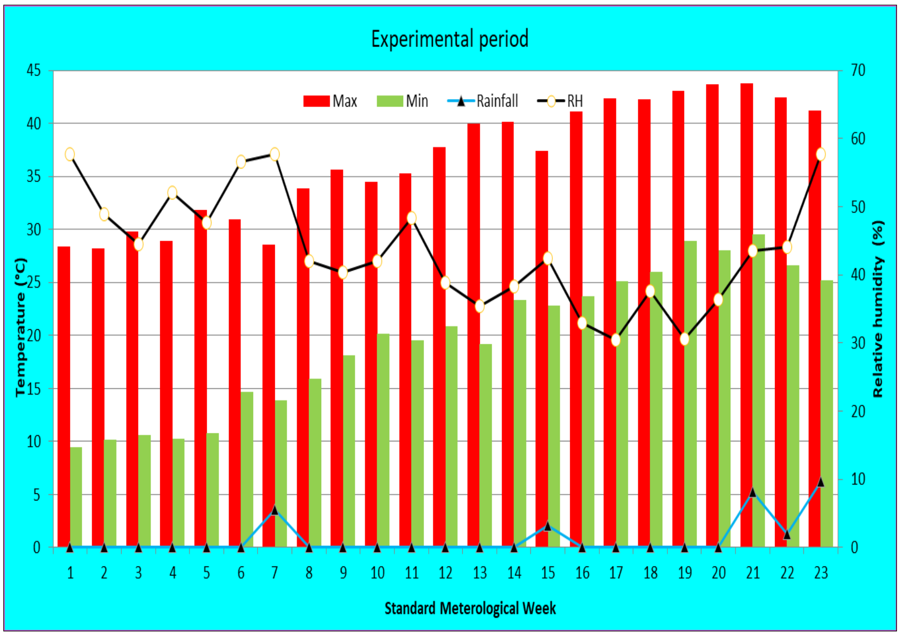


Fig. 1. Meteorological data of CICR, Nagpur during salinity study (2018).

Table 1. Experimental soil physico-chemical properties

Soil Parameters	Status
pH	07.80
EC (dSm^{-1})	00.26
Free calcium carbonate (%)	04.30
Organic carbon (%)	00.40
Available N (kg ha^{-1})	120.00
Available P (kg ha^{-1})	20.00
Available K (kg ha^{-1})	450.00
Available S (mg/kg)	15.00
B (mg/kg)	02.00
Cu (mg/kg)	03.50
Mn (mg/kg)	13.00
Zn (mg/kg)	00.60
Fe (mg/kg)	07.00
Ca (g/kg)	0.04
Mg (g/kg)	0.52
Na (g/kg)	2.24
Cl (g/kg)	0.06

and mixed well. The study was conducted in a net house, without any potential climate variations.

Planting material and sowing

Delinted cotton seeds of six cultivars such as, *G. herbaceum* (G -Cot 25, Jayadhar), *G. arboreum* (Phule dhanwantry, Roja) and *G. hirsutum* (Suraj, LRA-5166) were sown in pots under controlled conditions.

NaCl treatments

NaCl was weighed (58.50 g, 88 g and 117 g) was dissolved in 10 L of distilled water to prepare the respective treatment solutions. NaCl treatments began 21 days after sowing (DAS). Approximately 500 mL of NaCl solution corresponding to concentrations of 100 mM, 150 mM and 200 mM was applied per pot weekly, while control pots received deionized water. Pots were not drained throughout the study. The saline solutions were applied uniformly during each irrigation event.

Measurements and protocols

Morphological parameters recorded, including plant height, stalk diameter, average number of leaves, squares, flowers per plant, root length, monopodial and sympodial branches, leaf area, height-to-node ratio, total biomass. Biochemical parameters such as chlorophyll, carotenoids, protein, lipid peroxidation and total soluble sugars, as along with antioxidant parameters including nitrate reductase, proline, superoxide dismutase, catalase, peroxidase and relative water content, were recorded following standard protocols (12-21).

The recommended fertilizer dose for cotton (60:30:30 kg $N:P_2O_5: K_2O$ ha^{-1}) was applied. Half the N and the full P and K doses were applied at sowing, while the remaining N was applied in two split doses at 30 and 45 DAS. Potted plants were irrigated (50 mL) every three days during the seedling stage (100 mL) and every seven days after 45 DAS, based on soil moisture content. Soil moisture measured through probe. Standard plant protection measures were implemented throughout the study period. Aphid infestations observed during the vegetative stage were controlled by spraying neem oil (30 mL L^{-1}) twice, at 30 and 45 DAS.

Statistical Analysis

The study was arranged in a factorial completely randomized design (FCRD), comprising four treatments, two factors (cultivar × NaCl concentration) and three replications. Data were statistically analysed using OPSTAT software (http://14.139.232.166/opstat/). Ranking and grouping of treatments were performed using the AGRIS-AGDATA statistical package and the least significant difference (LSD) was calculated. Significance of differences was tested using the F-test and critical differences (CD) were calculated at a 0.05 probability level.

Results and Discussion

The present study investigated the morphological, biochemical, physiological and antioxidant responses of different cotton cultivars to varying NaCl concentrations.

Morphological responses

Salinity stress significantly influenced key morphological characteristics such as plant height, stalk diameter, leaves, square, flower, root length, monopodia and sympodia branches, leaf area, height node ratio and total biomass of cotton plants. The results revealed that both the cotton cultivars (C) and the NaCl (S) treatments significantly influenced plant height and stalk diameter; number of leaves at the harvesting stage; number of squares per plant at 30, 40 and 50 DAS; number of flowers per plant between 50 and 75 DAS; root length of cotton cultivars at the post-harvest stage (95 days after treatment (DAT)); monopodial and sympodial branches at 75 DAT; leaf area (LA) at 60 DAT; height to node ratio (HNR) at 90 DAS and total plant biomass at post-harvest (95 DAT) while the interaction (C × S) was not significant to all

Table 2. NaCl induced stress on cotton cultivars plant height and stalk diameter at 120 DAT

Cotton cultivar	Plant height (cm)				Stalk diameter (mm)			
	Control	100*mM*	150*mM*	200*mM*	Control	100*mM*	150*mM*	200*mM*
G-Cot 25	097.33	85.00	78.00	67.33	3.83	3.06	2.67	2.67
Jayadhar	107.67	89.67	79.00	69.33	4.17	3.50	2.67	2.33
Phule dhanwantary	061.33	33.67	29.66	27.00	2.67	1.83	1.17	1.00
Roja	067.67	47.33	41.33	31.67	2.83	2.03	2.00	1.17
Suraj	077.00	57.67	49.67	45.00	4.00	3.50	2.83	2.17
LRA-5166	070.66	46.67	42.33	37.66	3.83	3.50	3.00	2.03
Factors	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**
Cultivar (C)	4.68**	2.32	1.64	6.01	0.32**	0.16	0.11	11.72
NaCl (S)	3.82**	1.89	1.34		0.26**	0.13	0.09	
C × S	NS	4.64	3.28		NS	0.32	0.22	

Table 3. NaCl induced stress on cotton cultivars of average no. of leaves, squares and flowers per plant at 120 DAT

Cotton cultivar	Leaves (no.)				Squares (no.)				Flowers(no.)			
	Control	100*mM*	150*mM*	200*mM*	Control	100*mM*	150*mM*	200*mM*	Control	100*mM*	150*mM*	200*mM*
G-Cot 25	40.66	35.00	29.00	24.00	8.33	8.00	6.00	4.33	10.33	8.33	7.00	6.00
Jayadhar	54.33	44.00	35.00	32.67	8.00	7.00	7.00	6.33	10.67	10.00	9.00	8.66
Phule dhanwantary	42.33	26.00	23.33	17.00	5.66	5.67	3.67	3.33	07.33	6.33	6.00	4.67
Roja	45.66	23.66	22.33	20.33	5.33	4.67	4.00	3.33	06.67	6.00	5.33	4.33
Suraj	34.66	19.00	15.00	13.33	6.33	5.33	5.00	3.67	07.67	7.00	6.33	5.33
LRA-5166	37.00	22.66	20.00	14.67	6.33	5.33	5.00	4.00	09.00	7.33	7.00	6.67
Factors	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**
Cultivar (C)	4.26**	2.11	1.49	9.06	0.87**	0.43	0.31	9.84	0.67**	0.33	0.24	8.85
NaCl (S)	3.48**	1.72	1.22		0.71**	0.35	0.25		0.55**	0.27	0.19	
C × S	NS	4.22	2.99		NS	0.87	0.61		NS	0.67	0.47	

Table 4. NaCl induced stress on cotton cultivars of leaf area (60 DAT), height to node ratio and total plant biomass (95 DAT)

Cotton cultivar	Leaf area (cm)				Height to node ratio				Total plant biomass (g plant^{-1})			
	Control	100*mM*	150*mM*	200*mM*	Control	100*mM*	150*mM*	200*mM*	Control	100*mM*	150*mM*	200*mM*
G-Cot 25	38.50	25.17	23.50	19.50	1.61	1.56	1.49	1.43	27.71	34.65	35.48	46.69
Jayadhar	31.50	21.83	28.33	18.83	1.84	1.73	1.55	1.58	32.15	44.31	63.65	72.11
Phule dhanwantary	27.00	23.17	19.83	15.67	1.28	0.85	0.93	0.88	34.16	45.68	50.45	58.67
Roja	22.66	21.67	24.50	22.33	1.28	1.19	1.08	0.93	25.56	38.08	49.82	52.20
Suraj	32.17	27.33	21.83	23.17	1.43	1.57	1.38	1.38	24.23	45.33	51.00	57.54
LRA-5166	45.83	27.17	26.00	19.33	1.42	1.14	1.13	1.16	28.23	55.01	56.17	59.95
Factors	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**
Cultivar (C)	4.11*	2.04	1.44	17.22	0.17**	0.08	0.06	11.49	7.24**	3.59	2.54	14.52
NaCl (S)	3.36**	1.66	1.18		0.14**	0.07	0.05		5.91**	2.93	2.07	
C × S	NS	4.08	2.88		NS	0.17	0.12		NS	7.18	5.08	

morphological characteristics (Table 2-4).

Plant height declined progressively with increasing NaCl concentration. These findings align with a previous study in which it was reported that plant height decreased with increasing soil NaCl levels in 20 day old cotton plants (22). Similarly, all cultivars exhibited reduced stalk diameter under salinity stress compared to controls. The observed stalk diameter was also lower than typically seen during the normal growing season, suggesting that both seasonal variations and salinity stress contributed to the decline.

Control plants consistently produced a higher number of leaves compared to NaCl treated plants. Among the cultivars, Jayadhar consistently maintained a higher number of leaves under salinity stress. These findings align with the report that the number of leaves serves as an effective morphological indicator for assessing salt tolerance in plants. Across all cultivars, the number of squares per plant decreased with increasing NaCl levels (23). Salinity stress induced square drop before flowering more prominently in *arboreum* and *hirsutum* than in *herbaceum*.

These findings are consistent with that *herbaceum* performs well in saline rainfed areas (24). Additionally, the results align with square drop to disrupted nutrient influx under saline conditions (25). The mean data of various cultivars demonstrated that NaCl treatments reduced the number of flowers per plant, with NaCl treated plants showing fewer flowers than the control plants.

The results suggest that saline water irrigation not only reduces the number of squares and flowers but also alters the life cycle of the plant by shortening the vegetative stage and prolonging the flowering period. High salinity may inhibit anthesis, leading to reduced flower production. These findings are consistent with that saline water irrigation increases the transpiration rate, shortens the vegetative period and extends the flowering stage and its duration in cotton (26).

All NaCl treated plants exhibiting shorter root lengths compared to the control plants (Table 5). The lowest root lengths were recorded at the highest NaCl concentration (200 mM). Based on these results, *herbaceum* (Jayadhar and G-Cot 25) demonstrated greater tolerance to NaCl compared to *hirsutum* and *arboreum*, as evidenced by their lesser reductions in root length. These findings are consistent with laboratory studies using soft agar media, which also highlighted the deep root systems and active root lengths of *herbaceum* as key factors in mitigating the adverse effects of salinity. In contrast, the root lengths of *hirsutum* and *arboreum* cultivars were significantly reduced under high salinity levels. Similar conclusions were drawn by the recommended management strategies such as mulching combined with drip irrigation to alleviate the negative impacts of salinity on root growth (27).

Table 5. NaCl induced stress on cotton cultivars of root length, monopodia and sympodia per plant at 95 DAT

Cotton cultivar	Root length (cm)				Monopodial branches (no.)				Sympodial branches(no.)			
	Control	100*mM*	150*mM*	200*mM*	Control	100*mM*	150*mM*	200*mM*	Control	100*mM*	150*mM*	200*mM*
G-Cot 25	27.50	25.53	23.80	22.37	2.67	1.67	1.33	0.67	9.66	10.00	10.67	12.00
Jayadhar	23.97	23.33	22.40	18.73	4.00	2.33	1.66	1.67	7.66	08.67	9.33	12.67
Phule dhanwantary	24.67	20.57	18.13	16.80	3.67	2.00	1.33	1.00	6.00	06.67	8.33	09.33
Roja	22.10	19.13	17.63	15.10	4.66	2.66	2.67	1.33	6.66	07.67	9.33	11.33
Suraj	21.00	19.67	18.37	14.33	3.33	3.33	2.67	2.33	5.33	05.67	8.00	09.67
LRA-5166	25.00	19.00	12.77	06.93	3.66	2.66	1.66	1.33	5.33	05.67	6.33	07.67
Factors	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**	**C.D.**	**SE(d)**	**SE(m)**	**CV (%)**
Cultivar (C)	1.55**	0.77	0.54	6.14	0.56**	0.28	0.20	19.66	1.14**	0.56	0.40	15.47
NaCl (S)	1.27**	0.63	0.44		0.46**	0.23	0.16		0.93**	0.46	0.33	
C × S	3.10**	1.54	1.09		NS	0.55	0.39		NS	1.13	0.80	

The average number of monopodial branches was higher in NaCl treated plants compared to control plants. The data indicate that salinity stress increased the number of monopodial branches more significantly in *hirsutum* and *arboreum* than in *herbaceum*. This response may reflect the physiological adaptations of these species to salinity. The findings are consistent with earlier research reported that similar increases in monopodial branches in upland cotton under salinity stress (2, 28). Sympodial branches were generally higher in control plants compared to NaCl treated plants. The data suggest that salinity stress reduces sympodial branching, with *hirsutum* being more adversely affected than *herbaceum* and *arboreum*. Interestingly, some *herbaceum* and *arboreum* cultivars exhibited a lower reduction in sympodial branches under salinity stress, indicating greater tolerance. These findings align with that salinity stress impacts sympodial branching in cotton, with significant variability among species (28).

Leaf area decreased with increasing NaCl concentration when compared to the control, with the highest reduction observed at 200 mM NaCl. The reduction in LA across all cotton species suggests that salinity negatively impacts plant growth, but some cultivars, particularly Roja (*G. arboreum*), appear to exhibit better adaptation to saline conditions. These results are in agreement with previous studies, that reported similar salinity -induced reductions in leaf area in cotton plants (10, 29, 30).

In general, control plants exhibited a higher HNR compared to NaCl treated plants. The observed reduction in HNR across all treatments suggests that salinity has a negative impact on this growth parameter. However, Suraj (*G. hirsutum*) demonstrated the lowest reduction in HNR, indicating its relatively higher tolerance to NaCl stress. Similar findings have been reported in literature, where various stressors such as mepiquat chloride (Pix), herbicide drift, excess foliar fertilization and drought were shown to reduce HNR in cotton plants. These results highlight the ability of hirsutum to better withstand salinity stress compared to other species.

Control plants exhibited higher biomass content compared to NaCl treated plants, with the highest reduction observed at 200 mM NaCl. The data indicate that NaCl stress negatively impacts total plant biomass, with higher concentrations leading to greater reductions. However, under 200 mM NaCl treatment, some cultivars, such as Jayadhar, Phule dhanwantary and LRA-5166, demonstrated higher biomass compared to the control, indicating a degree of tolerance to salinity. G-Cot 25 and Roja were among the least tolerant cultivars, showing substantial biomass reduction under NaCl stress. Similar findings have been reported that a decrease in dry biomass with increasing salinity (31). High salinity also negatively affects shoot fresh weight and the shoot/root ratio and leads to stunted stalk growth and reduced shoot dry mass (27, 29, 32).

These results highlight the differential salinity tolerance among cotton cultivars, with certain species exhibiting better growth performance under saline conditions.

Physiological responses

Salinity stress induced notable changes in physiological parameters such as total chlorophyll, carotenoids and relative water content in cotton plants. Total chlorophyll and carotenoids of cotton leaves varied significantly ($p<0.05$) among cultivars at 30 DAT (Fig. 2). Total chlorophyll decreased with increasing NaCl concentrations across all cultivars tested, compared to the control plants. Among the NaCl treatments, the highest reduction in total chlorophyll was observed at 200 mM, followed by 150 mM, compared to the control. Among the cultivar, *G. hirsutum* exhibited the highest total chlorophyll control under NaCl treatment, indicating greater tolerance compared to *G. herbaceum* and *G. arboreum*. Carotenoids content was also highest in control plants and decreased with increasing NaCl concentration. Although carotenoid content declined in all cultivars, *G. herbaceum* showed the least reduction, suggesting higher tolerance. Based on carotenoids, *G. herbaceum* exhibited greater tolerance to NaCl compared to *G. arboreum* and *G. hirsutum*. These results align with findings that reported an increase in biochemical parameters such as carotenoids, chlorophyll a, chlorophyll b and anthocyanin with higher soil salinity (22). Conversely, noted that various biochemical and developmental responses in desi cotton cultivars changed under different salinity levels (33). Leaf chlorophyll reduction under salinity stress has also been observed in previous studies (31, 34).

In our study of cotton cultivars, significant differences were observed between cultivars and NaCl treatments for relative water content (RWC) at 60 DAT, but the interaction between cultivar and NaCl (Cultivar x NaCl) was not significant (Fig. 3). All cultivars showed a decrease in RWC with increasing salinity, with the most substantial reduction observed at 200 mM NaCl. Among the cultivars, *G. hirsutum* (Suraj) maintained the highest RWC under stress conditions, indicating greater tolerance. These findings align with previous studies reporting reduced RWC under saline conditions (34). It reinforces the conclusion that *G. hirsutum* has superior ability to retain water and maintain physiological functions under salt stress.

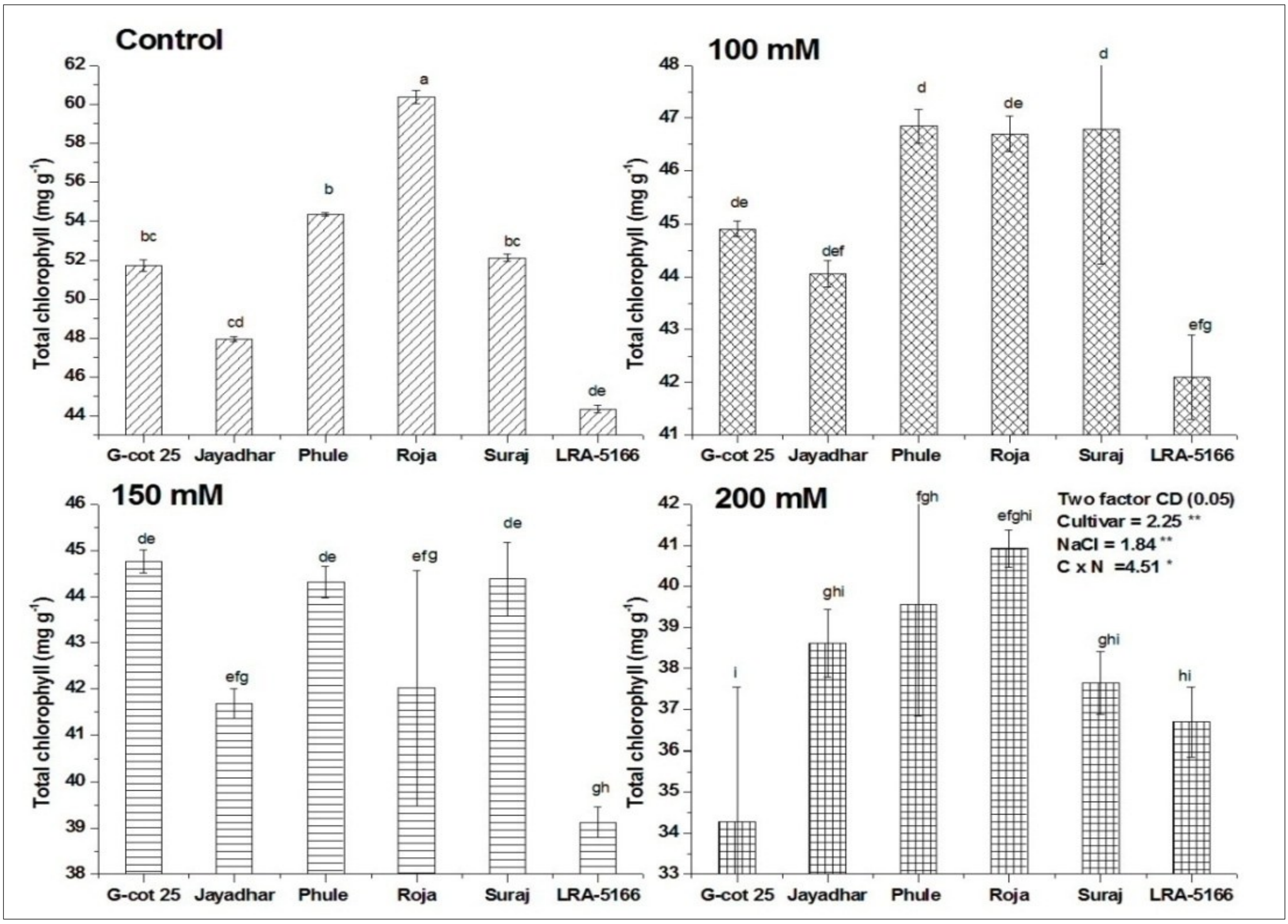
Biochemical responses

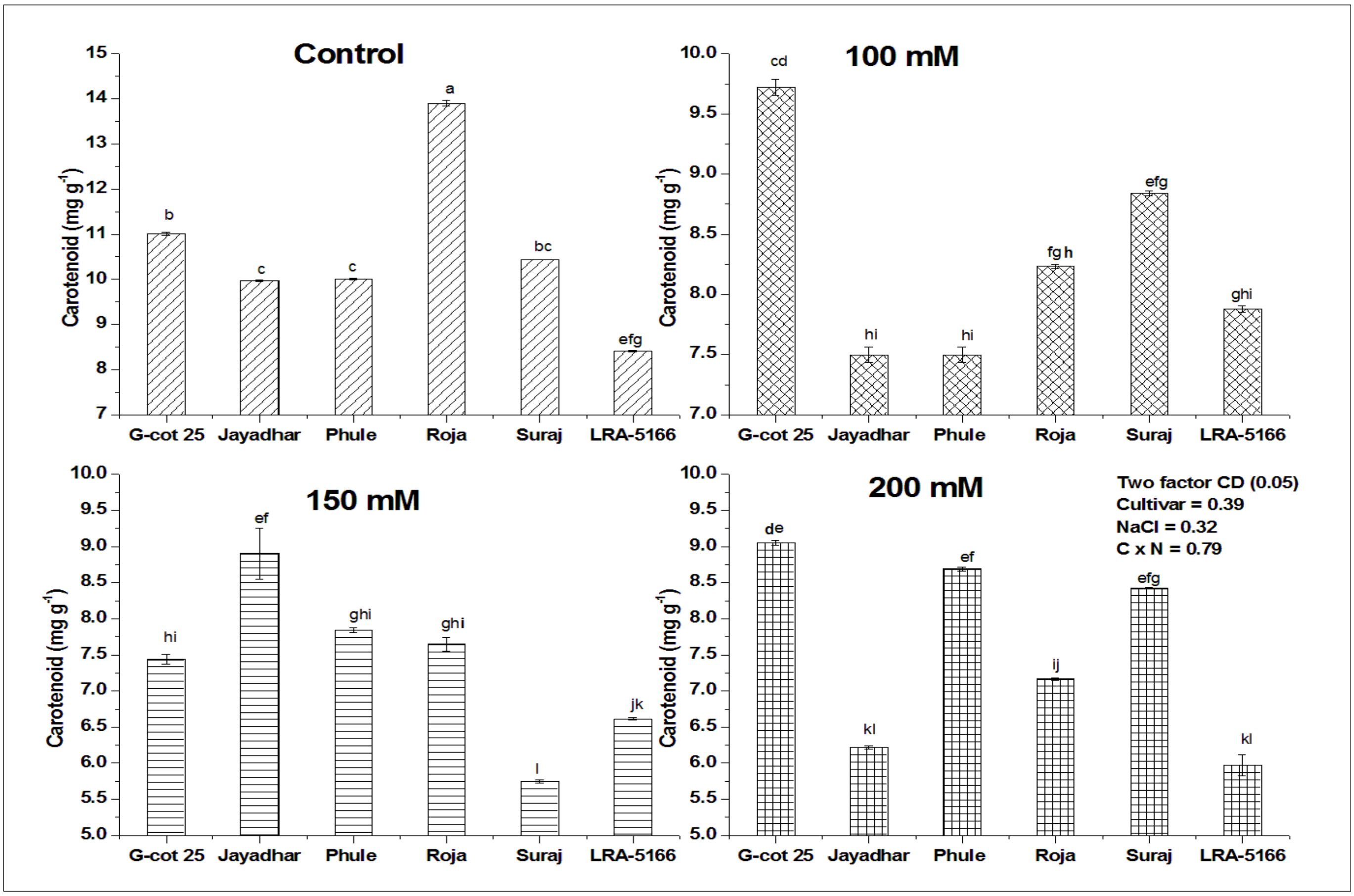
Salinity stress triggered notable alterations in the biochemical profile including protein, lipid peroxidation, total soluble sugar, nitrate reductase and proline accumulation.

Protein content, measured at 45 DAT, increased significantly ($p<0.05$) across all cultivars in response to NaCl treatments compared to controls (Fig. 2). This increase is likely due to the synthesis of stress-responsive proteins as part of the plant's defence mechanism. These results are in agreement with previous findings that demonstrated elevated protein levels under salt stress (35, 36).

Lipid peroxidation, measured as MDA content at 45 DAT among the cotton cultivars. A significant ($p<0.05$) increase in MDA was observed in response to NaCl treatment (Fig. 2). The minimum MDA levels were found in the control plants, while MDA increased with increasing NaCl concentration. *G. herbaceum* exhibited greater tolerance, as indicated by lower MDA accumulation, as it exhibited lower lipid peroxidation compared to *G. hirsutum* and *G. arboreum*. Our findings suggest that leaf MDA increases with increasing salinity, which aligns with similar observations in leaves and roots (10, 37).

Total soluble sugars (TSS), measured at 45 DAT, differed significantly ($p<0.05$) among cultivars and increased with NaCl


Control
100 mM
150 mM
200 mM
Total chlorophyll (mg g-1)
G-cot 25
Jayadhar
Phule
Roja
Suraj
LRA-5166
Two factor CD (0.05)
Cultivar = 2.25 **
NaCl = 1.84 **
C x N =4.51 *


Control
100 mM
150 mM
200 mM
Carotenoid (mg g-1)
G-cot 25
Jayadhar
Phule
Roja
Suraj
LRA-5166
Two factor CD (0.05)
Cultivar = 0.39
NaCl = 0.32
C x N = 0.79

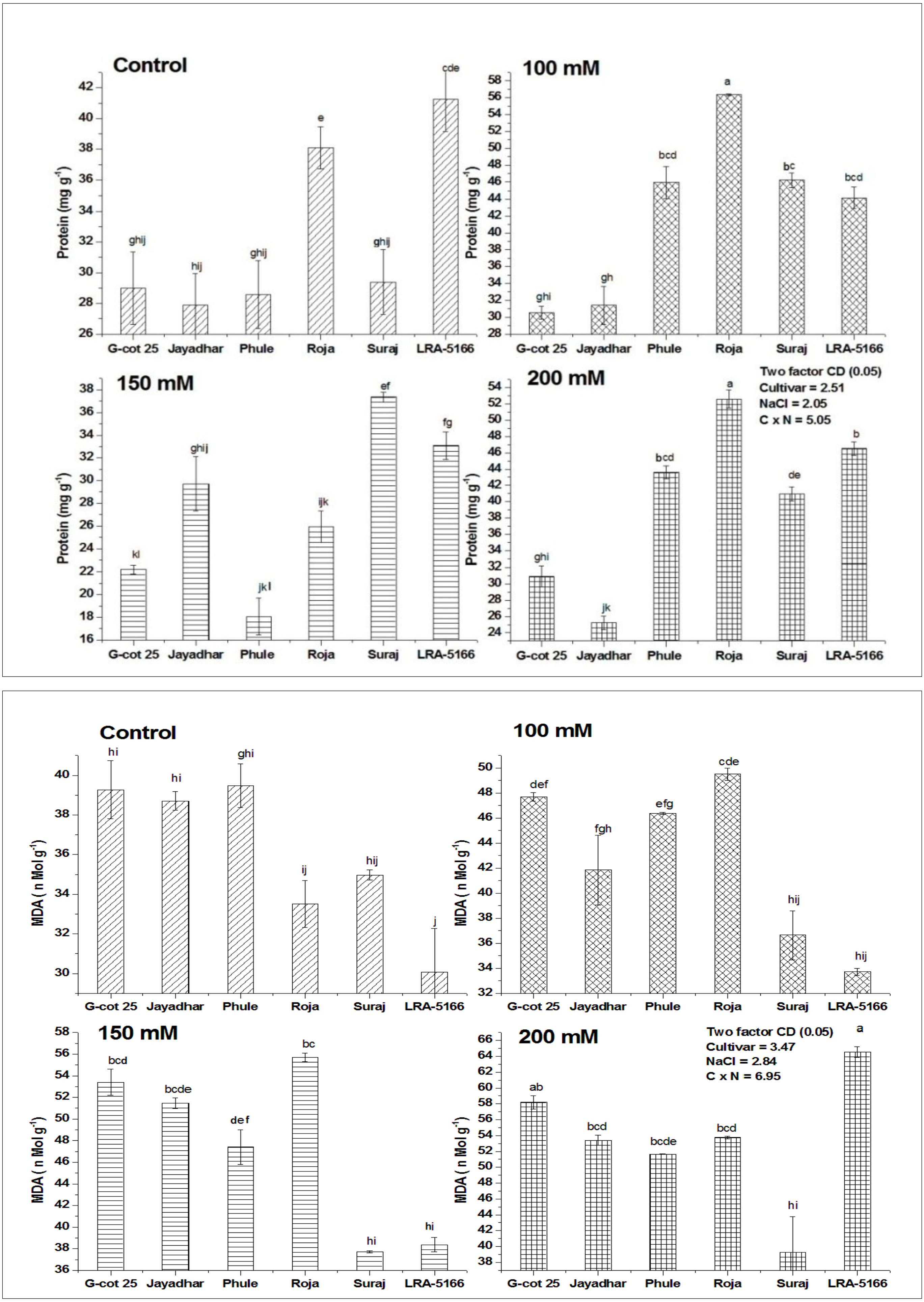
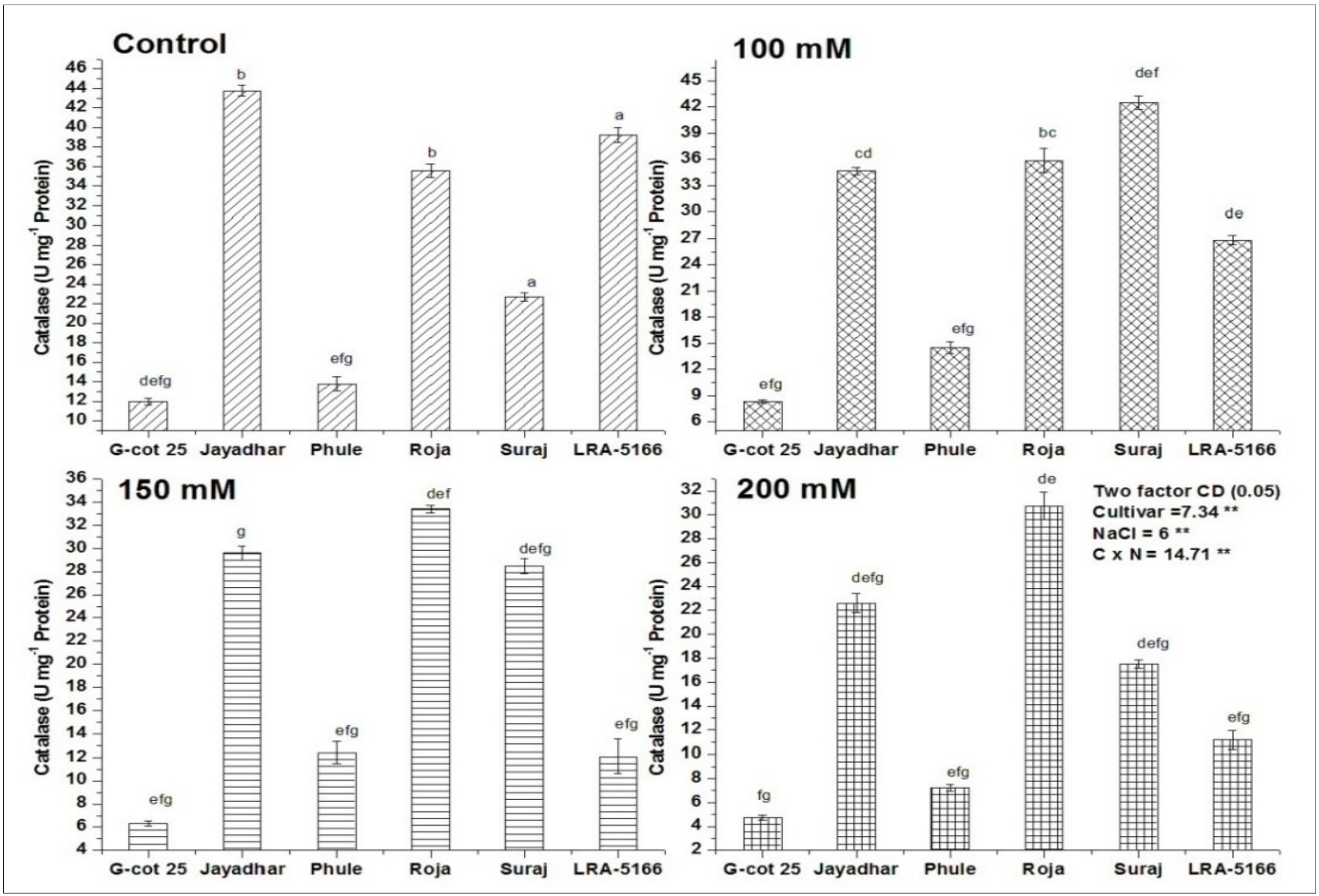
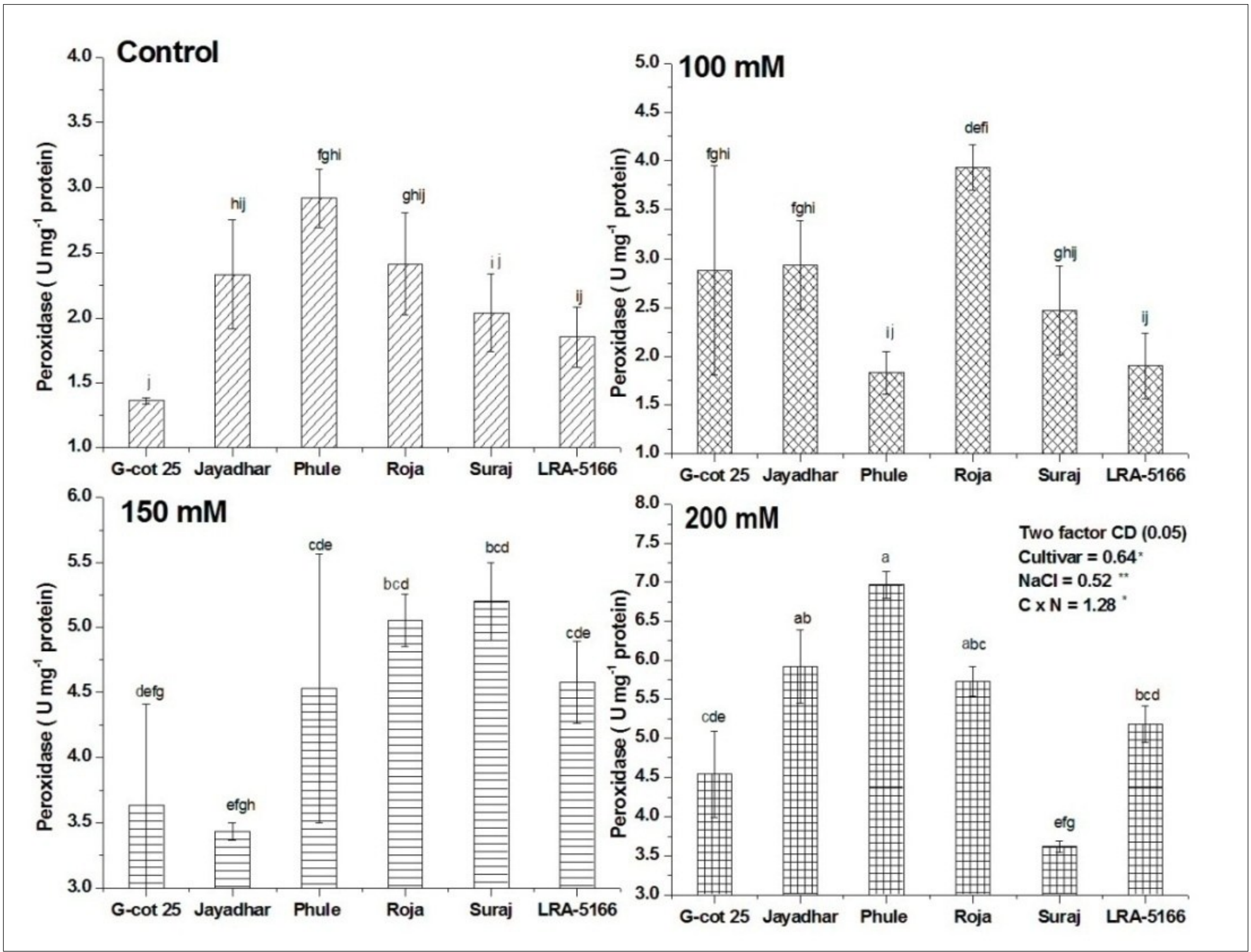


Fig. 2. NaCl induced stress on cotton cultivars of total Chlorophyll (30 DAT), carotenoid (30 DAT), protein (45 DAT) and lipid peroxidation (45 DAT).



Control
100 mM
150 mM
200 mM
Catalase (U mg-1 Protein)
G-cot 25
Jayadhar
Phule
Roja
Suraj
LRA-5166
Two factor CD (0.05)
Cultivar =7.34 **
NaCl = 6 **
C x N = 14.71 **



Control
100 mM
150 mM
200 mM
Peroxidase (U mg-1 protein)
G-cot 25
Jayadhar
Phule
Roja
Suraj
LRA-5166
Two factor CD (0.05)
Cultivar = 0.64*
NaCl = 0.52 **
C x N = 1.28 *

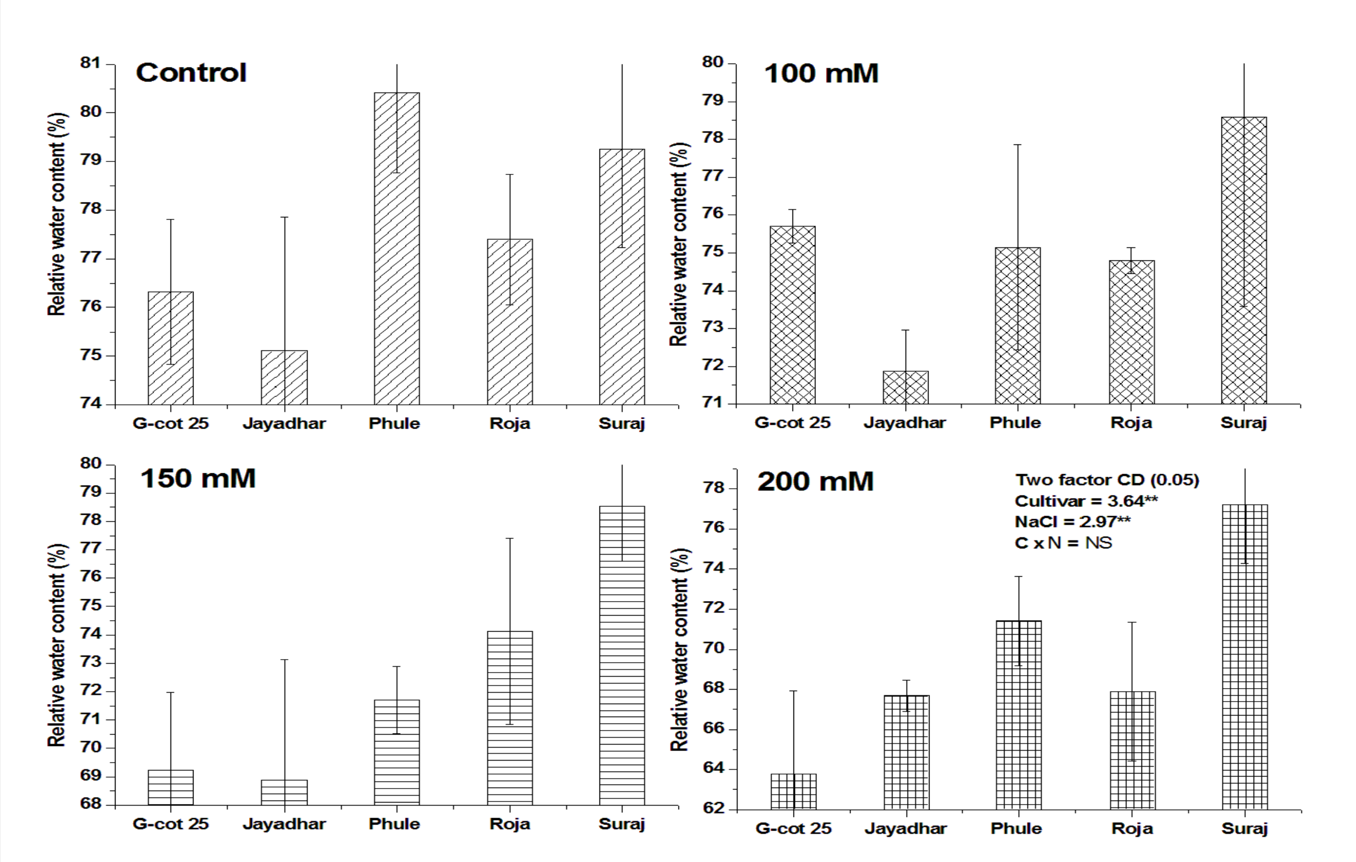
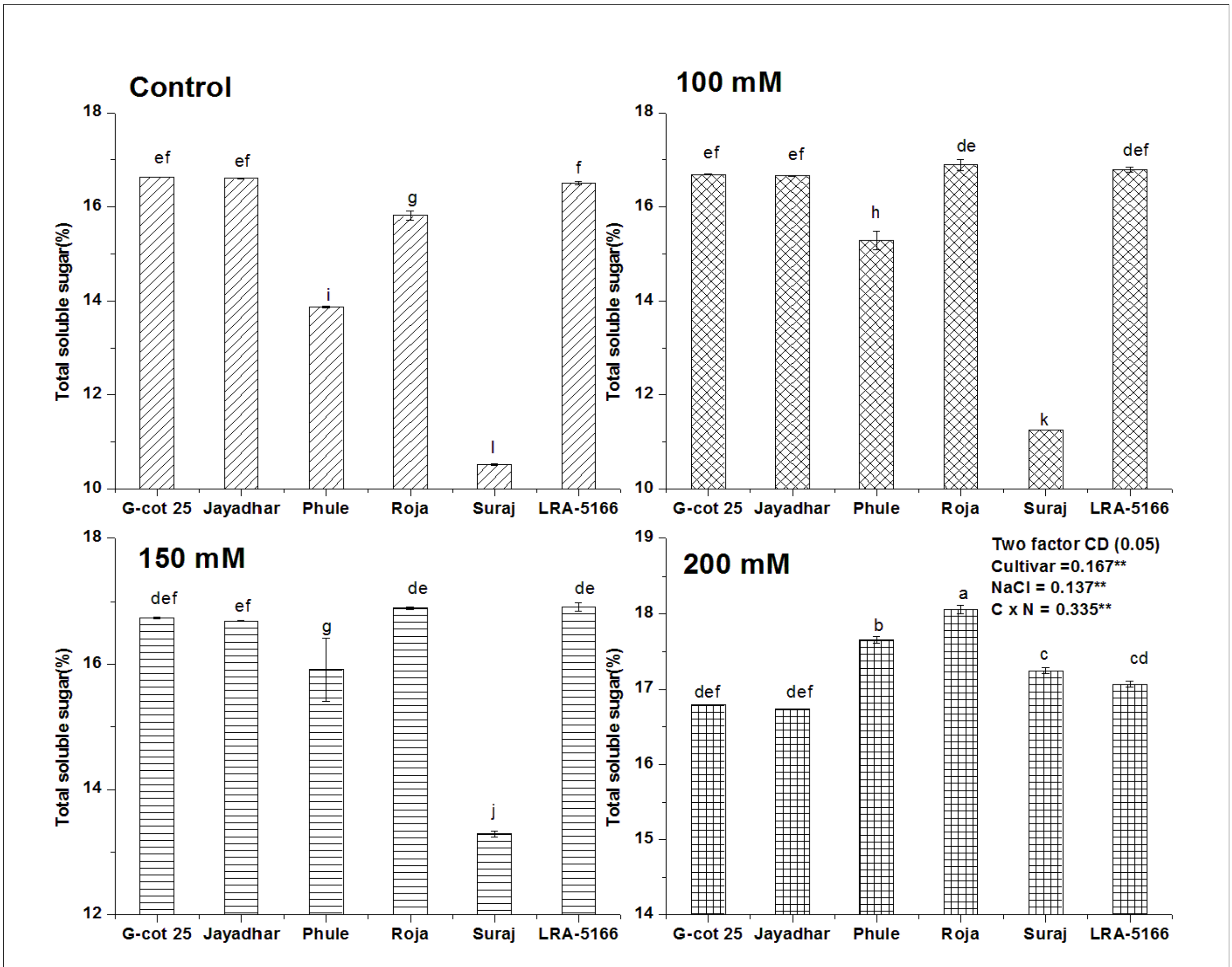
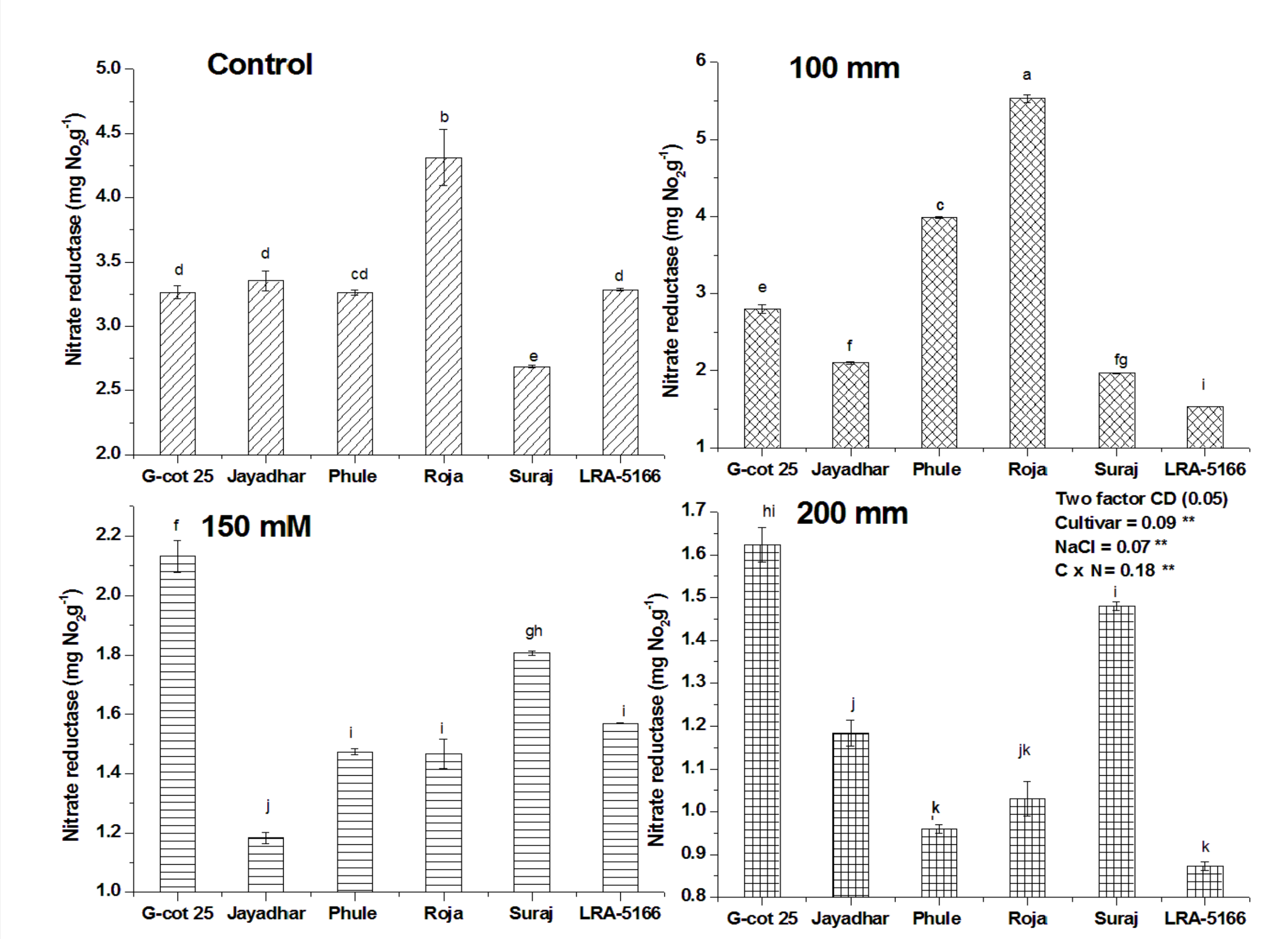
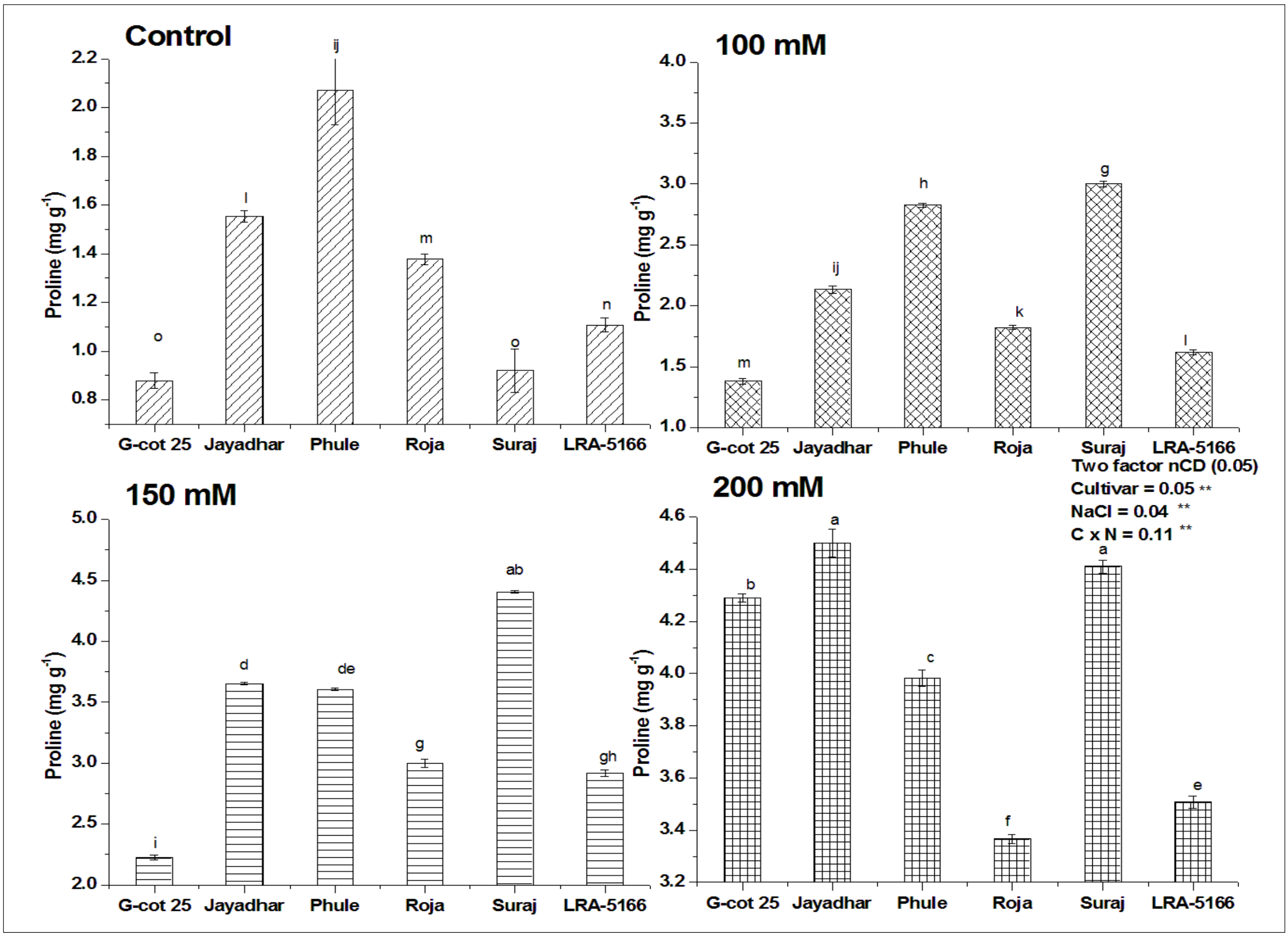


Fig. 3. NaCl induced stress on cotton cultivars of catalase, peroxidase (45 DAT) and relative water content (60 DAT).




Control
100 mm
150 mM
200 mm
Nitrate reductase (mg No2g-1)
G-cot 25
Jayadhar
Phule
Roja
Suraj
LRA-5166
Two factor CD (0.05)
Cultivar = 0.09 **
NaCl = 0.07 **
C x N= 0.18 **


Control
100 mM
150 mM
200 mM
Proline (mg g-1)
G-cot 25
Jayadhar
Phule
Roja
Suraj
LRA-5166
Two factor nCD (0.05)
Cultivar = 0.05 **
NaCl = 0.04 **
C x N = 0.11 **

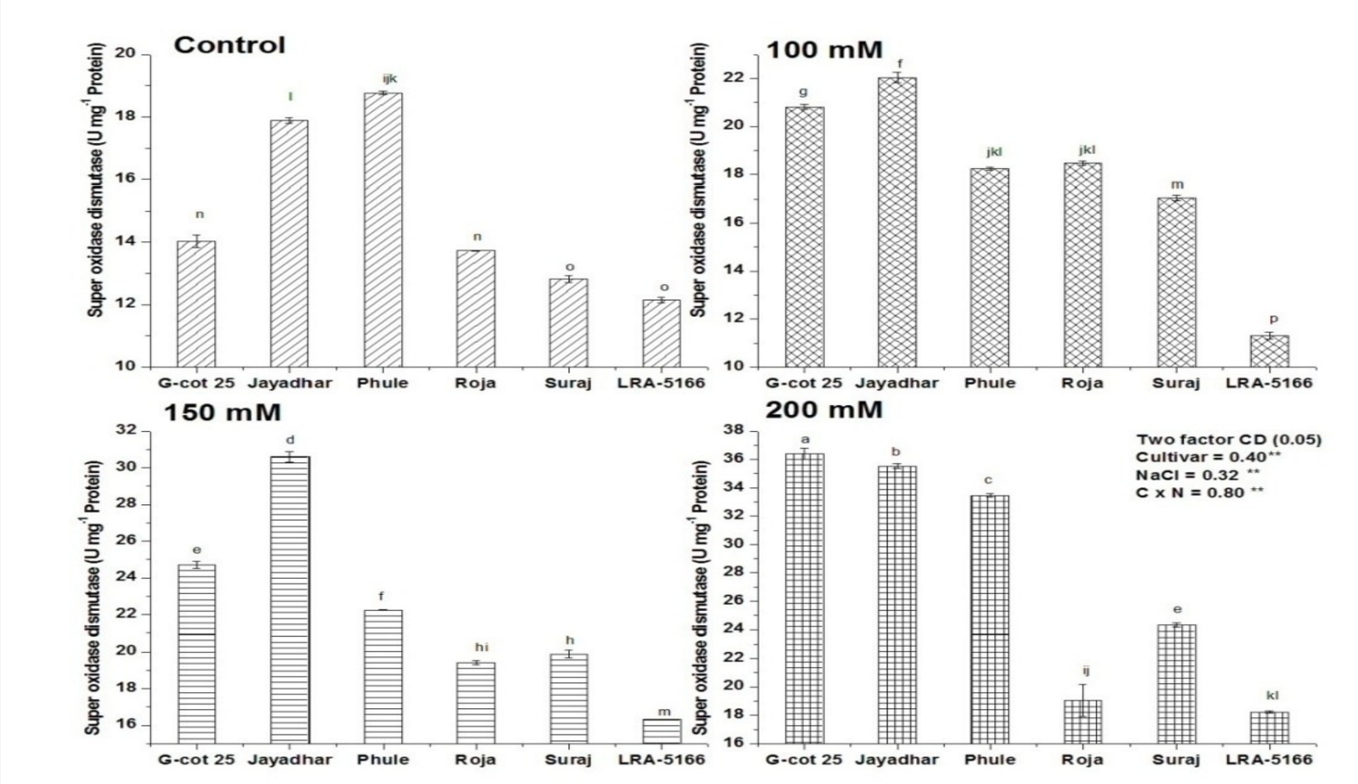


Fig. 4. NaCl induced stress on cotton cultivars of total soluble sugar, nitrate reductase, proline (45 DAT) and super oxidase dismutase (60 DAT).

treatment compared to controls (Fig. 4). Control plants had the lowest TSS levels, while salt-treated plants showed increased TSS, likely due to osmotic adjustment mechanisms that help maintain cell turgor under stress. Based on TSS content stability, *G. herbaceum* showed the highest tolerance, maintaining relatively stable sugar levels under salinity stress. These findings corroborate previous results highlighting TSS as a marker of osmotic adjustment in salt-stressed plants (36).

Nitrate reductase activity (NRA), also measured at 45 DAT, showed significant reductions ($p<0.05$) decline with increasing NaCl concentrations across all cultivars (Fig. 4). NRA was highest in control plants and lowest at 200 mM NaCl. Among the cultivars, *G. herbaceum* maintained higher NRA, suggesting better metabolic functioning under salinity compared to the other species. These results are in line with earlier findings that reported reduced nitrate and nitrite reductase activities due to saline irrigation and excessive nitrogen application (38).

Proline accumulation, an important osmoprotectant, increased significantly ($p<0.05$) across all cultivars with increasing NaCl concentrations (Fig. 4). Proline content was lowest in control plants and highest under 200 mM NaCl, particularly in *G. hirsutum* (Suraj), which exhibited the greatest proline accumulation. This indicates a strong stress mitigation response, consistent with earlier reports and suggests that proline serves as an effective marker of salt tolerance in cotton (36).

Antioxidant responses

Salinity stress markedly enhanced the activity (superoxidase dismutase, catalase and peroxidase) of key antioxidant enzymes in cotton. The activity of superoxide dismutase (SOD) differed significantly among the NaCl treatments at 45 DAT (Fig. 4). SOD activity was found to be the lowest in control plants, but it increased (1.4 to 2.6 times) with the increasing concentration of NaCl in all cultivars. Based on SOD activity, *G. herbaceum* (Jaydhar and G cot-25) exhibited the highest tolerance (2.0 to 2.6 time) to salt stress compared to *G. hirsutum* (1.5 to 1.8 time) and *G. arboreum* (1.4 to 1.7 time). Increased antioxidant enzyme activity, including SOD, glutathione reductase and proteins, has been linked to salt tolerance in cotton cultivars. Under saline conditions, tolerant cultivars generally exhibit higher SOD (G-Cot 25) compared to sensitive ones (Roja). Previous studies support the notion that the salt tolerance of cotton varieties is enhanced by the increased activity of antioxidant enzymes such as SOD under salt stress conditions (34, 39).

The effect of NaCl on catalase activity (CAT) was significant, showing a reduction ($p<0.05$) in CAT activity across all cotton cultivars at 45 DAT (Fig. 3). CAT activity decreased in response to NaCl treatment in all cultivars when compared to the control. Based on CAT activity results, *G. arboreum* (Roja) exhibited the highest tolerance to salt stress compared to *G. hirsutum* and *G. herbaceum*. Similar findings were reported that showed reduction in catalase activity under salt stress (35). These results highlight the differential responses of cotton cultivars to salt stress and suggest that certain cultivars, like Roja and Phule dhanwantary, may be more adaptable to NaCl-induced oxidative stress due to their ability to maintain or minimize CAT. CAT decreases under high salt stress. It detoxifies the hydrogen peroxide (H_2O_2) into water and oxygen, which protects plants from oxidative stress. Sometimes it may due to the ionic toxicity. However, in this study LRA-5166 and Jaydhar recorded deviation in terms of CAT. The peroxidase (POD) activity in cotton cultivars varied significantly ($p<0.05$) at 45 DAT (Fig. 3). In control plants, POD activity was lowest, but it increased in response to NaCl treatment (0.8 to 2.2 time). Based on POD activity, *G. herbaceum* (G cot-25) exhibited the highest tolerance to NaCl stress compared to *G. hirsutum* and *G. arboreum*. These results align with the findings that had an increase in POD activity under salt stress, indicating that POD activity may contribute to the plants' response to oxidative stress caused by NaCl exposure (35).

Under 200 mM NaCl, G-Cot 25 maintained higher SOD activity, while Roja showed the least response. Similarly, Roja had minimum CAT decrease and LRA-5166 recorded maximum decrease. Conversely, POD increased under salinity with maximum Phule dhanwantary and minimum recorded with Suraj. Overall, *G. herbaceum* is considered highly tolerant to NaCl stress based on its SOD, CAT and POD data of this study. Our results demonstrate that NaCl stress significantly affected various traits, with clear variations observed between cultivars and NaCl treatments.

Based on an integrative assessment of morphological, physiological, biochemical and antioxidant responses, *G. herbaceum* cultivar Jayadhar demonstrated the highest tolerance to salinity. It exhibited minimal reductions in leaf number, root length, sympodial branches and total biomass under NaCl stress, along with greater stability in carotenoids content, total soluble sugars, nitrate reductase activity and lower levels of lipid peroxidation. These traits, in combination with elevated antioxidant enzyme activities particularly peroxidase and superoxide dismutase collectively highlight its enhanced resilience to NaCl-induced stress across multiple functional parameters.

Conclusion

In conclusion, *G. herbaceum* cultivars, particularly Jayadhar, demonstrated superior tolerance to NaCl-induced stress compared to *G. hirsutum* and *G. arboreum*, as reflected in their morphological, biochemical and antioxidant responses. The increase in protein content, proline accumulation and antioxidant activity in these cultivars suggests they possess adaptive mechanisms to cope with salt stress, making them promising candidates for cultivation in saline-prone areas. Further research into the genetic and molecular basis of salt tolerance in cotton will help develop more resilient cultivars for sustainable agriculture in saline environments.

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Authors' contributions

DK conducted the experiments and prepared the figures. AM conceptualized the study and drafted the manuscript. DB performed the statistical analysis and contributed to manuscript editing. PK interpreted the antioxidant analysis and assisted with manuscript editing. RS performed biochemical activity. CR performed antioxidant activity. VN provided cultivars, guided and supported the study. All authors reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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