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

Fundamental and Applied Agriculture

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

Vol. 9(3), pp. 195 – 203: 2024, doi: https://doi.org/10.5455/faa.216212

AGRICULTURE | ORIGINAL ARTICLE

Chitosan Enhances Drought Tolerance in Maize (*Zea mays* L.) by Promoting Growth and Chlorophyll Content While Reducing Hydrogen Peroxide Levels

Abdul Kadir[1], Arifa Setu[1], Shayla Sharmin[1], Mohammad Anowar Hossain[1] ✉, Md. Tahjib-Ul-Arif[1], Muhammad Javidul Haque Bhuiyan[1], Yoshiyuki Murata[2]

[1] Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

[2] Graduate School of Environmental and Life Science, Okayama University, Kita-ku, Okayama 700-8530, Japan

ARTICLE INFO

Article history
Received: 11 Aug 2024
Accepted: 28 Sep 2024
Published online: 30 Sep 2024

Keywords
Drought stress, Chitosan, Antioxidant enzymes, Maize, Drought tolerance

Correspondence
Mohammad Anowar Hossain
✉: anowar.biochem@bau.edu.bd

ABSTRACT

Drought is one of the major constraints for maize (*Zea mays* L.) cultivation at the global level, as maize is sensitive to drought. To elucidate the impact of chitosan (CS) on improving drought tolerance in maize, the morphological and biochemical features of a drought-stressed maize variety (CAVARI 3696) at the germination and seedling stages were analyzed. The experiments performed at both stages were comprised of four different treatments in triplicate, viz., control (C), drought (D), drought with 50 ppm chitosan (D+50 CS) and 50 ppm chitosan only (50 CS). At the germination stage, drought stress significantly declined seed germination percentage and the growth and biomass of seedlings, while CS supplementation under drought enhanced germination and growth significantly. In the seedling stage, drought-stressed maize plants exhibited a significant reduction in chlorophyll contents (both Chl *a* and Chl *b*) but elevation in H_2O_2 buildup and lipid peroxidation measured as malondialdehyde (MDA) level. Drought stress also enhanced proline accumulation and improved catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) enzyme activities in maize plants. However, chitosan raised chlorophyll levels and suppressed H_2O_2, MDA, and proline content in maize plants under drought stress. Moreover, CS supplementation reduced the activities of the antioxidant enzymes under drought. These results suggest that chitosan can be used to improve the growth performance of maize under drought through enhancement in defense responses against the buildup of reactive oxygen species and associated oxidative injury.

1. Introduction

Drought stress is one of the most severe abiotic stress factors that adversely affect plant growth and development and constrain crop production in arid and semi-arid areas (Farooq et al., 2009; Ilyas et al., 2021). The severity and duration of the stressful conditions determine the detrimental effects of drought on crop production (Farooq et al., 2009). Due to global warming, drought stress is anticipated to be enhanced, thereby imposing a significant threat to world food security (Ilyas et al., 2021).

Reduced water availability has been reported to drop crop growth and yield by disturbing plant water relations, reducing CO_2 assimilation and disrupting enzyme activity (Farooq et al., 2009). To avoid drought stress, plants cut water losses by reducing transpiration and developing deep root systems to improve water uptake (Zargar et al., 2017). Among the determinants of growth reduction under drought, the excess accumulation of reactive oxygen species (ROS) is critical which considerably damages the structural and functional integrity of cellular organelles and the whole plant (Hasanuzzaman et al., 2020; Ilyas et al., 2021).

Plants have evolved defense mechanisms to relieve the harmful effects of excess accumulated ROS, maintain the stability of cellular metabolism and improve productivity (Hasanuzzaman et al., 2020; Ilyas et al., 2021).To adapt to stress conditions, plants possess several mechanisms including the accumulation of compatible solutes (e.g., sugars, proline) and activation of enzymatic (catalase; CAT; peroxidase; POD; ascorbate peroxidase; APX) and non-enzymatic antioxidant systems (Hasanuzzaman et al., 2020; Rajput et al., 2021; Ilyas et al., 2021). All these

Cite This Article

Kadir A, Setu A, Sharmin S, Hossain MA, Tahjib-Ul-Arif M, Bhuiyan MJH, Murata Y. 2024. Chitosan Enhances Drought Tolerance in Maize (*Zea mays* L.) by Promoting Growth and Chlorophyll Content While Reducing Hydrogen Peroxide Levels. *Fundamental and Applied Agriculture*, 9(3): 195–203. https://doi.org/10.5455/faa.216212

mechanisms assist plants in protecting important cellular ingredients such as proteins, enzymes and nucleic acids and enhancing stress tolerance (Hasanuzzaman et al., 2020; Ilyas et al., 2021).

Maize is an important food and feed crop in many countries. In Bangladesh, maize has recently gained much attention due to its enormous demand in the fish and animal feed industry (Mottaleb et al., 2018). It has emerged as the most important cereal crop after rice in Bangladesh, and its production of more than 4 million metric tons in the fiscal year 2021–2022 makes it the fastest-expanding cereal in the country (BBS, 2022). Maize is a sensitive crop to drought stress, particularly at the reproductive stages of development (Hussain et al., 2019). Due to climate change, it is speculated that drought stress will become a major threat to maize yields and will decrease world maize production (Kim and Lee, 2023).

One of the methods of mitigating the adverse effects of abiotic stresses on plants and improving their performance is employing biostimulants (Nephali et al., 2020). These elicitors are chemicals or various biological factors that can induce physiological changes in plants (Zhao et al., 2005). Several compounds, such as chitosan (CS), have been identified with eliciting properties that stimulate plants' reactions to stresses and their defense mechanisms (Bautista-Baños et al., 2006). CS is a biopolymer with non-toxic, biodegradable and biocompatible properties (Dragostin et al., 2016). Chitosan is obtained through the deacetylation of chitin from the exoskeleton of crustaceans (Zhao et al., 2019). Several studies have proven the role of chitosan in increasing plants' tolerance to several forms of abiotic stresses such as low-temperature, drought, and salt stress and enhancing plant growth (Guan et al., 2009; Malerba and Cerana, 2016; Li et al., 2017; Geng et al., 2020; Zhang et al., 2021). It has been reported that CS supplementation alleviates the adverse effects of drought stress by upregulating the antioxidant system, improving relative water content and enhancing photosynthetic activities (Li et al., 2017; Mustafa et. al., 2022; Hidangmayum et al., 2023).

Therefore, the current study was designed to explore the potential roles of CS in the mitigation of drought effects as well as in the improvement of the growth of maize plants under drought conditions. With this aim, several parameters were evaluated upon exposing plants to drought stress with and without CS treatment. The findings of this study will provide insight into the role of CS in the improvement of growth and productivity of the economically important maize crop under drought stress conditions.

2. Materials and Methods

2.1. Plant material and experiment in the germination stage

To assess the role of chitosan in mitigating the detrimental effect of drought, a pot experiment was conducted on maize (*Zea mays* L. var. hybrid maize, CAVARI 3696) in the net house of the Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh. Uniform-sized maize seeds were sorted out and surface sterilized using 5% sodium hypochlorite and 2% Tween-20 for 25 min, followed by three times washing with distilled water (dH_2O). Seeds were then placed in Petri dishes and four groups of treatments were applied i.e., control (C) with distilled water (non-stress condition); drought stress (D); drought stress with 50 ppm chitosan spray (D+50 CS); and distilled water with 50 ppm chitosan spray (50 CS). In the germination stage, drought condition was induced by applying polyethylene glycol (PEG-6000) solution (10%) at a 3-day interval, where every single dose contained 50 mL solution. The drought stress was imposed for nine days.

2.2. Germination percentage

The number of sprouted and germinated seeds was counted daily, commencing from the 4th to the 9th day. After 9 days, the final count was done, and the germination percentage was calculated by the following formula:

$$GP\ (\%) = \frac{G}{T} \times 100$$

Where, GP = germination percentage, G = total number of seeds germinated, and T = total number of seeds placed for germination.

2.3. Analysis of plant growth parameters

To determine shoot length (SL), the length from the shoot base to the leaf tip was measured. Similarly, the length from the root base to the root tip was measured to determine the root length (RL). Three seedlings were collected and weighed to determine the fresh weight (FW) of the shoot and root. The dry weight (DW) of the shoot and root was determined after oven drying at 60 °C for four days. The FWs and DWs of shoot and root were expressed as mg per seedlings.

2.4. Seedling stage experiment and treatments

Uniform-sized maize seeds were surface sterilized and washed with distilled water (dH_2O) as described earlier. Subsequently, the seeds were presoaked in dH_2O for 24 hours and kept at room temperature for 3 days to induce germination. Then, uniformly germinated seeds were placed in earthen pots (23.5 cm in height and 25.5 cm in diameter) containing 12 kg of soil (silt loam, pH of 6.18) and allowed to grow in a net house (average temperature, 25 °C and relative humidity, 60%). The soil was prepared by adding the following nutrients: urea (0.5 g/Kg), triple super phosphate (0.3 g/kg), muriate of potash (0.3 g/Kg) and gypsum (0.3 g/Kg). One third urea per pot was applied during soil preparation and later in two instalments at 25-30-day intervals to ensure the proper supply of nitrogen throughout the crop life span. The 45-day-old seedlings were separated into four treatment groups as described earlier i.e., "C", "D", "D+50 CS", and "50 CS". At the seedling stage, drought conditions were induced by a limited water supply for a certain period and further no water for plants. For the foliar spray of chitosan, it was dissolved in 1.0% acetic acid to get a stock solution and 50 mL of solution was sprayed in each pot over both the upper and lower parts of the leaves. The pH of the chitosan solution was adjusted to 6.5 with NaOH. The drought stress period lasted for 2 weeks.

2.5. Determination of chlorophyll content

The method established by Coombs et al. (1985) was used to determine the content of chlorophyll. A fresh leaf sample (50 mg) was taken into a test tube containing 10 mL of 80% acetone, covered by aluminium foil, and preserved in the dark for 7 to 10 days. Spectrophotometric readings were taken at 645 nm and 663 nm wavelengths using a UV/Vis spectrophotometer (Shimadzu, UV-1201; Japan) and the result was expressed as mg g^{-1} FW of leaf.

2.6. Determination of malondialdehyde (MDA) content

The MDA content was measured according to the method of Zhang and Huang (2013). Fresh leaves (0.1 g) were homogenized with 1 mL of 5% trichloroacetic acid (TCA) using a mortar and pestle maintaining a 4 ºC temperature. Then the extracts were collected and centrifuged at 11,500×g for 15 min at 4 ºC. After centrifugation, the supernatant was transferred to a new tube and mixed with 20% TCA containing 0.5% thiobarbituric acid (TBA). The mixture was boiled at 95 ºC for 15 min. After cooling quickly, the mixture was centrifuged at 11,500×g for 12 min. The absorbance of the mixture was measured at 532 nm wavelength using a UV/Vis spectrophotometer.

2.7. Determination of hydrogen peroxide (H_2O_2) level

The amount of H_2O_2 was measured following the method of Velikova et al. (2000). Fresh leaf samples (0.1 g) were homogenized in a mortar with pestle using 1 mL of 0.1% trichloroacetic acid (TCA) at 4 °C. After centrifugation at 10,000×g for 15 min, the supernatant was kept in the dark for 1 h after mixing with phosphate buffer (10 mM, pH 7.0) and potassium iodide (1 M) in the ratio of 0.5 mL: 0.5 mL: 1 mL. The absorbance of the resulting solution was recorded at 390 nm using a UV/Vis spectrophotometer.

2.8. Determination of proline

Proline content was determined following the modified method of Bates et al. (1973). A leaf sample (50 mg) was homogenized in a chilled mortar with a pestle using 10 mL of 3% sulfosalicylic acid. The homogenate was centrifuged (4500×g for 10 min) and then filtered through filter paper. Two mL filtrate was pipetted into a test tube, followed by 2 mL of acid ninhydrin and 2 mL of glacial acetic acid, and then heated for 1 hour at 100 °C. The reaction was started by adding 4 mL of toluene. The absorbance of the collected toluene was measured at 520 nm using a UV/Vis spectrophotometer.

2.9. Determination of antioxidant enzyme activity

The activity of CAT (EC: 1.11.1.6) was measured according to Aebi (1984). The activities of POD (EC: 1.11.1.7) and APX (EC: 1.11.1.11) were measured as described by Nakano and Asada (1981). A fresh leaf sample (50 mg) was collected and homogenized with 3 mL of 50 mM phosphate buffer (pH 8.0) (PB) in an ice-chilled mortar and pestle. The homogenate was centrifuged at 11,000×g for 10 minutes. The clear supernatant was used for assaying enzyme activity. For the CAT activity assay, 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 POD activity, 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 were mixed. For APX assay, 0.6 mL of PB, 0.1 mL of EDTA, 0.1 mL of H_2O_2 and 0.1 mL of ascorbate were mixed to prepare the reaction mixture. The reaction was started by adding 0.1 mL of enzyme extract and changes in absorbance were recorded immediately at 240 nm, 470 nm, and 290 nm for the CAT, POD and APX assays, respectively, at 30s intervals for two minutes using a UV/Vis spectrophotometer.

2.10. Statistical analysis

A one-way analysis of variance (ANOVA) was performed using Minitab 17.0. Different alphabetical letters denote statistically significant differences observed among the treatments at $p < 0.05$, according to Fisher's LSD Test. The data given in the figures and tables are all means with standard errors (n = 3).

3. Results

3.1. Effect of CS on germination and growth attributes of maize under drought stress

The protective role of CS on the germination capacity and growth of maize under drought stress was evaluated by determining the germination percentage (GP) of seeds and several growth parameters of seedlings (Fig. 1a-g). The GP was recorded higher under control conditions compared to drought conditions (Fig. 1a). The highest GP was observed in control (94.67%), whereas, the lowest (40%) was in the drought condition (Fig. 1a). However, there was a significant increase in GP in maize under drought with exogenous application of 50 ppm CS compared to those in drought without CS (Fig. 1a).

Maize seedlings exposed to drought exhibited a significant decrease in shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) by 28.45%, 35.79%, 26.47%, 35.71%, 38.66% and 23.77%, respectively, compared to that of under non-stress control treatment (Fig. 1b-g). However, exogenous application of CS to drought-stressed plants relieved the lethal effects of drought by enhancing the SL, RL, SFW, RFW, SDW, and RDW by 52.64%, 34.92%, 32%, 77.77%, 84.78%, and 50.53%, respectively, in comparison with that of only drought-stressed plants (Fig. 1b-g). Non-stressed plants supplemented with exogenous CS also showed higher shoot and root growth and biomass than only drought-stressed plants (Fig. 1b-g).

3.2. Effect of CS on the morphological appearance and chlorophyll content of maize plants under drought stress

A difference in the morphological appearance was observed in maize plants at the vegetative stage under different treatment conditions (Fig. 2). Plants exposed to drought stress showed retarded growth, less leaf production, and a variation in leaf colour compared to non-stressed control plants (Fig. 2). However, plants applied

with CS under drought stress showed morphological appearance comparable to control plants (Fig. 2). The growth of maize plants was also better under only CS treatment than drought-stressed plants (Fig. 2).

The Chl content of maize leaves was analyzed to determine the role of CS in protecting photosynthetic machinery under drought stress (Fig. 3a-b). Drought stress significantly decreased Chl *a* and Chl *b* content compared to plants grown in control conditions (Fig. 3a-b). On the other hand, supplementation of CS enhanced the Chl *a* and Chl *b* content in drought-stressed plants (Fig. 3a-b). However, CS (50 ppm) alone significantly increased the Chl *a* and Chl *b* content compared to drought-stressed maize plants (Fig. 3a-b).

3.3. Effect of exogenous CS on lipid peroxidation and proline content under drought stress

Membrane lipid peroxidation in plants was detected by measuring the levels of H_2O_2 and MDA. In the present study, the accumulation of H_2O_2 and MDA markedly increased under drought conditions (Fig. 4a-b). However, the application of CS to drought-stressed plants resulted in reductions in H_2O_2 and MDA content (Fig. 4a-b). The highest H_2O_2 and MDA levels were recorded under drought conditions and the lowest in controls and drought with CS (Fig. 4a-b). On the other hand, only CS (50 ppm) treatment also showed significantly lower H_2O_2 and MDA levels compared to drought-stressed plants (Fig. 4a-b).

In the present study, drought stress significantly increased proline content in maize plants compared to non-stress controls (Fig. 4c). The highest proline content was detected in the plants exposed to drought conditions (Fig. 4c). However, CS supplementation under drought conditions significantly reduced the proline content (Fig. 4c). The application of CS without drought also showed a significant reduction in proline level compared to the drought conditions (Fig. 4c).

3.4. Effect of exogenous CS on antioxidant enzyme activity under drought stress

The maize plants exposed to drought stress showed a significant increase in the activities of antioxidant enzymes CAT, POD and APX (Fig. 5a-c) A general trend of increased CAT, POD and APX activities was found in the drought-stressed maize plants compared to those of the non-stressed controls (Fig. 5a-c). However, the activities of CAT, POD and APX enzymes significantly declined with CS application under drought stress (Fig. 5a-c). Application of CS in non-stress conditions also reduced CAT, POD and APX activities compared to the drought stress conditions (Fig. 5a-c).

4. Discussion

The improved crop cultivation technologies focus not only on enhancing crop productivity and quality but also minimizing the risks posed to the natural environment. Several studies have demonstrated the use of biostimulants as one of the emerging strategies for enhancing crop productivity by promoting growth and resilience to the changing climate (Nephali et al., 2020). It is well reported that CS supplementation upregulates many physiological processes, and here we investigated the effect of CS supplementation on drought tolerance of maize.

Growth and morphological parameters are effective indicators for assessing the influence of biotic and abiotic stress factors on plant development (Hanaka et al., 2021). In the current experiment, drought stress inhibited seed germination (Fig. 1a). CS application, on the other hand, successfully ameliorated the negative impact of drought on the germination of maize seeds (Fig. 1a). A significant increase in germination% with CS supplementation was previously reported in drought-stressed and also salt-stressed plants (Hameed et al., 2014; Ling et al., 2022; Khaleduzzaman et al., 2021). Growth parameters i.e., SL, RL, SFW, RFW, SDW, and RDW of the drought-stressed maize seedlings were inhibited significantly (Fig. 1b-g). However, the growth and biomass of drought-stressed plants were significantly restored when treated with CS. This result is consistent with the previous findings on rice, white clover, mungbean etc. (Moolphuerk et al., 2022; Ling et al., 2022; Hidangmayum et al., 2023). The hindered growth of maize plants under drought stress may be the consequence of the decrease in water relations and nutrient uptake, alteration in metabolism, and increase in ROS content which causes lipid peroxidation, protein degradation and membrane leakage (Hasanuzzaman et al., 2020; Ilyas et al., 2021).

Plant growth is the key determinant of crop yield and more than 90% of biomass is derived from photosynthetic products. (Li et al., 2017; Yamori, 2020). Chlorophyll (Chl) represents an important pigment and an essential part of the primary reaction of photosynthesis (Li et al., 2017). In the current study, drought stress decreased Chl *a* and Chl *b* contents (Fig. 3a-b). This decline in photosynthetic pigments could be one of the major causes of retarded growth observed in drought-stressed plants in our study. Chloroplast is the prime site for ROS production (Munné-Bosch et al., 2001; Hasanuzzaman et al., 2020). Under various abiotic stresses like drought, ROS overaccumulation, due to the unbalanced ROS generation and ROS scavenging, leads to oxidative stress which is the main cause of impaired chloroplast functions and chlorophyll pigment damage (Munné-Bosch et al., 2001; Hasanuzzaman et al., 2020). However, in our study, CS supplementation to the drought-stressed maize plants raised Chl *a* and Chl *b* contents (Fig. 3a-b). Drought ameliorative effects of CS in terms of enhanced chlorophyll pigments were reported earlier in alfalfa, wheat, tomato etc. (Behboudi et al., 2019; Mustafa et al., 2022; Demehin et al., 2024). This improved Chl content in the current study might be one of the causes of retrieved growth-related attributes of drought-stressed maize plants after CS supply. For the proper functioning of chloroplasts and, in turn, for crop productivity, the uptake of essential metals is vital (Solymosi and Bertrand, 2012). Chitosan application under drought stress has been reported to improve mineral uptake by plants (Abu-Muriefah, 2013; Bistgani et al., 2023; Shinde et al., 2024). Improved uptake of essential elements may help in increasing the number of chloroplasts per cell, thus contributing to increased synthesis of chlorophyll (Hidangmayum et al., 2019).

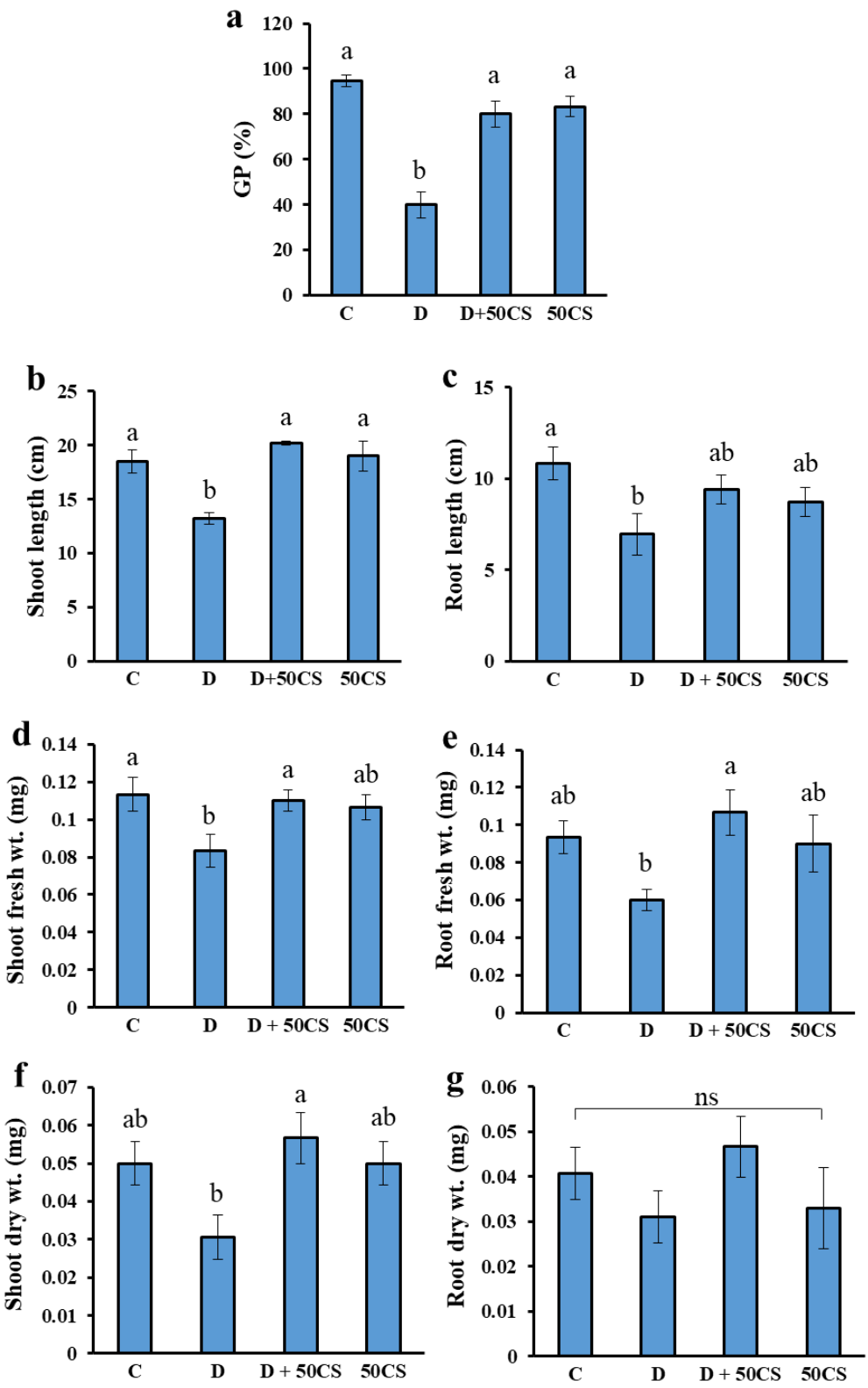

Figure 1. Effect of CS on (a) germination percentage, (b) shoot length, (c) root length, (d) fresh weight of shoot, (e) fresh weight of root, (f) dry weight of shoot and (g) dry weight of root of maize plants grown under four different treatment levels viz. “C” (control); “D” (drought); “D+50CS” (drought + 50 ppm chitosan); “50CS” (50 ppm chitosan). The bar indicates the mean ± SE of three replicates. Different letters obtained from Fisher's LSD test indicate significant differences among treatments at p <0.05. “ns" indicates not significant (p <0.05)

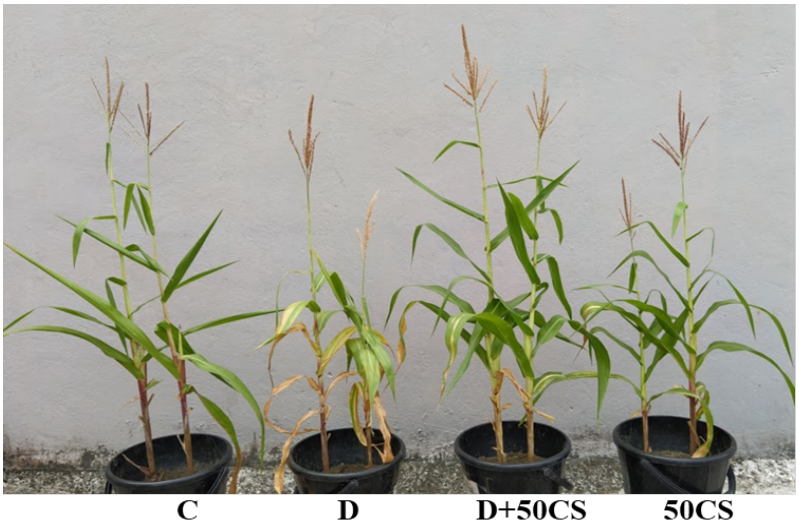

Figure 2. Effect of drought stress and CS on the phenotypes of maize plants after exposure for two weeks to four different treatment levels, viz., “C” (control), “D” (drought), “D+50CS” (drought + 50 ppm chitosan), and “50CS” (50 ppm chitosan)

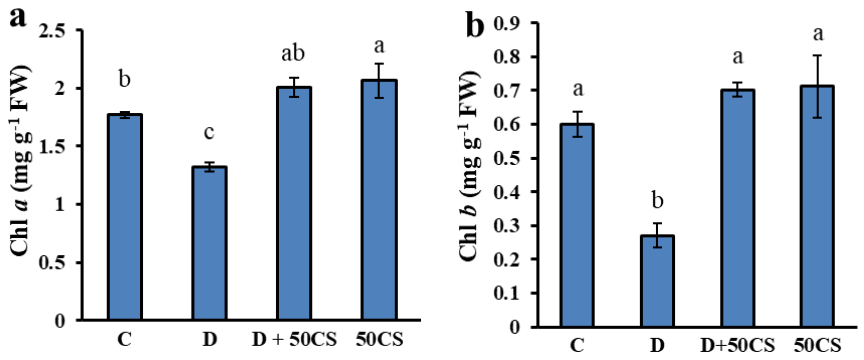

Figure 3. Effect of CS on (a) Chl *a* and (b) Chl *b* content in the leaves of maize plants grown under four different treatment levels, viz., “C” (control), “D” (drought), “D+50CS” (drought + 50 ppm chitosan), and “50CS” (50 ppm chitosan). The bar indicates the mean ± SE of three replicates. Different letters obtained from the Fisher's LSD test indicate significant differences among treatments at $p < 0.05$.

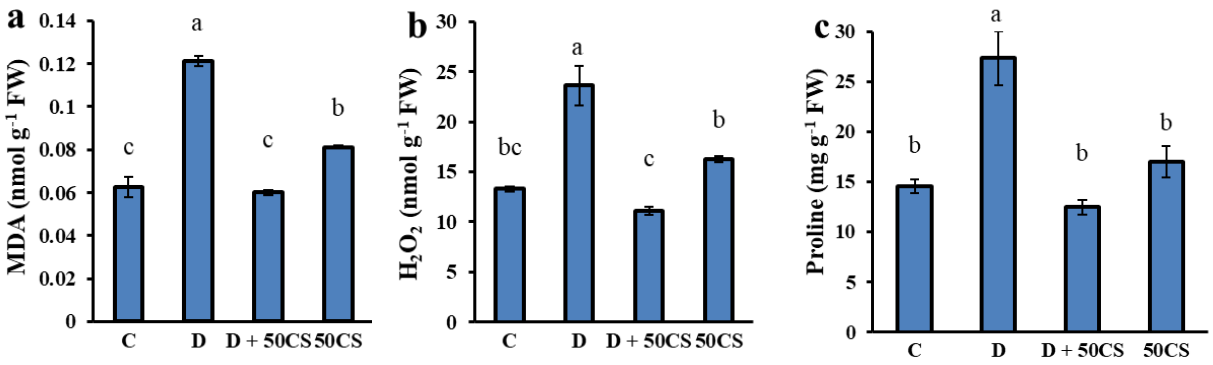

Figure 4. Effect of CS on (a) MDA, (b) H_2O_2 and (c) proline contents in the leaves of maize plants grown under four different treatment levels, viz. “C” (control), “D” (drought), “D+50CS” (drought + 50 ppm chitosan), and “50CS” (50 ppm chitosan). The bar indicates the mean ± SE of three replicates. Different letters obtained from the Fisher's LSD test indicate significant differences among treatments at $p < 0.05$.

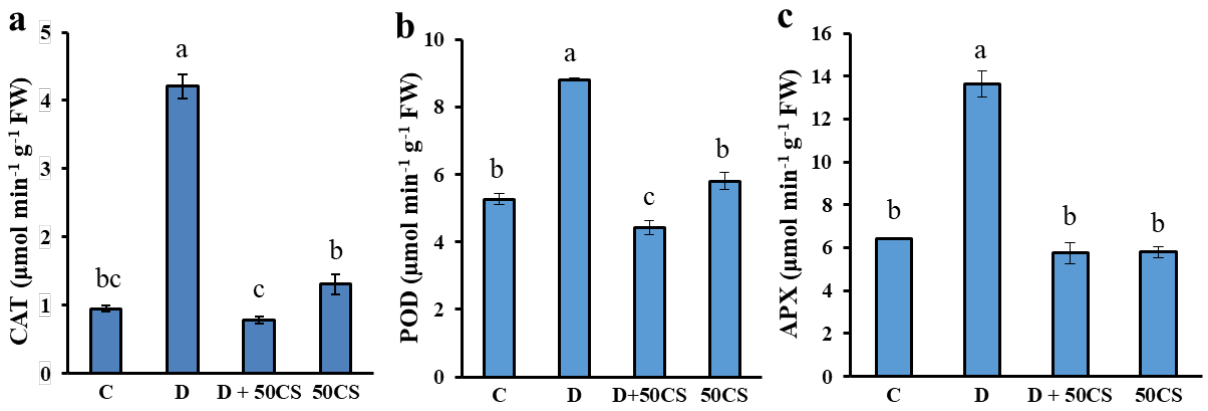

Figure 5. Effect of CS on the activity of (a) CAT, (b) POD and (c) APX enzymes in the leaves of maize plants grown under four different treatment levels, viz., "C" (control), "D" (drought), "D+50CS" (drought + 50 ppm chitosan), and "50CS" (50 ppm chitosan). The bar indicates the mean ± SE of three replicates. Different letters obtained from the Fisher's LSD test indicate significant differences among treatments at $p < 0.05$.

One of the adverse consequences of ROS-induced oxidative stress is the peroxidation of membrane lipids (Hasanuzzaman et al., 2020). MDA, a byproduct of lipid peroxidation, is widely measured as a marker for oxidative stress-related membrane lipid damage (Soltabayeva et al., 2021). In the current work, a significant buildup of MDA and H_2O_2 was observed in drought-stressed maize leaves indicating enhanced lipid peroxidation triggered by ROS accumulation (Fig. 4a-b). On the other hand, supplementation of CS to maize leaves relieved drought-induced oxidative stress effects, as represented by the decreased MDA and H_2O_2 levels (Fig. 4a-b). This outcome is supported by other findings in maize, sorghum, mungbean, tomato etc. (Rabêlo et al., 2019; Ávila et al., 2023; Hidangmayum et al., 2023; Demehin et al., 2024).

A common physiological mechanism adopted by plants to counter the adverse effects of abiotic stresses is the accumulation of low-molecular-weight organic compounds, compatible solutes such as proline (Hossain, et al., 2014; Nephali et al., 2020; Mansour and Salama, 2020). As an osmoprotectant, proline facilitates osmotic adjustment, enables water absorption, stabilizes subcellular structures and also scavenges reactive oxygen species (ROS) (Hossain et al., 2014; Nephali et al., 2020; Mansour and Salama, 2020). In the present investigation, an elevation in proline content was observed in drought-stressed maize plants compared to non-stressed plants (Fig. 4c). However, CS application declined the proline content under drought conditions (Fig. 4c). Chitosan supply to drought-stressed plants has been reported to induce the further accumulation of proline than drought-stressed plants without chitosan (Rabêlo et al., 2019; Hidangmayum et al., 2023; Demehin et al., 2024). In safflower, high concentrations of chitosan increased proline contents but low concentrations (0.05–0.4%) decreased proline levels with increasing water-deficit stress (Mahdavi et al., 2011). The application of chitosan on sorghum grown under water-deficit conditions contributed to a reduction in the proline content (Ávila et al., 2023). Therefore, Hidangmayum et. al. (2019) and Shinde et. al. (2024) suggested that the effect of chitosan under drought may vary due to plant species and other factors. Moreover, an increase in the production of chlorophyll may lead to a decrease in the synthesis of proline under drought with CS since both proline and photosynthetic pigments are synthesized from the same substrate (Paleg and Aspinall, 1981; Hidangmayum et al., 2019).

Antioxidant enzymes are crucial players in protecting plants from oxidative stress-induced cellular damage by scavenging ROS (Rajput et al., 2021; Ghorbel et al., 2024). Plants possess several antioxidant enzymes associated with ROS scavenging such as CAT, APX and POD (Rajput et al., 2021; Ghorbel et al., 2024). CAT decomposes H_2O_2 into water and molecular oxygen (O_2), APX utilizes ascorbate as a specific electron donor to scavenge H_2O_2 to water, while POD works in the extra-cellular space for scavenging H_2O_2 (Rajput et al., 2021; Ghorbel et al., 2024). Prompt detoxification of ROS by regulating antioxidant defense is one of the key adaptive mechanisms of plants for drought tolerance (Ilyas et al., 2021; Rajput et al., 2021). In the current study, the activities of CAT, POD and APX increased in plants exposed to drought stress (Fig. 5a-c). The elevated activities of these antioxidant enzymes indicated the defense measures adopted by plants against raised H_2O_2 levels triggered by drought stress. On the other hand, drought-stressed plants treated with CS resulted in reductions in CAT, POD and APX activities (Fig. 5a-c). This outcome contradicts several reports where chitosan treatment significantly improved CAT and APX activities than untreated plants under drought stress (Hidangmayum et al., 2023; Ávila et al., 2023). However, chitosan was also detected to reduce POD activity under water-deficit conditions (Ávila et al., 2023). In water-stressed white clover, CS improved CAT and POD activities but did not affect APX activity (Ling et al., 2022). Increased APX activity but decreased CAT activity was reported in maize exposed to water-deficit conditions with chitosan (Rabêlo et al., 2019). The reduced antioxidant enzyme activity with CS supplementation might be triggered by the ROS scavenging ability presented by CS. Yang et al. (2009) proposed that chitosan may act as an antioxidant to enhance resistance to oxidative stress during drought. Chitosan may possess antioxidant activity because of the presence of hydroxyl and amino groups in its structure which offers an effective scavenger of ROS (Xie et al., 2001; Sun et al., 2008; Yang et al., 2009).

5. Conclusion

Agricultural production must be raised to meet the increasing global food demand. Therefore, novel approaches, such as the utilization of biostimulant, need to be developed to improve agricultural practices and crop production. Maize is an important cereal crop and its utilization is increasing rapidly worldwide. Drought stress severely affects the normal physiological processes of maize plants at every growth stage, ultimately limiting yield. Water evaporation and consequently drought stress is expected to accelerate in the future. Biostimulants like chitosan and its oligomers have been used in plants to confer resistance against abiotic stresses such as drought, salt stress and heavy metal toxicity. Their efficacy in enhancing ROS scavenging systems and ultimately improving the performance of plants under stress has attracted researchers to offer a more varied application and continue to explore this novel biopolymer. In the present experiment, CS application significantly enriched chlorophyll *a* and *b* contents and thereby boosted vegetative growth while these parameters were reduced in maize under drought. The levels of oxidative stress markers such as H_2O_2 and MDA dropped under drought conditions because of CS supplementation. Accumulation of osmolyte (proline) and the activities of antioxidant enzymes CAT, POD and APX were increased under drought stress but CS application reduced their levels. The diminished antioxidant enzyme activities in the presence of CS point to the ROS scavenging activities offered by CS. Therefore, it can be concluded that CS is a potential biostimulator to enhance plant growth and tolerance to oxidative stress under drought by suppressing ROS levels and related oxidative damage.

Acknowledgement

The authors are thankful to Dr. Hari Pada Seal (Professor, Department of Agricultural Chemistry, BAU, Mymensingh) for providing the chitosan.

Conflict of Interests

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

References

Abu-Muriefah SS. 2013. Effect of chitosan on common bean (*Phaseolus vulgaris* L.) plants grown under water stress conditions. International Research Journal of Agricultural Science and Soil Science, 3(6): 192-199.

Aebi H. 1984. Catalase *in vitro*. In Methods in enzymology (Vol. 105, pp. 121-126). Academic press.

Ávila RG, Magalhães PC, Vitorino LC, Bessa LA, de Souza KRD, Queiroz RB, Jakelaitis A, Teixeira MB. 2023. Chitosan induces sorghum tolerance to water deficits by positively regulating photosynthesis and the production of primary metabolites, osmoregulators, and antioxidants. Journal of Soil Science and Plant Nutrition, 23(1): 1156-1172.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant and soil, 39(1): 205-207.

Bautista-Baños S, Hernandez-Lauzardo AN, Velazquez-Del Valle MG, Hernández-López M, Barka EA, Bosquez-Molina E, Wilson CL. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop protection, 25(2): 108-118.

BBS. 2022. Yearbook of Agricultural Statistics. Bangladesh Bureau of Statistics (BBS), Statistics and Informatics Division (SID), Ministry of Planning; Government of the People's Republic of Bangladesh: Dhaka, Bangladesh

Behboudi F, Tahmasebi-Sarvestani Z, Kassaee MZ, Modarres-Sanavy SAM, Sorooshzadeh A, Mokhtassi-Bidgoli A. 2019. Evaluation of chitosan nanoparticles effects with two application methods on wheat under drought stress. Journal of Plant Nutrition, 42(13): 1439-1451.

Bistgani ZE, Hashemi M, Akbari P, Mumivand H. 2023. Influence of drought stress and foliar application of chitosan on nutrient accumulation and phenolic composition of *Thymus daenensis* Celak. Crop Science, 63(2): 921-935.

Coombs J, Hind G, Leegood RC, Tieszen LL, Vonshak A. 1985. Analytical techniques, Techniques in Bioproductivity and Photosynthesis (Second Edition). Elsevier, pp. 219-228

Demehin O, Attjioui M, Goñi O, O'Connell S. 2024. Chitosan from Mushroom Improves Drought Stress Tolerance in Tomatoes. Plants, 13(7): 1038.

Dragostin OM, Samal SK, Dash M, Lupascu F, Pânzariu A, Tuchilus C, Ghetu N, Danciu M, Dubruel P, Pieptu D, Vasile C. 2016. New antimicrobial chitosan derivatives for wound dressing applications. Carbohydrate polymers, 141: 28-40.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. 2009. Plant drought stress: effects, mechanisms and management. Agronomy for sustainable development, 29: 185-212.

Geng W, Li Z, Hassan MJ, Peng Y. 2020. Chitosan regulates metabolic balance, polyamine accumulation, and Na^+ transport contributing to salt tolerance in creeping bentgrass. BMC Plant Biology, 20: 1-15.

Ghorbel M, Olayen W, Brini F. 2024. Roles of enzymatic antioxidants in stress response and signaling in plants. In Defense-Related Proteins in Plants (pp. 413-468). Academic Press.

Guan YJ, Hu J, Wang XJ, Shao CX. 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. Journal of Zhejiang University Science B, 10: 427-433.

Hameed A, Sheikh MA, Hameed A, Farooq T, Basra SMA, Jamil A. 2014. Chitosan seed priming improves seed germination and seedling growth in wheat (*Triticum aestivum* L.) under osmotic stress induced by polyethylene glycol. Philipp Agric Sci, 97(3): 294-299.

Hanaka A, Majewska M, Jaroszuk-Ściseł J. 2021. Study of the influence of abiotic and biotic stress factors on horticultural plants. Horticulturae, 8(1): 6.

Hasanuzzaman M, Bhuyan, MB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V. 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants, 9(8): 681.

Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A. 2019. Application of chitosan on plant responses with special reference to abiotic stress. Physiology and molecular biology of plants, 25: 313-326.

Hidangmayum A, Dwivedi P, Kumar P, Upadhyay SK. 2023. Seed priming and foliar application of chitosan ameliorate drought stress responses in mungbean genotypes through modulation of morpho-physiological attributes and increased antioxidative defense mechanism. Journal of Plant Growth Regulation, 42(10): 6137-6154.

Hossain MA, Hoque, MA, Burritt DJ, Fujita M. 2014. Proline protects plants against abiotic oxidative stress: biochemical and molecular mechanisms. In Oxidative damage to plants (pp. 477-522). Academic press.

Hussain HA, Men S, Hussain S, Chen Y, Ali S, Zhang S, Zhang K, Li Y, Xu Q, Liao C, Wang L. 2019. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Scientific Reports, 9(1): 3890

Ilyas M, Nisar M, Khan N, Hazrat A, Khan AH, Hayat K, Fahad S, Khan A, Ullah A. 2021. Drought tolerance strategies in plants: a mechanistic approach. Journal of Plant Growth Regulation, 40: 926-944.

Khaleduzzaman M, Hossain MA, Bhuiyan MJH, Mahmud S, Tahjib-Ul-Arif M, Murata Y. 2021. Chitosan mitigates salt stress in rice by enhancing antioxidant defense system. Fundamental and Applied Agriculture, 6(4): 336-348.

Kim KH, Lee BM. 2023. Effects of climate change and drought tolerance on maize growth. Plants, 12(20): 3548.

Li Z, Zhang Y, Zhang X, Merewitz E, Peng Y, Ma X, Huang L, Yan Y. 2017. Metabolic pathways regulated by chitosan contributing to drought resistance in white clover. Journal of proteome research, 16(8): 3039-3052.

Ling Y, Zhao Y, Cheng B, Tan M, Zhang Y, Li Z. 2022. Seed priming with chitosan improves germination characteristics associated with alterations in antioxidant defense and dehydration-responsive pathway in white clover under water stress. Plants, 11(15): 2015.

Mahdavi B, Modarres Sanavy SAM, Aghaalikhani M, Sharifi M, Dolatabadian A. 2011. Chitosan improves osmotic potential tolerance in safflower (*Carthamus tinctorius* L.) seedlings. Journal of Crop Improvement, 25(6): 728-741.

Malerba M, Cerana R. 2016. Chitosan effects on plant systems. International journal of molecular sciences, 17(7): 996.

Mansour MMF, Salama KHA. 2020. Proline and abiotic stresses: responses and adaptation. Plant ecophysiology and adaptation under climate change: mechanisms and perspectives II: mechanisms of adaptation and stress amelioration, 357-397.

Moolphuerk N, Lawson T, Pattanagul W. 2022. Chitosan mitigates the adverse effects and improves photosynthetic activity in rice (*Oryza sativa* L.) seedlings under drought condition. Journal of Crop Improvement, 36(5): 638-655.

Mottaleb KA, Kruseman G, Erenstein O. 2018. Determinants of maize cultivation in a land-scarce rice-based economy: The case of Bangladesh. Journal of Crop Improvement, 32(4): 453–476.

Munné-Bosch S, Jubany-Marí T, Alegre L. 2001. Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. Plant, cell & environment, 24(12): 1319-1327.

Mustafa G, Shehzad MA, Tahir MHN, Nawaz F, Akhtar G, Bashir MA, Ghaffar A. 2022. Pretreatment with chitosan arbitrates physiological processes and antioxidant defense system to increase drought tolerance in alfalfa (*Medicago sativa* L.). Journal of Soil Science and Plant Nutrition, 22(2): 2169-2186.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22(5): 867.880

Nephali L, Piater LA, Dubery IA, Patterson V, Huyser J, Burgess K, Tugizimana F. 2020. Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. Metabolites, 10(12): 505.

Paleg LG, Aspinall D. 1981. The physiology and biochemistry of drought resistance in plants. Academic Press, New York

Rabêlo VM, Magalhães PC, Bressanin LA, Carvalho DT, Reis COD, Karam D, Doriguetto AC, Santos MHD, Santos Filho PRDS, Souza TCD. 2019. The foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water deficit in maize, improving the antioxidant system and increasing photosynthesis and grain yield. Scientific reports, 9(1): p.8164.

Rajput VD, Harish, Singh RK, Verma KK, Sharma L, Quiroz-Figueroa FR, Meena M, Gour VS, Minkina T, Sushkova S, Mandzhieva S. 2021. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. Biology, 10(4): 267.

Shinde NA, Kawar PG, Dalvi SG. 2024. Chitosan-Based Nanoconjugates: A Promising Solution for Enhancing Crop Drought-Stress Resilience and Sustainable Yield in the Face of Climate Change. Plant Nano Biology, 100059.

Soltabayeva A, Ongaltay A, Omondi JO, Srivastava S. 2021. Morphological, physiological and molecular markers for salt-stressed plants. Plants, 10(2): 243.

Solymosi K, Bertrand M. 2012. Soil metals, chloroplasts, and secure crop production: a review. Agronomy for Sustainable Development, 32: 245-272.

Sun T, Yao Q, Zhou D, Mao F. 2008. Antioxidant activity of N-carboxymethyl chitosan oligosaccharides. Bioorganic & medicinal chemistry letters, 18(21): 5774-5776.

Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant science, 151(1): 59-66.

Xie W, Xu P, Liu Q. (2001). Antioxidant activity of water-soluble chitosan derivatives. Bioorganic & Medicinal Chemistry Letters, 11(13): 1699-1701.

Yamori W. 2020. Photosynthesis and respiration. In Plant factory (pp. 197-206). Academic Press.

Yang F, Hu J, Li J, Wu X, Qian Y. 2009. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. Plant Growth Regulation, 58: 131-136.

Zargar SM, Gupta N, Nazir M, Mahajan R, Malik FA, Sofi NR, Shikari AB, Salgotra RK. 2017. Impact of drought on photosynthesis: Molecular perspective. Plant Gene, 11: 154-159.

Zhang G, Wang Y, Wu K, Zhang Q, Feng Y, Miao Y, Yan Z. 2021. Exogenous application of chitosan alleviates salinity stress in lettuce (*Lactuca sativa* L.). Horticulturae, 7(10): 342.

Zhang Z, Huang R. 2013. Analysis of malondialdehyde, chlorophyll proline, soluble sugar, and glutathione content in Arabidopsis seedling. Bio-protocol, 3(14): e817

Zhao J, Davis LC, Verpoorte R. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnology advances, 23(4): 283-333.

Zhao J, Pan L, Zhou M, Yang Z, Meng Y, Zhang X. 2019. Comparative physiological and transcriptomic analyses reveal mechanisms of improved osmotic stress tolerance in annual ryegrass by exogenous chitosan. Genes, 10(11): 853.