ISSN 2518–2021 (Print) 2415–4474 (Electronic)

Fundamental and Applied Agriculture

Journal home page: https://www.f2ffoundation.org/faa/index.php/home

Vol. 11 (1), pp. 44 – 52: 2026, doi: https://doi.org/10.5455/faa.327777

Plant Science | ORIGINAL ARTICLE

Enhancement of Growth, Photosynthetic Pigments, and Antioxidant Defense in Salt-Stressed Chilli (*Capsicum annuum* L.) by Chitosan

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ARTICLE INFO

Article history
Received: 15 Dec 2025
Accepted: 12 Feb 2026
Published online: 31 Mar 2026

Keywords
Antioxidant defense, Chitosan, Chilli, Oxidative stress, Photosynthetic pigments, Salinity tolerance

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ABSTRACT

Soil salinity is a critical abiotic factor that confines chilli (*Capsicum annuum* L.) growth and productivity by inducing oxidative damage and disrupting physiological and biochemical processes. This study investigated the effectiveness of exogenous chitosan in enhancing salinity tolerance in two chilli varieties, i.e., local variety Kajol and hybrid variety Dhanuya. Seedlings of both varieties were grown under four different conditions encompassing combinations of with and without salt stress (100 mM NaCl) and foliar application of chitosan (25 ppm). Salinity stress hindered plant height, branch and leaf counts, flower production, and photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and total chlorophyll) in both varieties. It also induced pronounced proline accumulation and boosted the activities of antioxidant enzymes catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX), reflecting enhanced oxidative stress. In contrast, foliar application of chitosan under saline conditions markedly alleviated the detrimental effects of salinity by improving growth attributes and preserving photosynthetic pigment levels. Supplementation of chitosan moderated proline accumulation and regulated antioxidant enzyme activities, thus sustaining cellular redox homeostasis under salt stress conditions. Chitosan application alone also promoted plant growth, improved photosynthetic pigment synthesis, and modulated antioxidant enzyme activities compared with the untreated control. These findings demonstrate that exogenous chitosan is an effective and environmentally friendly biostimulant for improving antioxidant defense responses, enhancing salinity tolerance, and sustaining chilli production in salt-affected environments.

1. Introduction

Chilli is an important spice and vegetable crop that belongs to the Solanaceae family and is much sought after because of its pungent taste, nutritional properties like vitamins A and C, and its diverse uses in medicine. In Bangladesh, chilli is an important spice that is used in its raw, dried, or powdered form to add taste and aroma to various food preparations (Rahman et al., 2023). Chilli is cultivated throughout the year in Bangladesh, with winter varieties contributing about 70% of the total production, mainly grown between October and April (Rahman et al., 2023). The agricultural land used for the crop is about 239,203 acres, producing about 149,473 metric tons of chilli annually, which is an important contribution to the spice sector (Rahman et al., 2023). The cultivation of chilli has a large economic value as it produces more revenue per unit area than many cereal crops, creates rural employment, improves nutritional security, and helps to reduce the expenditure of foreign currency due to reduced imports (Al-Imran et al., 2024; Rahman et al., 2023).

Salinity stress is recognized as a significant abiotic limitation to agricultural productivity worldwide, particularly in arid, semi-arid, and coastal regions, where soil salinization adversely affects crop development through osmotic imbalance, ion toxicity (notably Na^+ and Cl^-), and nutritional deficiencies (Munns, 2002). In Bangladesh, the spread of salinity in coastal and southern regions, intensified by rising sea levels, tidal waves, shrimp aquaculture, and diminished freshwater availability, has rendered extensive areas of cultivable land unsuitable for sensitive crops, thereby posing a critical threat to food security and the livelihoods of rural communities. Chilli (*Capsicum annuum* L.), identified as a glycophyte and exhibiting moderate to high sensitivity to salinity, suffers notable yield reductions even at moderate salinity levels, rendering it particularly susceptible in saline-affected regions of Bangladesh, where farmers frequently depend

Cite This Article
Roy A, Ray SR, Maria MA, Tahjib-Ul-Arif M, Sharmin S, Bhuiyan MJH, Hossain MA. 2026. Enhancement of Growth, Photosynthetic Pigments, and Antioxidant Defense in Salt-Stressed Chilli (*Capsicum annuum* L.) by Chitosan. *Fundamental and Applied Agriculture*, 11(1): 44–52. https://doi.org/10.5455/faa.327777

on indigenous varieties that lack tolerance (Dutta et al., 2025). High salinity levels can create a negative effect on chilli and lead to low seed germination rates, lower seedling vigour, and plants that grow poorly due to stunted growth (e.g., reduced height, lower leaf area and biomass), lower photosynthetic rates as a result of chlorophyll breakdown, disrupted nutrients or nutrient uptake (e.g., high levels of Na^+), delayed flowering and fruit ripening times, smaller fruit sizes, fewer fruit produced and overall significant reductions in the quality and quantity of yield that can be sold (Zamljen et al., 2022). These physiological impairments often result in yield reductions of more than 10–50%, depending on the level of salinity stress experienced, which further increases the economic difficulties faced by chilli farmers in Bangladesh who live in these affected areas.

Chitosan, a biodegradable polysaccharide derived from chitin through deacetylation, is a potent biostimulant in agriculture that increases plant tolerance to various abiotic stresses through physiological and biochemical processes (Hidangmayum et al., 2023; Rojas-Pirela et al., 2024; Sharif et al., 2018). When applied to plants, chitosan, in its role as a foliar spray or seed treatment, acts as an elicitor to trigger plant defense mechanisms, such as the upregulation of antioxidant enzymes like superoxide dismutase, catalase, and peroxidase, to scavenge free radicals like ROS, thus protecting the plant from oxidative damage and lipid peroxidation (Stasińska-Jakubas & Hawrylak-Nowak, 2022). Moreover, chitosan stimulates osmotic adjustment in plants by increasing osmolytes such as proline and sugars, increasing nutrient uptake, improving photosynthesis, and modulating gene expression to improve plant growth while preserving biomass under stressful conditions (Rojas-Pirela et al., 2024; Negm et al., 2020). Chitosan, with its biostimulant properties, is a potent agent in mitigating abiotic stresses like salinity, drought, etc., in plants, thus serving as a safe alternative to chemical protectants while increasing plant stability (Diao et al., 2017). Under salinity stress, chitosan increases antioxidant activity in plants, helping them counteract ion toxicity.

The optimum amount of chitosan needs to be applied to achieve the maximum crop yield, and at the same time, an excessive amount of chitosan results in toxicity. However, it is very important to know the exact amount of chitosan required to achieve crop growth under salinity stress conditions. Determining the precise concentration of chitosan is critical, as the threshold between growth stimulation and phytotoxicity remains a narrow and largely unexplored gap in chilli. This research aims to investigate the mechanisms by which exogenous chitosan plays role in mitigating the negative effects triggered by salinity. The primary focus was to examine the modulation in activities of key antioxidant enzymes such as ascorbate peroxidase, peroxidase, and catalase in response to chitosan under salt stress. Tracking of these biochemical markers will assist in understanding how chitosan facilitates in the plant's internal detoxification system to scavenge reactive oxygen species (ROS), thereby maintaining cellular homeostasis and protecting the photosynthetic apparatus under high-salt pressure. However, to date, very few studies have been conducted on chilli plants regarding the ameliorative effect of chitosan under abiotic stresses, including salinity. The efficacy of exogenous chitosan as an effective biostimulant in chilli under salinity stress needs to explore. Therefore, the present study was designed to examine the effect of chitosan on the growth performance, photosynthetic pigment production, and antioxidant defense capacity of chilli under salinity stress, thus in improving salinity tolerance.

2. Materials and Methods

2.1. Experimental site and planting material

The experiment was carried out on the rooftop of the Department of Biochemistry and Molecular Biology at Bangladesh Agricultural University (BAU), Mymensingh. Two chilli varieties: local variety- Kajol and hybrid variety- Dhanuya were collected from reliable sources and used in this study.

2.2. Experimental design and treatments

The experiment was conducted using plastic pots arranged in a Completely Randomized Design (CRD). The experiment comprised four treatments with three replications for both varieties: the control (C) condition was maintained by growing plants under the natural environment, only applying normal water and normal doses of fertilizers. The 100 mM saline (S) condition was created by applying 100 mM NaCl solution prepared by water. A total of 5 liters of saline solution was prepared and applied to each pot over a period of 5 days (1 liter/day per pot), starting from 25 DAS. Saline with Chitosan (S+CTS) condition was created by applying a foliar spray of chitosan (25 ppm) on the plants already subjected to 100 mM saline stress. The chitosan was applied periodically (2-3 times) after salinity stress induction and before the flowering stage. The chitosan (CTS) condition was maintained by applying a foliar spray of Chitosan (25 ppm) to the plants under control conditions (naturally growing plants) at the same intervals.

2.3. Determination of morphological traits

Different morphological parameters (i.e., plant height, number of branches per plant, number of leaves per plant, number of flowers per plant, etc.) that are associated with the quantity and quality of chilli were recorded.

2.4. Determination of antioxidant enzyme activity

For enzyme extraction, fresh leaf samples (0.05 g) were homogenized in 3 mL of 50 mM potassium phosphate buffer (PB) (pH 8.0). The homogenate was centrifuged at 12,000×*g* for 10 min at 4 °C, and the supernatant was used to determine the activities of catalase, peroxidase, and ascorbate peroxidase. The activity of CAT (EC: 1.11.1.6) was measured according to Aebi (1984). The reaction mixture was made by mixing 0.7 mL of PB, 0.1 mL of EDTA, and 0.1 mL of H_2O_2. In the assay of POX (EC: 1.11.1.7) and APX (EC: 1.11.1.11) activities, the method described by Nakano and Asada (1981) was followed. In case of POX activity, the reaction mixture was prepared with 0.6 mL of 50 mM PB, 0.1 mL of EDTA, 0.1 mL of H_2O_2, and 0.1 mL of guaiacol. For assaying APX activity, 0.6 mL of PB, 0.1 mL of EDTA, 0.1mL of H_2O_2, and 0.1 mL of ascorbate were added to the reaction mixture. The changes in absorbance were recorded immediately after adding 0.1 mL of enzyme extract at 30-second intervals for 2 minutes. The absorbances were read at 240 nm, 470nm, and 290 nm for assaying CAT,

POX, and APX activities, respectively, using a UV/Vis spectrophotometer (Shimadzu, UV-1201; Japan).

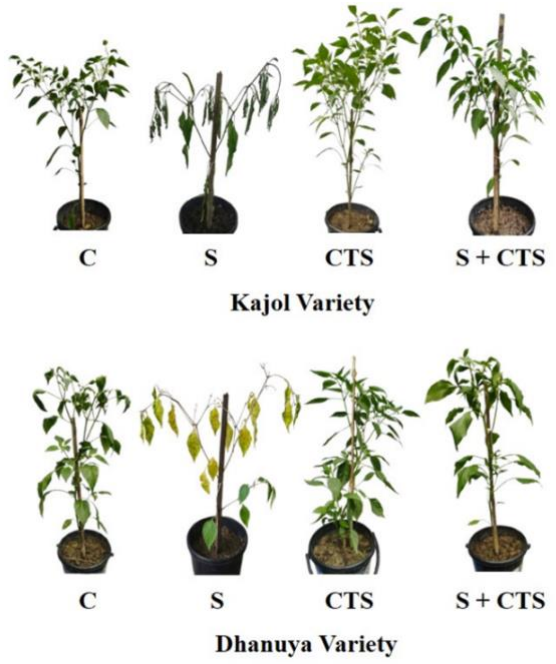

Figure 1. Effect of salinity stress and chitosan on the phenotypes of two chilli varieties, viz. Kajol and Dhanuya under four different treatment conditions, viz. "C" (Control); "S" (Salinity); "CTS" (Chitosan); and "S+CTS" (Salinity plus Chitosan).

2.5. Determination of proline

Proline concentration in fresh leaf samples was assessed using Bates et al. (1973). A total of 0.05 g of the sample was ground with 1 mL of 3% sulfosalicylic acid, adding another 3 mL during grinding. The homogenate was transferred to a falcon tube and centrifuged at 5000 rpm for 10 minutes. Then, 2 mL of the supernatant was collected in a screw cap tube, followed by 500 µL of acid ninhydrin reagent and 500 µL of glacial acetic acid. The mixture was heated at 100°C for 45 minutes and cooled in an ice bath. Next, 3 mL of toluene was added and shaken for 15 minutes to separate the chromophore. The absorbance of the toluene layer was recorded at 520nm using a UV-Vis spectrophotometer (Shimadzu, UV-1201; Japan), with a reagent blank as reference. A standard curve was created using analytical grade proline at concentrations of 0.005 to 0.025 mg/mL. Proline content was determined by comparing absorbance values to the standard curve.

2.6. Determination of total chlorophyll

To determine the chlorophyll content, a method developed by Coombs et al. (1985) was followed. The fresh leaf sample of 0.05 g was dipped into a screw-capped test tube containing 80% acetone and preserved in the dark for 7-10 days. The absorbance readings were recorded at 645 and 663 nm using a UV/Vis spectrophotometer (Shimadzu, UV-1201; Japan), and the result was expressed as mg g^{-1} fresh weight (F.W.) of leaf.

2.7. Data analysis

A one-way analysis of variance (ANOVA) was performed using Minitab 22.0 statistical software. Different alphabetical letters denote statistically significant differences observed among the treatments at $p < 0.05$, according to Tukey's Honestly Significant Difference (HSD) test. The data given in the figures and tables are all means with standard errors (n = 3).

3. Results

3.1. Phenotypic appearance

Salinity caused reduction in plant growth, leaf production, and flowering in both chilli varieties compared with their respective control plants during the experimental period (Fig. 1). However, morphological growth under stressed conditions noticeably recovered with the foliar application of chitosan in both varieties (Fig. 1).

3.2. Number of flowers and branches per plant

Under salt stress, a drastic reduction was observed in flower production in both varieties compared to their respective controls (Fig. 2A-B). In the local variety Kajol, the number of flowers dropped from approximately 73 to nearly 28 under saline conditions, whereas in the hybrid variety Dhanuya, the reduction was from 65 to about 28.

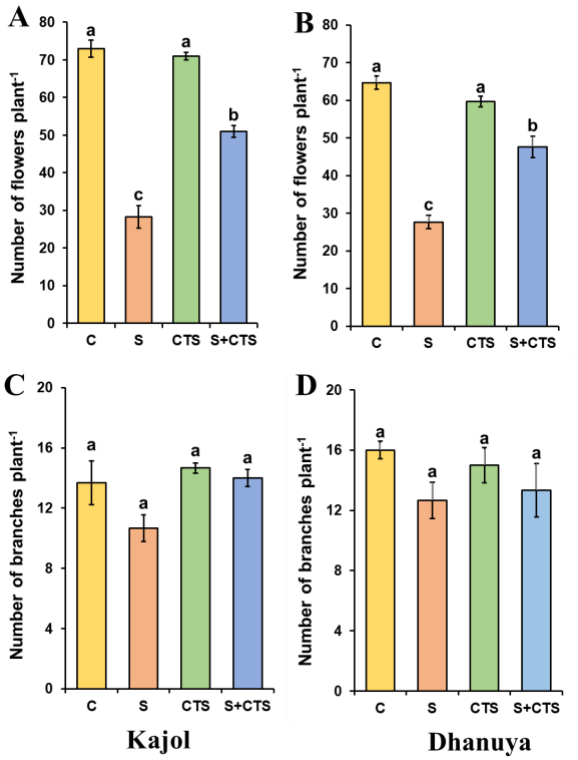

Figure 2. Effect of chitosan on (A-B) number of flowers per plant and (C-D) number of branches per plant of two chilli varieties, viz. Kajol (A, C) and Dhanuya (B, D) under four different treatment conditions, viz. "C" (Control); "S" (Salinity); "CTS" (Chitosan); and "S+CTS" (Salinity plus Chitosan). The bars indicate the mean ± SE of three replicates (n = 3). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$.

However, the foliar application of chitosan (S+CTS) alleviated the adverse effect of stress by increasing the number of flowers significantly in both varieties compared to stressed plants without chitosan (Fig. 2A-B). Salinity also exerted a negative influence on the number of branches per plant in both chilli genotypes (Fig. 2C-D). On the other hand, the application of chitosan enhanced the branching capacity in both varieties under salt stress compared to only stressed plants (Fig. 2C-D). The application of only chitosan maintained flower and branch production comparable to controls (Fig. 2A-D).

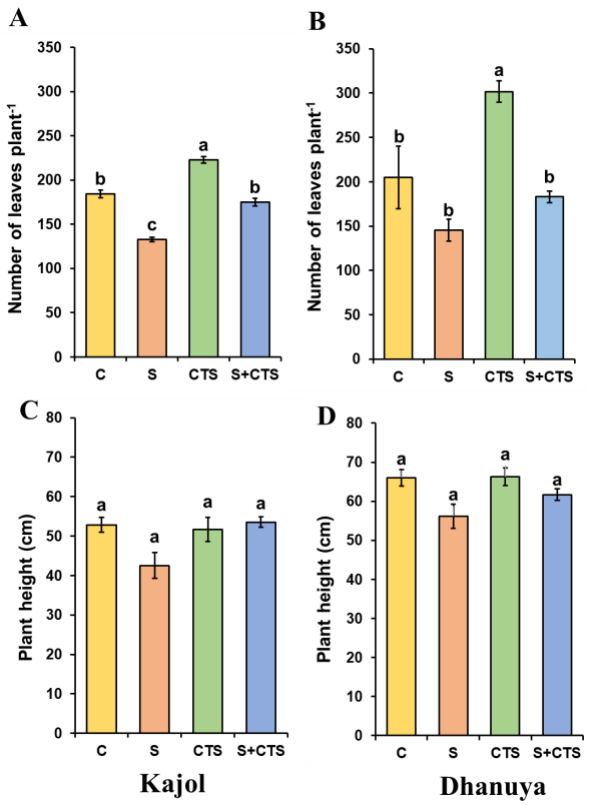

Figure 3. Effect of chitosan on (A-B) number of leaves per plant and (C-D) plant height of two chilli varieties, viz. Kajol (A, C) and Dhanuya (B, D) under four different treatment conditions, viz. "C" (Control); "S" (Salinity); "CTS" (Chitosan); and "S+CTS" (Salinity plus Chitosan). The bars indicate the mean ± SE of three replicates (n = 3). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$

3.3. Number of leaves per plant and plant height

The number of leaves per plant showed a significant variation under different treatment conditions (Fig. 3A-B). Salinity stress resulted in a substantial decline in foliar growth in both varieties. In the hybrid variety Dhanuya, the leaf count dropped from approximately 205 to 145, whereas in the local variety Kajol, it decreased from about 184 to 133. However, the foliar application of chitosan (S+CTS) effectively mitigated the deleterious effects of salt stress by significantly promoting leaf proliferation compared to plants exposed to stress with no chitosan (Fig 3A-B). The spray of chitosan alone also increased leaf numbers in both varieties compared to controls (Fig. 3A-B).

Plant height, a primary indicator of vegetative vigor, was restricted by salinity in both chilli varieties (Fig. 3C-D). In Dhanuya, the height decreased from approximately 66 cm to 56 cm, while in Kajol, it was reduced from nearly 53 cm to 43 cm. On the other hand, the supplementation of chitosan enhanced plant height in both genotypes under salt stress, almost restoring it to the control level (Fig 3C-D). The only chitosan spray caused no significant alterations in plant height relative to controls (Fig. 3C-D).

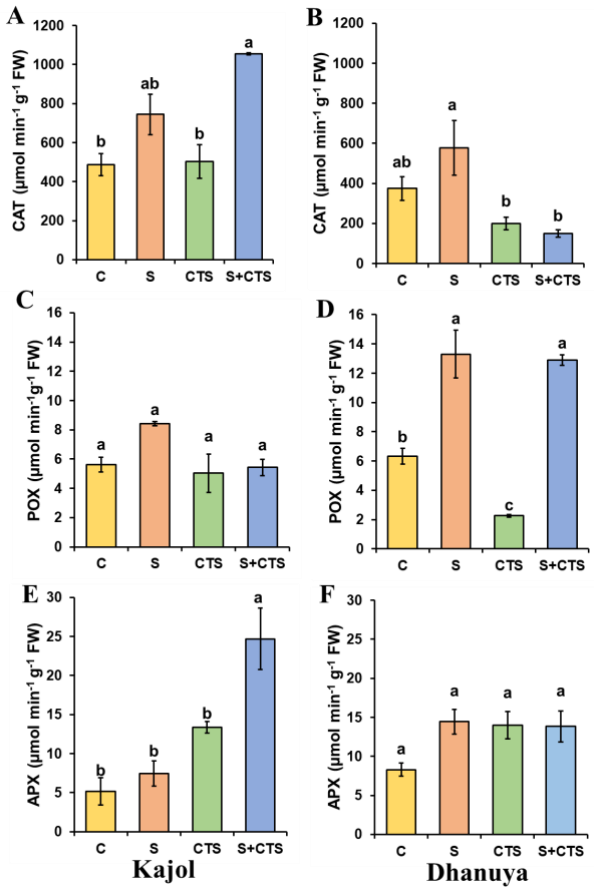

Figure 4. Effect of chitosan on the activities of (A-B) catalase (CAT), (C-D) peroxidase (POX), and (E-F) ascorbate peroxidase (APX) enzymes of two chilli varieties, viz. Kajol (A, C, E) and Dhanuya (B, D, F) under four different treatment conditions, viz. "C" (Control); "S" (Salinity); "CTS" (Chitosan); and "S+CTS" (Salinity plus Chitosan). The bars indicate the mean ± SE of three replicates (n = 3). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$.

3.4. Biochemical parameters

3.4.1. Antioxidant enzyme activity

Various salinity stress triggered an increase in CAT, POX and APX activities in both varieties as a protective response against oxidative damage (Fig. 4A-F). In the hybrid variety Dhanuya, CAT activity rose from approximately 375 to nearly 577 µmol/min/g FW under stress, whereas in the local variety Kajol, the increase was from 487 to around 745 µmol/min/g FW (Fig. 4A-B). Salt stress caused a rise in POX activity from 6 to nearly 13 µmol/min/g FW in Dhanuya, while in Kajol, it increased from 6 to around 8 µmol/min/g FW (Fig. 4C-D). The activity

of APX, a crucial enzyme in the ascorbate-glutathione cycle, was upregulated from 8 to 14 µmol/min/g FW for the variety Dhanuya, whereas from 5.2 to 7.5 µmol/min/g FW for Kajol variety, under stress (Fig. 4E-F).

Interestingly, the application of chitosan resulted in a significant reduction of CAT levels but preserved POX and APX activities in Dhanuya under salinity, suggesting that chitosan effectively mitigated the oxidative stress, thereby reducing the plant's need for excessive enzymatic defense. In contrast, chitosan application under salt stress increased CAT and APX activities, and maintained POX activity in Kajol compared to salt stress with no chitosan conditions (Fig. 4A, 4C, 4E). On the other hand, only chitosan application decreased CAT and POX activities in Dhanuya but sustained in Kajol, whereas increased APX activity in both varieties compared to controls (Fig. 4A-F).

3.4.2. Proline content

Proline accumulation is a vital physiological response to osmotic stress induced by salinity. The experimental data revealed that salinity stress led to a significant increase in proline content in both chilli varieties compared to their respective controls (Fig. 5A-B). For the local variety Kajol, the accumulation rose from 41 mg/g to around 121 mg/g FW (Fig. 5A). In the hybrid variety Dhanuya, the proline concentration increased sharply from approximately 85 mg/g FW to 133 mg/g FW under salt stress (Fig. 5B).

On the other hand, the application of chitosan under salt stress maintained elevated proline levels in both genotypes relative to stress conditions without chitosan, helping the plants sustain turgor pressure and protect cellular structures from dehydration (Fig. 5A-B). The supply of chitosan under control conditions increased proline levels in Kajol variety, but decreased in Dhanuya variety compared to their respective controls (Fig. 5A-B).

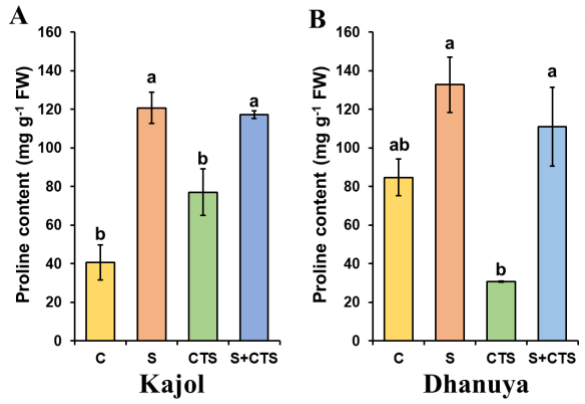

Figure 5. Effect of chitosan on proline content of two chilli varieties, viz. Kajol (A) and Dhanuya (B) under four different treatment conditions, viz. “C” (Control); “S” (Salinity); “CTS” (Chitosan); and “S+CTS” (Salinity plus Chitosan). The bars indicate the mean ± SE of three replicates (n = 3). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$.

3.4.3. Determination of chlorophyll content

Salinity stress led to a noticeable reduction in both chlorophyll *a* and *b* levels and total chlorophyll contents in both genotypes compared to controls (Fig. 6A-F). In the hybrid variety Dhanuya, chlorophyll *a* decreased from approximately 0.57 mg/g to 0.49 mg/g, while in the local variety Kajol, it dropped from 0.56 mg/g to around 0.48 mg/g (Fig. 6A-B). A similar inhibitory effect was observed for chlorophyll *b* (Fig. C-D). Total chlorophyll content under saline conditions declined in the variety Kajol from 1.1 mg/g to nearly 0.93 mg/g FW and in the variety Dhanuya from 1.2 mg/g FW to 0.86 mg/g FW (Fig. 6E-F).

However, the application of chitosan effectively mitigated this degradation. Chitosan treatment under salt stress almost restored the chlorophyll *a*, chlorophyll *b* and total chlorophyll levels to near-control values in both varieties, suggesting a better physiological mechanism to protect their photosynthetic apparatus from salt-induced oxidative damage through the assistance of chitosan (Fig. 6A-F). Supplementation of chitosan under non-stress conditions also maintained high chlorophyll levels comparable to controls in both varieties (Fig. 6A-F).

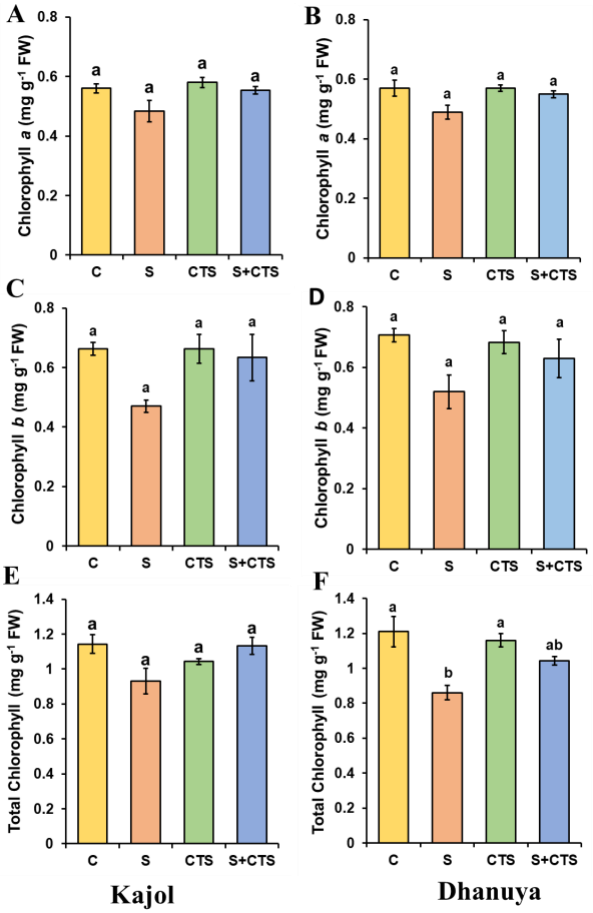

Figure 6. Effect of chitosan on (A-B) chlorophyll *a*, (C-D) chlorophyll *b*, and (E-F) Total chlorophyll of two chilli varieties, viz. Kajol (A, C, E) and Dhanuya (B, D, F) under four different treatment conditions, viz. “C” (Control); “S” (Salinity); “CTS” (Chitosan); and “S+CTS” (Salinity plus Chitosan). The bars indicate the mean ± SE of three replicates (n = 3). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$.

4. Discussion

In the present study, salt stress greatly decreased the flower count in both chilli varieties (Fig. 2A-B). This

decrease might be due to the buildup of harmful Na^+ and Cl^- ions, which decreases osmotic potential and interferes with the flowering transition process (Flowers & Colmer, 2015). Elevated salinity induces hormonal disruptions, notably raising abscisic acid (ABA) concentrations, resulting in early flower drop and aging (Wang et al., 2001). Moreover, the decline in photosynthetic efficiency due to salt stress restricts the supply of carbohydrates necessary for flower development, as recently noted by (Al-Naggar et al., 2017). In the current research, the decreased flower count caused by salinity was notably restored through foliar chitosan application in both Dhanuya and Kajol varieties (Fig. 2A-B). The mitigation of salt stress-induced growth inhibition by exogenous chitosan application has also been reported in previous studies involving chilli, tomato, lettuce, maize, wheat, and other crop species (Özkurt & Bektaş, 2022; Zhang et al., 2021; Shen et al., 2024; Ma et al., 2022). A stimulating influence of chitosan on reproductive characteristics was noted, which is consistent with recent discoveries in several horticultural species (Pichyangkura & Chadchawan, 2015). Chitosan serves as an effective biostimulant that boosts the absorption of vital nutrients such as nitrogen, phosphorus, and potassium, essential for flower induction (Moenne & González, 2021). Additionally, chitosan has been shown to enhance the expression of genes associated with flowering and boost the plant's antioxidant capacity, thereby delaying stress-induced flower aging and improving yield consistency in challenging environments (Hidangmayum et al., 2023).

The results of the current study showed that salinity stress caused a notable decrease in plant height and branch and leaf count in the local variety Kajol and the hybrid variety Dhanuya (Fig. 2C-D, 3A-D). This growth suppression is a common reaction to osmotic stress caused by salt, which restricts water availability and decreases turgor pressure, crucial for cell elongation and lateral bud formation (Flowers & Colmer, 2015). Salinity reduces the production of growth-enhancing hormones such as auxins and cytokinins, while elevating ethylene synthesis, thereby inhibiting vegetative growth (Hidangmayum et al., 2023). In addition, ion toxicity caused by excessive sodium buildup disrupts metabolic activities, directly obstructing the plant's capacity to grow new branches (Yasuor et al., 2017). Nonetheless, the use of foliar chitosan greatly reduced these negative impacts (Fig. 2C-D, 3A-D). Chitosan application resulted in a significant rise in plant height and branching density in both varieties when compared to their individual salt-stressed controls (Fig. 2C-D, 3A-D). This stimulation of growth is probably a result of chitosan acting as a bio-stimulant, which boosts the net photosynthetic rate and enhances the absorption of vital macro and micronutrients (Pichyangkura & Chadchawan, 2015). Chitosan and its derivatives enhance the plant's defense and growth processes by influencing carbon and nitrogen metabolism (Moenne & González, 2021).This outcome is consistent with the findings reported in previous studies on tomato, maize, soybean, and other crops (Ullah et al., 2020; Jiao et al., 2024; Sadeghipour, 2021) Furthermore, by improving the antioxidant defense system, chitosan mitigates oxidative damage to cellular membranes, facilitating improved cell growth and vegetative development in saline environments (Moenne & González, 2021).

The functions of essential antioxidant enzymes, such as catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX), were notably altered in both chilli types during salinity stress and subsequent chitosan application (Fig. 4A-F). This regulation is an essential physiological reaction to combat the excess generation of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), which causes oxidative harm to cellular membranes and proteins under salt stress (Pichyangkura & Chadchawan, 2015). In the present study, catalase activity exhibited a differential response between the two cultivars (Fig. 4A-B). Under salinity stress alone, both Kajol and Dhanuya showed an upregulation of CAT activity to scavenge the accumulated H_2O_2. However, upon the foliar application of chitosan under saline conditions (S+CTS), Kajol demonstrated a massive, synergistic increase in CAT activity (Fig. 4A), suggesting a highly responsive defense system. Conversely, in Dhanuya, the combination of salinity and chitosan resulted in a suppression of CAT activity compared to the salt stress alone (Fig. 4B). This reduction indicates that chitosan might have mitigated the primary oxidative stress or activated alternative hydrogen peroxide-scavenging pathways in Dhanuya, thereby reducing the dependency on CAT for ROS detoxification (Zhang et al., 2021)

Similarly, POX activity, which plays a dual role in H_2O_2 detoxification and cell wall lignification (Diao et al., 2017), was significantly influenced by treatments (Fig. 4C-D). Salinity stress alone induced a sharp rise in POX levels in both varieties (Fig. 4C-D). When chitosan was applied to salt-stressed plants, Dhanuya sustained a high level of POX activity, whereas Kajol maintained a moderate baseline (Fig. 4C-D). Ascorbate peroxidase activity, which functions specifically within the ascorbate-glutathione cycle to reduce H_2O_2 in chloroplasts and cytosol, was highly responsive to the foliar application of chitosan (Fig. 4E-F). Chitosan application boosted APX activity in both varieties, with the most pronounced enhancement observed in the salt-stressed Kajol variety (Fig. 4E-F). Oligosaccharides derived from chitosan can influence the expression of genes linked to the ascorbate-glutathione cycle, thereby directly boosting APX activity to safeguard the photosynthetic machinery from oxidative surges (Bigham Soostani et al., 2025). The results indicated that chitosan improves ROS scavenging processes in plants under saline environments.

The distinct performance of these three core enzymes highlighted that while both varieties employ antioxidant mechanisms to survive salt stress, the hybrid variety Dhanuya and the local variety Kajol utilize different enzymatic strategies. Chitosan successfully acts as an effective biostimulant, but it differentially modulates the CAT, POX, and APX coordination depending on the specific crop variety to effectively optimize the overall metabolic balance under environmental stress (Usman et al., 2025). The findings that chitosan contributes to mitigating adverse effects of salt stress by suppressing ROS accumulation and membrane injury are supported by previous reports on rapeseed, tomato, bean, maize, wheat, etc. (Ullah et al., 2020; Hajihashemi, 2022; Turk et al., 2019; Mulaudzi et al., 2022)

To mitigate salt stress, plants accumulate compatible solutes such as proline to maintain cytoplasmic osmotic balance and enhance cellular water uptake (Van Zelm et al., 2020; Balasubramaniam et al., 2023). In the present

study, salinity stress led to a notable rise in proline levels in the leaves of both chilli varieties, Kajol and Dhanuya (Fig. 5A-B). Proline serves as an essential osmoprotectant and signaling compound that aids plants in sustaining osmotic equilibrium and safeguarding cellular components from reactive oxygen species (ROS) during salt stress (Hayat et al., 2012). An increase in proline levels is a frequent physiological response in horticultural crops to counteract the decrease in osmotic potential due to elevated soil salinity (Tang et al., 2023). Additionally, the application of chitosan to leaves was observed to sustain elevated proline levels in plants under salt stress while averting excessive buildup in non-stressed controls (Fig. 5A-B). This indicates that chitosan functions as an elicitor that enhances the plant's metabolic readiness. Oligosaccharides derived from chitosan promote the expression of genes related to the biosynthesis of compatible solutes, such as proline (Moenne & González, 2021). Chitosan application improves membrane stability and recovery of chilli plants from salt-induced physiological stress by regulating proline levels, reinforcing its function as an effective bio-stimulant in saline conditions (Bigham Soostani et al., 2025). Chitosan enhances ability to withstand salinity stress in tomato (*Solanum lycopersicum* L.) by enhancing non-enzymatic antioxidants including proline (Mohamed et al., 2023).

Plant growth largely depends on photosynthesis, in which chlorophyll is the primary photosynthetic pigment (Simkin et al., 2022). Chlorophyll content is directly associated with plant health, as chlorophyll *a* and chlorophyll *b* are the principal pigments responsible for capturing light energy during photosynthesis (Pareek et al., 2017; Jiménez-Lao et al., 2021). The levels of photosynthetic pigments, namely chlorophyll *a*, chlorophyll *b*, and total chlorophyll, were markedly decreased due to salinity stress in both Kajol and Dhanuya chilli varieties (Fig. 6A-F). This decline is mainly due to the activation of the chlorophyllase enzyme caused by salt, which breaks down chlorophyll molecules, and the concurrent inhibition of vital enzymes that play a role in pigment biosynthesis (Ashraf & Harris, 2013). Salinity stress disrupts chloroplast ultra-structure and induces the generation of reactive oxygen species (ROS), resulting in chlorophyll photo-oxidation (Hasanuzzaman et al., 2020). In contrast, chitosan applied to the leaves greatly lessened the breakdown of chlorophyll pigments in saline environments (Fig. 6A-F). This outcome is consistent with the findings of prior research on tomato, maize, soybean, and other crops (Ullah et al., 2020; Jiao et al., 2024; Sadeghipour, 2021). Chitosan serves as an effective biostimulant that enhances nutrient absorption, especially magnesium M^{2+}, which is a key component of the chlorophyll molecule (Zohoungbogbo et al., 2025). Oligosaccharides derived from chitosan promote the production of chlorophyll biosynthesis precursors and boost the function of antioxidant enzymes that safeguard chloroplasts against oxidative harm (Pichyangkura & Chadchawan, 2015). Additionally, chitosan application sustains a greater relative chlorophyll content by enhancing the expression of genes associated with photosynthesis and minimizing sodium ion buildup in leaves (Hidangmayum et al., 2023). Therefore, the outcomes suggested that chitosan contributes in reducing salt-induced degradation of photosynthetic pigments.

5. Conclusion

The current research aimed to assess the effect of foliar chitosan treatment on two varieties of chilli, Kajol and Dhanuya, subjected to salinity stress. The study examined the morphological, physiological, and biochemical responses of chilli after exposure to salt stress to assess the chitosan's potential for mitigating stress-induced injuries. The findings showed that salinity stress considerably impeded the vegetative and reproductive development of both chilli varieties. Morphological parameters such as plant height, branch count, and flowers per plant were significantly diminished under saline conditions. This reduction in growth was linked to a notable decline in photosynthetic pigments, particularly chlorophyll *a*, chlorophyll *b*, and overall chlorophyll content. Biochemically, the plants reacted to salt-induced oxidative stress by accumulating proline and enhancing the activity of antioxidant enzymes like catalase, peroxidase, and ascorbate peroxidase. Although these internal mechanisms offered a degree of protection, they were inadequate in preventing significant growth loss. However, chitosan application facilitated morphological recovery, minimized chlorophyll breakdown, optimized proline and enzyme levels to maintain better cellular homeostasis and yield potential under stress. In conclusion, chitosan can be applied as a highly effective and eco-friendly strategy in chilli cultivation in salt-affected regions to alleviate salinity stress and improve crop productivity and quality for sustainable agriculture.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

The authors would like to acknowledge their gratitude towards the university authority for the support from Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Author contributions

Conceptualization, SRR and TUA; methodology, SRR, TUA, MAM and AR; formal analysis, SRR, AR and TUA; investigation, writing-original draft preparation, AR, SRR, MAM, TUA, SS, JHV and MAH; writing, review and editing, AR, SRR, TUA, SS, MJB and MAH; supervision, SRR and TUA. All authors have read and agreed to the published version of the manuscript.

Funding

The authors would like to extend their sincere appreciation to the Bangladesh Agricultural University Research System (BAURES) for funding this research (Project No. 2024/2318/BAU).

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