Online Journal of Biological Sciences

Original Research Paper

Differential Response of Chitosan-Pretreated Rice Cultivars at the Seedling Stage to Drought Stress on Growth and Leaf Metabolites

[1]Wasinee Pongprayoon, [2]Sataporn Deeying, [3]Sakchai Hongthong, [4]Gholamreza Khaksar, [5]Nichaphat Kanoksinwuttipong and [2]Chakkree Lekklar

[1]Department of Biology, Faculty of Science, Burapha University, Chonburi, Thailand
[2]Division of Agricultural Technology, Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao, Thailand
[3]Division of Chemistry, Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao, Thailand
[4]Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
[5]Division of Cannabis Health Sciences, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram, Thailand

Article history
Received: 03-05-2024
Revised: 12-07-2024
Accepted: 18-07-2024

Corresponding Author:
Chakkree Lekklar
Division of Agricultural Technology, Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao, Thailand
Email: chakkree.lek@rru.ac.th

Abstract: The escalating prevalence of drought stress presents a significant obstacle to global rice cultivation, particularly in arid regions affected by climate change-induced water scarcity. Despite extensive research on the efficacy of exogenously applied chitosan in enhancing drought tolerance across various crops, there remains a gap in understanding the differential responses of chitosan-treated rice seedlings among different cultivars and different chitosan concentrations during pre-drought and drought stress conditions. This study aims to address this gap by investigating the effects of chitosan application on the stress responses of four distinct rice cultivars, namely Khao Dowk Mali 105 (KDML105), Suphanburi 1 (SB1), Riceberry (RB), and RD49 during before and drought stress conditions. The study observed significant variations in phenotypic traits, particularly shoot and root weights, among the cultivars, following chitosan treatment (10, 20, and 40 ppm) during simulated drought stress. The results indicate that the application of 10 ppm of chitosan considerably improves the growth of shoots in the KDML105 under conditions of drought stress. This improvement is associated with an increase in the levels of fructose and glucose in the leaves. Chitosan treatment in SB1 showed higher concentrations of amino acids, including proline, that facilitate the development of roots in drought conditions. Conversely, RB and RD49 demonstrated adverse effects on growth parameters, with elevated H_2O_2 levels indicating oxidative stress. This study highlights the diverse reactions of several rice cultivars to chitosan pretreatment when subjected to drought stress.

Keywords: Chitosan, Cultivar, Drought, Metabolite, Rice (*Oryza sativa* L.)

Introduction

Rice (*Oryza sativa* L.) is a globally consumed staple food crop. The expanding global population and the high demand for food have led to increased efforts to explore sustainable agricultural methods to produce enough rice to meet consumer demands (Khush, 2005). To this end, an 87% increase in production is needed (Samal *et al*., 2018). However, abiotic environmental stressors, including salinity, drought, excessive temperatures, the presence of harmful metal ions, and exposure to UV radiation have adversely affected crop production and led to a significant yield loss (Ahmad *et al*., 2014; Singh *et al*., 2015). Drought is a prevalent constraint in global agricultural productivity (Takahashi *et al*., 2020). In recent times, numerous areas across the globe have experienced drought, which has been worsened by the effects of climate change (Turral *et al*., 2011; Wassmann *et al*., 2009; Konopianov *et al*., 2024).

As a response to drought stress, Reactive Oxygen Species (ROS) production is stimulated in plants. These ROS cause significant harm to plants experiencing stress by peroxidation of membrane lipids and directly

interacting with various macromolecules. This ultimately results in decreased plant development and yield (Takahashi *et al*., 2020). Osmotic adjustment and cellular compatible solute accumulation in plant cells are known as prime physiological adaptations to drought conditions mainly through turgor maintenance and the protection of specific cellular functions by defined solutes. Soluble sugars and proline are among the common solutes that play major roles in osmotic adjustment. The primary physiological responses of plant cells to dry conditions are osmotic adjustment and cellular-compatible solute accumulation, which are primarily accomplished via turgor maintenance and the defense of particular cell functions by defined solutes. Soluble sugars and proline are important solutes that contribute significantly to osmotic adjustment (Ghosh *et al*., 2021; Hayat *et al*., 2012). These stress responses to drought may allow short-term adaptation to temporary deficits in water. Nevertheless, if the stress persists, it dramatically decreases plant growth and productivity. Elicitors are chemical substances derived from both biotic and abiotic origins that can induce stress responses in plants, leading to increased production and accumulation of secondary metabolites (Guru *et al*., 2022). Yeast extracts, fungal carbohydrates, and chitosan are commonly employed as elicitors. Chitosan is a polycationic polymer synthesized by alkaline N-deacetylation of chitin, a structural substance found in many invertebrates, especially the exoskeletons of crustaceans including shrimp and crabs. Giraldo *et al*. (2023) review that agriculture commonly uses chitosan as a biostimulant to enhance plant growth and protect against damage (Pichyangkura and Chadchawan, 2015; Chamnanmanoontham *et al*., 2015). The interaction between chitosan and plant cells initiates when chitosan binds to specific cell receptors. This binding triggers the release of secondary messengers, including hydrogen peroxide (H_2O_2), Calcium ion (Ca^{2+}), Nitric Oxide (NO), and phytohormones (jasmonate, ethylene, and abscisic acid), within the cell (Pichyangkura and Chadchawan, 2015; Román-Doval *et al*., 2023). These messengers would then induce physiological responses including improving membrane stability and activating the antioxidant system, regulating callose synthesis and programmed cell death, inducing antioxidant enzymes, and enhancing stomatal closure and regulating water usage (Román-Doval *et al*., 2023). Chitosan has the potential to induce positive responses in plants against various environmental stresses (Malerba and Cerana, 2015); Hidangmayum *et al*., 2019; Mukarram *et al*., 2023; Phothi and Theerakarunwong, 2017). Scientific research has provided substantial evidence that applying chitosan can enhance drought tolerance in different crops, including the methods of application and the main mechanisms by which chitosan conferred drought resistance (Boonlertnirun *et al*., 2007; Dolatkhah Dashtmian *et al*., 2023). These reports include cowpea (foliar application on seedlings, improving growth and yield; Farouk and Amany (2012), pepper (foliar application on seedlings, reducing the water usage; Bittelli *et al*., 2001), grapevine (dipping of stem cutting before planting, maintaining chlorophyll content; Górnik *et al*., 2008), *Thymus diagenesis* Celak (spraying before the flowering stage, increasing flowering and full bloom; Emami Bistgani *et al*., 2017). In addition, a study conducted by Li *et al*. (2017) found that applying chitosan to white clover (*Trifolium repens*) seedlings resulted in an increase in the synthesis of metabolites that respond to drought stress.

The application of chitosan, with a degree of deacetylation of 96.62% and a molecular weight of approximately 100,000 KDa, led to a notable decrease in leaf rolling and the percentage of damaged leaves during water-deficit stress. Chitosan treatment prior to drought stress provided the best results, with the lowest leaf rolling scores and damaged leaf percentage (Boonlertnirun *et al*., 2007) Additionally, Moolphuerk and Pattanagul (2020) examine the effect of pretreating rice seedlings with chitosans of varying molecular weights (MW), including low, medium and high MW, on their response to drought stress. Rice seedlings were pretreated with chitosans through seed priming and foliar spray. They discovered that chitosan with a low molecular weight was especially successful in enhancing beneficial effects, such as enhancing root growth, preserving higher water content, and increasing antioxidant activities (Moolphuerk *et al*., 2022). Recently, a phosphoproteomic investigation was conducted on ‘KDML105’ rice, a drought-sensitive cultivar subjected to osmotic stress to identify phosphoproteins and differently expressed proteins in response to chitosan pretreatment under osmotic stress were found. A total of around 2,000 phosphoproteins, with significant changes in 60 proteins in response to chitosan treatment under osmotic stress, were identified. The proteins played a part in defense responses, signaling, metabolic processes, transport, transcription, and increased shoot growth. This maintained the plant's photosynthetic pigments and made it more resistant to drought through intricate networks involving signal transduction and secondary metabolism (Pongprayoon *et al*., 2022).

Furthermore, to elucidate the mechanisms by which chitosan improves abiotic stress tolerance in rice seedlings induced by salinity in different rice cultivars, two salt-sensitive and two salt-tolerant rice cultivars were treated with 25-50 ppm of chitosans, respectively. Chitosan at 50 ppm mitigated the reduction in shoot length, shoot dry weight, and photosynthetic pigments caused by salt stress, particularly in the salt-sensitive cultivars, suggesting rice cultivars have varied physiological responses different to chitosan to combat salinity. Furthermore, to clarify the ways in which chitosan enhances the ability of rice seedlings to

withstand abiotic stress caused by salinity in various rice varieties, two rice cultivars that are sensitive to salt and two rice cultivars that are tolerant to salt were exposed to 25-50 ppm of chitosan, respectively. Chitosan, when applied at a concentration of 50 ppm, alleviated the negative effects of salt stress on growth parameters and photosynthetic pigments. This effect was particularly noticeable in rice cultivars that are sensitive to salt stress. These findings indicate that different rice cultivars exhibit diverse physiological responses to chitosan in order to mitigate the harmful effects of salinity (Khaleduzzaman *et al*., 2021).

However, previous studies have not comprehensively addressed the differential responses of various rice cultivars to chitosan in pretreatment at different concentrations under drought stress conditions. There is a need for a deeper understanding of how different rice cultivars react in terms of growth and metabolite changes at the seedling stage when subjected to drought stress and chitosan treatment. Therefore, the aim of this study is to investigate the differential response of chitosan-pretreated rice cultivars at the seedling stage to drought stress, focusing on growth and leaf metabolites. By comparing various cultivars, this research seeks to identify specific responses that could inform better management practices and the development of more resilient rice varieties.

Materials and Methods

Plant Materials, Growing Conditions, Chitosan and Drought Stress Treatments

Rice (*O. sativa* L.) seeds of 4 cultivars consisting of Khao Dawk Mali 105 (KDML105), Suphanburi 1 (SB1), Riceberry (RB), and RD49 were provided by Suphan Buri Rice Research Centre, Rice Department, Ministry of Agriculture and Cooperatives, Thailand. The seeds were surface sterilization by immersing them in 70% ethanol for 1 min, then in a 3% sodium hypochlorite solution (NaOCl) for 15 min, and finally washed four times with sterile distilled water for 5 min each. The chitosan preparation and treatment involved using Oligomeric (O) chitosan with an 80% degree of deacetylation, referred to as O80. This chitosan was purchased from Olizac Technologies in Pathum Thani, Thailand, and was made according to the method described in Limpanavech *et al*. (2008). Briefly, 0.2 g of chitosan was dissolved in 100 mL of 200 mm acetic acid. The chitosan is effectively dissolved in this acidic solution, making it easier to use for subsequent applications. After being completely mixed with 0.5% acetic acid, the chitosan solution was diluted with deionized water to reach the desired concentrations for the treatments, which ranged from 10-40 ppm. The pH of the resulting chitosan solution was carefully adjusted to approximately 5.5, ensuring it remained mildly acidic and suitable for application. Rice seeds were first soaked with O80 chitosan at 10, 20, and 40 ppm for 48 h. Then soaked seeds were geminated in plastic trays filled with sand for 14 days before transferring to a modified WP No. 2 nutrient solution (Vajrabhaya and Vajrabhaya, 1991). Subsequently, chitosan, with the equivalent concentration, containing Triton-×100 (0.01% v/v), was applied twice to the 14- and 28-day-old seedling leaves by extensively spraying until complete saturation was reached. The control treatment was conducted by applying a spray of distilled water. For the drought stress treatment, rice seedlings that were applied with chitosan at the second time for 2 days (30-day-old seedling) were transferred to nutrient solution that contained 2% (w/v) Polyethylene Glycol 4000 (PEG4000) as drought stress (Fig. 1). All rice seedlings were cultivated in a greenhouse with photosynthetic photon flux density of 400±50 μmoL/m^2/s and a temperature shift at 32°C ±2°C/28°C ±2°C during day/night intervals.

Experimental Design and Data Collection

The experiment was designed as a Completely Randomized Design (CRD) with 4 replicates. Rice seedlings were harvested before being subjected to drought stress (called WP14) and after 7 days of drought stress (called PEG7) to analyze physiological and biochemical parameters. Plants were separated into aerial parts and roots to measure the weight of the shoot and root, both when they were fresh and when they were dried. To determine the dry weight, the shoot and root tissues were then dried using a hot air oven set at 60°C until constant weight.

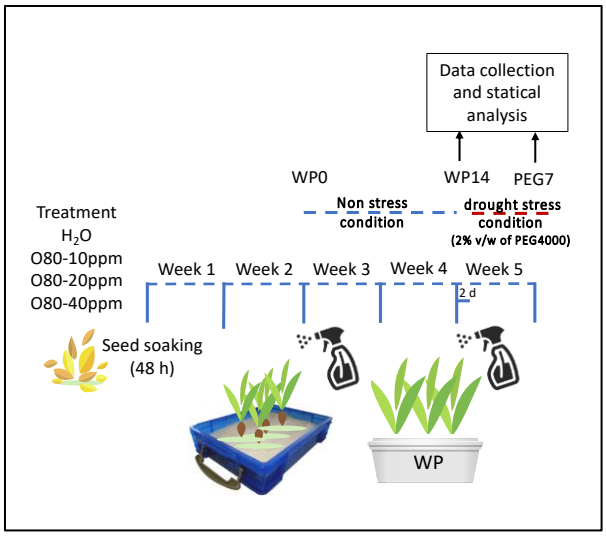

Fig. 1: The schematic diagram of the experimental procedure of chitosan treatment in this study

Determination of the Hydrogen Peroxide Content

The quantification of hydrogen peroxide (H_2O_2) was performed following the methodology described by Lekklar *et al*. (2019). The reaction mixture consisted of 1000 µL of enzyme extract and 300 µL of 0.3% $Ti_2(SO_4)_3$ (w/v) in 20% H_2SO_4 (v/v). The mixture was then subjected to 8000× g (15 min). The microplate spectrophotometer (Multiscan GO; Thermo Fisher Scientific) at a wavelength of 410 nm was carried out to quantify the intensity of the yellow color that was generated in the reaction (Jana and Choudhuri, 1982). The measured absorbance values were compared to a standard curve of H_2O_2 concentration. The standard curve was generated using 5 known concentrations of H_2O_2, following the same procedure as described before.

Determination of Soluble Sugar Contents

Soluble sugar in leaf (fructose, glucose, and sucrose) was analyzed with gas chromatography with a mass spectrophotometer (GC-MS) following methods described by Davies (1988); and Jiménez-Martín *et al*. (2012).

Calibration Standard

The standard for the quantitation of sugar is used mixture of sugar standards for the standard calibration curve. The mixture standard includes D-(-)-fructose, D-(+)-glucose, and sucrose were diluted two folds for 7 levels (156.25 ug/L -100 mg/L) with deionized water. The derivatization reaction of the mixed sugar standard proceeds as the derivatization reaction of the sugar sample in the next mention.

Sample Preparation

The sample preparation for sugar analysis using gas chromatography-mass spectrometer (GC/MS) as a measuring technique was divided into two steps including (1) extraction of sugar from rice samples by liquid/liquid extraction and derivatization reaction of extracted sugar to trimethylsilyl derivatives for GC/MS analysis. The extraction of sugar consisted of adding 1ml of extraction solution (25% acetonitrile in deionized water) to 10 mg of dried rice samples. After that the mixtures were agitated for 2 min and extracted by ultrasonic homogenizer sonicator for 20 min at room temperature. The extracted samples were stored at room temperature for 30 min and subsequently received centrifugation at 10,000 rpm for 10 min. Then, 100 µL of supernatant of each extracted sample was added into a GC vial screw cap containing 25 µL of internal standard (meso-erythritol, 20 mg/L in final concentration). The mixtures were dried by concentrator at 60°C for 1 h. The derivatization reaction of extracted sugar samples consisted of the addition of 25 µL of bis[trimethylsilyl] trifluoroacetamide (BSTFA) containing 1% Trimethylchlorosilane (TMCS) and 125 µL of Dimethylformamide (DMF) to the dried sugar sample to form trimethylsilyl derivatives. After that the mixtures were homogenized for 30 sec by vortex and allowed to stand at room temperature for 30 min before injecting into the GC/MS instrument. The GC/MS analysis used four microliters for the determination of trimethylsilyl sugar derivatives in rice samples.

GC/MS Conditions

The GC/MS analysis of sugar derivatives was achieved by using the mass spectrometer as the detector with DB-5MS+DG (30 meters, 0.25 mm ID, and 0.25 µm df) connected to the HP-5MS (15 meters, 0.25 mm ID, and 0.25 um df) capillary column from Agilent and with (EI) ionization of scan mode. The oven temperatures were programmed as follows; initiation at 140°C held for 1 min, then gradationally ramped to 180°C (50°C/min), 235°C (7°C/min) held for 2 min and 325°C (70°C/min) held for 4.5571 min for a total run time of 17.5 min. The temperature at the injector and detector were set as 250-240°C, respectively. Helium was employed as a carrier gas with a 22.2 mL/min flow rate.

Free Amino Acids Analysis

One hundred milligrams of dried leaf were grounded and extracted by 4 mL of 25% acetonitrile in 0.1 mol/L HCl. Subsequently, the mixture experienced sonication for 20 min and was then subjected to centrifugation at a force of 9,000× g for 20 min. Analyzed in this study was a volume of 100 µl of supernatant using the EZ: Faast (easy-fast amino acid sample testing kit) method as described by Jiménez-Martín *et al*. (2012), with some modifications according to Pongprayoon *et al*. (2022). The GC-MS analysis involved injecting a 2 µL aliquot of derivatized amino acids into an HP-5MS column using pulsed split mode with a 1:5 split ratio at 280°C. Helium served as the carrier gas at a constant flow rate of 1.4 mL/min. The oven temperature was programmed to increase from 130°C to 190°C at 6°C/min, followed by a rise to 230°C at 30°C/min for 5 min and finally to 325°C for 6 min. The mass spectrometer was set with the transfer line at 325°C, the ion source at 240°C, and the quadrupole at 180°C and it operated in the selected ion monitoring mode.

Statistical Analysis

The data were analyzed using SPSS statistical software (version 28). The treatment means conducted ANOVA and significant differences from the control data were determined using Duncan's New Multiple Range Test (DMRT) at $p<0.05$. Error bars indicate the standard error, while significant differences are represented by superscripted letters above each column in the figures. For Principal Component Analysis (PCA) of soluble sugar in the leaf, the average of each treatment from all rice cultivars was analyzed and visualized using SRplot (Tang *et al*., 2023)

Results

Growth Induced by Chitosan During Before and Drought Stress Conditions

We investigated the effect of increasing concentrations of chitosan treatment (10, 20, and 40 ppm) on the shoot and root fresh and dry weights of rice seedlings grown in nutrient solution before being subjected to drought (WP no. 2 nutrient solution: WP14) and simulated drought stress (WP. no.2 added with 2% PEG4000: PEG7). As represented in Fig. (2), under WP14 condition, the chitosan treatment at 40 ppm positively affected the Shoot Fresh Weight (SFW) and Shoot Dry Weight (SDW) for the KDML105 by approximately two folds compared to the control treatment (Fig. 2A-B). However, for the other three cultivars (SB1, RB, and RD49), chitosan treatment did not affect the measured parameters under WP14 conditions. The SFW and SDW of these cultivars were not significantly different under chitosan treatments compared to the control (water treatment). Under PEG7 conditions, similar findings were observed for the KDML105 and SB1 cultivars. For the KDML105, chitosan treatment at 10 ppm significantly enhanced the SFW and SDW by approximately 1.6 times and 1.8 times, respectively compared to the control treatment. For SB1, chitosan treatment did not affect the measured parameters similar to the WP14 conditions. However, notable impacts of chitosan treatment were detected in RB and RD49 plants exposed to PEG7 conditions. In comparison to the control plants, In RB, the SFW and SDW exhibited a reduction of around 29% and 28% respectively, as compared to the plants that were not treated with chitosan. In RD49, the SFW and SDW exhibited a reduction of around 42% and 34%, respectively, as compared to the plants that were not treated with chitosan (Fig. 2C-D).

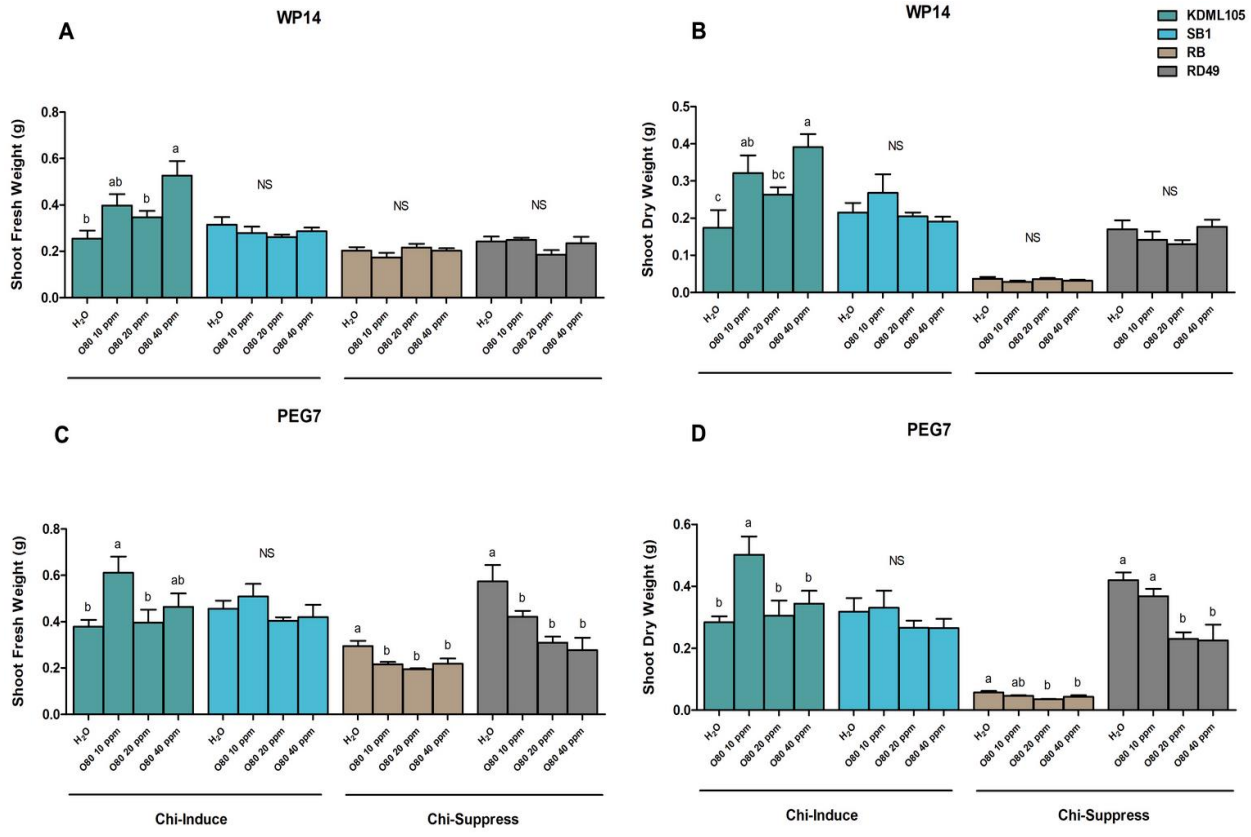

Fig. 2: Effect of chitosan treatment on the shoot fresh and dry weights from four different cultivars grown before being subjected to stress (A and B) and under stress conditions (C and D). Bars represent the mean ± standard error (SE) of four independent replicates. Bars labeled with different letters above them show statistically significant differences as analyzed by Duncan's multiple range test ($p<0.05$), whereas 'NS' indicates non-significant differences. WP14: Two-week-old rice seedlings pretreated with chitosan and grown with nutrient solution for 14 days. PEG7; Thirty-day-old rice seedlings pretreated with chitosan were transferred to a nutrient solution combination with 2% PEG4000 for 7 days

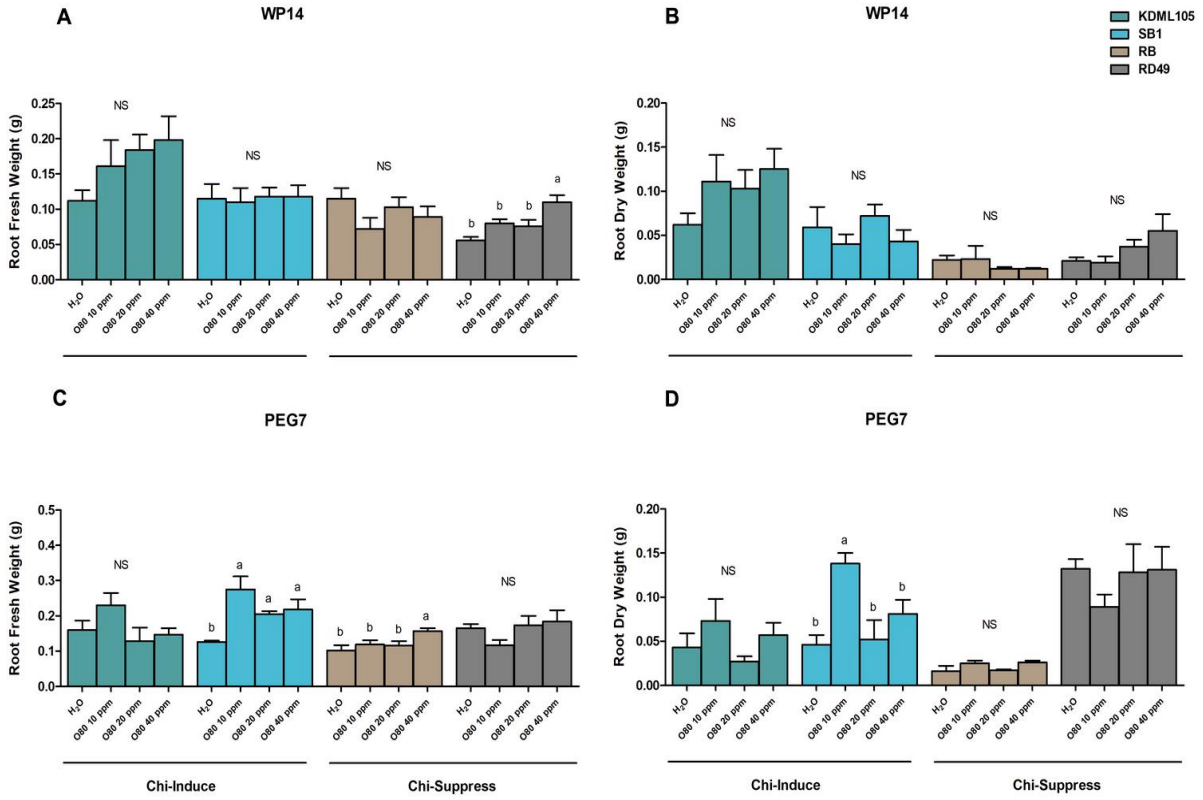

Fig. 3: Effect of chitosan treatment on the root fresh and dry weights from four different cultivars grown before stress (A-B) and under stress conditions (C-D). Bars represent the mean ± standard error (SE) of four independent replicates. Bars labeled with different letters above them show statistically significant differences as analyzed by Duncan's multiple range test ($p<0.05$), whereas 'NS' indicates non-significant differences. WP14: Two-week-old rice seedlings pretreated with chitosan and grown with nutrient solution for 14 days. PEG7; Thirty-day-old rice seedlings pretreated with chitosan were transferred to a nutrient solution combination with 2% PEG4000 for 7 days

No significant variations were seen in the Root Fresh Weight (RFW) and Root Dry Weight (RDW) between the chitosan treatment and the control for KDML105, SB1, and RB cultivars under WP14 conditions. Nevertheless, in the case of the RD49, the application of chitosan at a concentration of 40 ppm resulted in a twofold increase in RFW compared to the control treatment. However, this treatment did not have a significant effect on RDW, as shown in Fig. (3A-B). When chitosan was applied to plants under PEG7 conditions, there were no significant changes in RFW and RDW for KDML105 and RD49 compared to the control treatment. However, for SB1 and RB, chitosan treatment resulted in a significant increase in RFW by approximately 1.8 and 1.5 times, respectively, compared to the control treatment (Fig. 3C). In the case of RDW, the application of chitosan at a concentration of 10 ppm had a favorable impact on the measured value only for the SB1 cultivar, with a threefold increase. However, no significant effect was detected for the other three cultivars (Fig. 3D).

Hydrogen Peroxide Content of Rice Leaves Induced by Chitosan During Before and Drought Stress Conditions

We measured the H_2O_2 contents of the seedlings of four cultivars under WP14 and PEG7 conditions. Under WP14 conditions for KDML105, SB1, and RB cultivars, the H_2O_2 contents did not differ significantly in chitosan-treated samples compared to the control. However, for the RD49, chitosan treatment increased the H_2O_2 contents (Fig. 4A). During PEG7 conditions, the H_2O_2 content of chitosan-treated KDML105 and SB1 seedlings was not significantly different from the control, while in RD49, a significant increase in the H_2O_2 content (38.4%) was observed when treated with chitosan at 40 ppm (Fig. 4B).

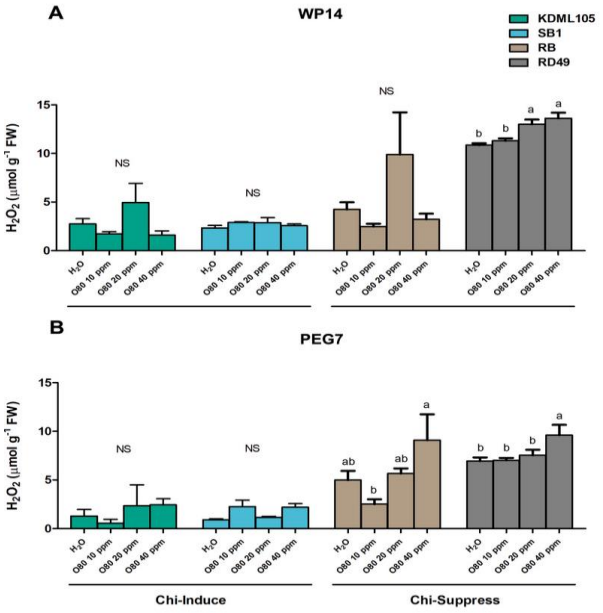

Fig. 4: Effect of chitosan treatment on H_2O_2 contents of rice leaves grown before stress (A) and under stress conditions (B). Bars represent the mean ± standard error (SE) of four independent replicates. Bars labeled with different letters above them show statistically significant differences as analyzed by Duncan's multiple range test ($p<0.05$), whereas 'NS' indicates non-significant differences. WP14: Two-week-old rice seedlings pretreated with chitosan and grown with nutrient solution for 14 days. PEG7; Thirty-day-old rice seedlings pretreated with chitosan were transferred to a nutrient solution combination with 2% PEG4000 for 7 days

Soluble Sugar Content of Rice Leaves Induced by Chitosan During Before and Drought Stress Conditions

We examine the impact of chitosan on the level of soluble sugars (fructose, glucose, and sucrose) in leaves, in particular under WP14 and PEG7 conditions, using GC-MS analysis. When KDML105 and RD49 rice plants were treated with chitosan at a concentration of 40 ppm under WP14 conditions, the fructose levels in the rice leaves reduced by around 27% and 34% respectively, compared to plants that were not treated (Fig. 5A). Notably, when exposed to a concentration of 10 ppm, KDML105 rice that had been treated showed a considerable rise in fructose levels in the leaves under the PEG7 condition. On the other hand, all RB rice treated with chitosan concentration showed a lower amount of fructose under the PEG7 condition (Fig. 5B). The glucose contents of all rice cultivars, except for SB1, were altered by chitosan under the WP14 condition. Furthermore, all levels of chitosan resulted in a reduction in glucose contents in RD49, with a decrease ranging from 8-25%. The concentration of 10 ppm exhibited the most significant decrease, reaching the lowest level compared to the control (Fig. 5C). Chitosan exhibited an impact on glucose levels in all cultivars except SB1 under PEG7 conditions. In particular, 10 ppm of chitosan increased glucose in KDML105 by 26.9%. Nevertheless, at 40 ppm, chitosan slightly decreased glucose levels (14.8%). Interestingly, the reduction of glucose contents in RB rice leaves was evaluated in all chitosan treatments (15%), particularly at 40 ppm, which had the lowest level compared to the control (Fig. 5D). Figure (5E-F) represents the sucrose levels in leaves when exposed to WP14 and PEG7 conditions, respectively. No statistically significant differences were observed between the chitosan-treated plants (KDML105 and SB1, Chi-induce group) and the plants that were not treated with chitosan. In contrast, for RB and RD49 (the chi-suppress group), chitosan treatments resulted in a significant decrease in sucrose levels, particularly at 40 ppm, with RB and RD49 showing the greatest decreases of around 12.2 times and 6.6 times, respectively, compared to the control treatment.

PCA was performed on the relative contents of fructose, glucose, and sucrose to obtain an overview of the differences in the stress responses of the four cultivars. Principal components 1 and 2 explained 52.6% and 29.3% of the overall variation, respectively. Notably, a clear separation was observed. KDML105 and SB1 were clustered together and were separated from RB and RD49 (Fig. 6) This finding is consistent with our previous observation that chitosan treatment under simulated drought stress enhanced the SFW and SDW of KDML105 (Fig. 2C-D) and RFW and RDW of SB1 (Fig. 3C-D). However, for the other two cultivars (RB and RD49), the SFW and SDW significantly declined under drought stress with chitosan treatment (Fig. 2C-D). Taken together, PCA separated KDML105 and SB1 from RB and RD49, suggesting that the fructose, glucose, and sucrose contents might play a key role in the drought stress response of different cultivars induced by chitosan treatment.

The Effect of Chitosan-Treated Rice During Drought Stress in Relation to Free Amino Acid Contents in Leaves

We profiled the shoot amino acid contents that included Alanine (ALA), α-Aminobutyric acid (ABA), Arginine (ARG), Aspartic acid (ASP), Cystathionine (CTH), Cysteine (CYS), Glutamic acid (GLU), Glutamine (GLN), Glycine (GLY), Histidine (HIS), Hydroxylysine (HLY), Isoleucine (ILE), Leucine (LEU), Lysine (LYS), Methionine (MET), Ornithine (ORN), Phenylalanine (PHE), Proline (PRO), Sarcosine (SAR), Serine (SER), Threonine (THR), Tryptophan (TRP), Tyrosine (TYR) and Valine (VAL) of the chitosan-treated rice seedlings under drought stress conditions. The average data of the effect of chitosan-treated rice during drought stress in all rice cultivars were displayed in supplementary materials are available upon request to the corresponding author. For the Chi-Suppress group (RB and RD49), the amino acid contents were not significantly different under chitosan treatment compared to the control (Fig. 7).

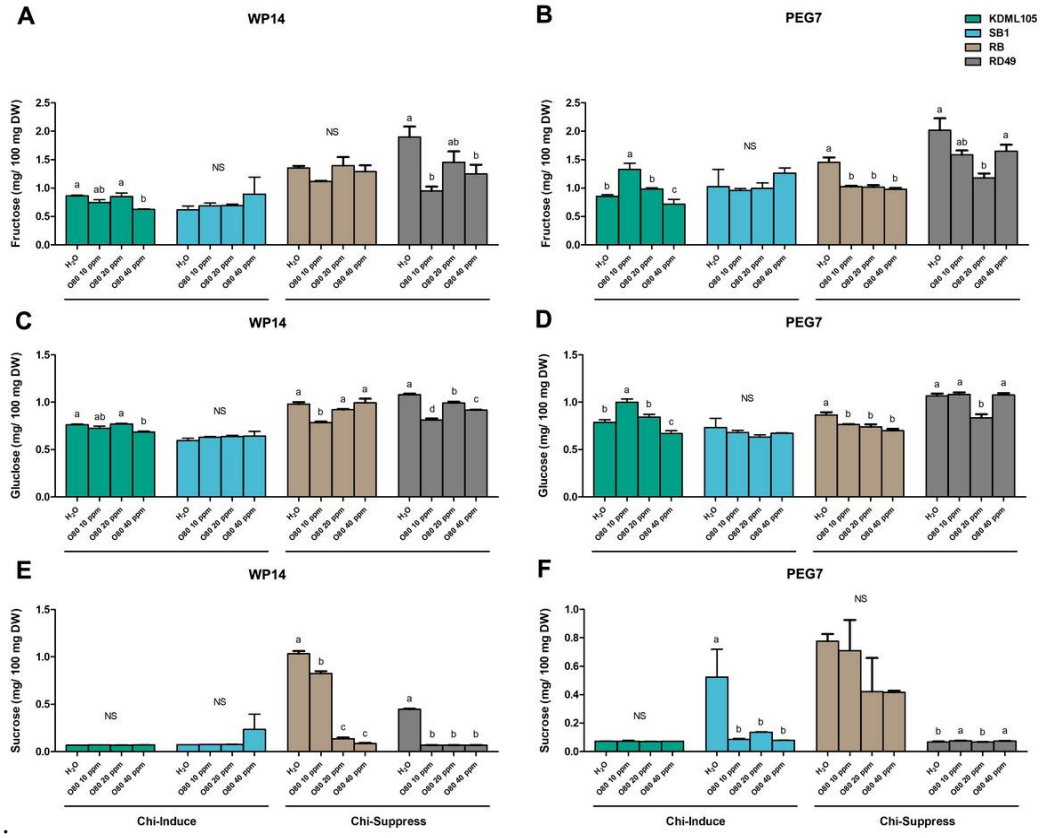

Fig. 5: Effect of chitosan treatment on Soluble sugar contents of rice leaves grown before stress (A, C and E) and under stress conditions (B, D and E). Bars represent the mean ± standard error (SE) of four independent replicates. Bars labeled with different letters above them show statistically significant differences as analyzed by Duncan's multiple range test ($p<0.05$), whereas 'NS' indicates non-significant differences. WP14: Two-week-old rice seedlings pretreated with chitosan and grown with nutrient solution for 14 days. PEG7; Thirty-day-old rice seedlings pretreated with chitosan were transferred to a nutrient solution combination with 2% PEG4000 for 7 days

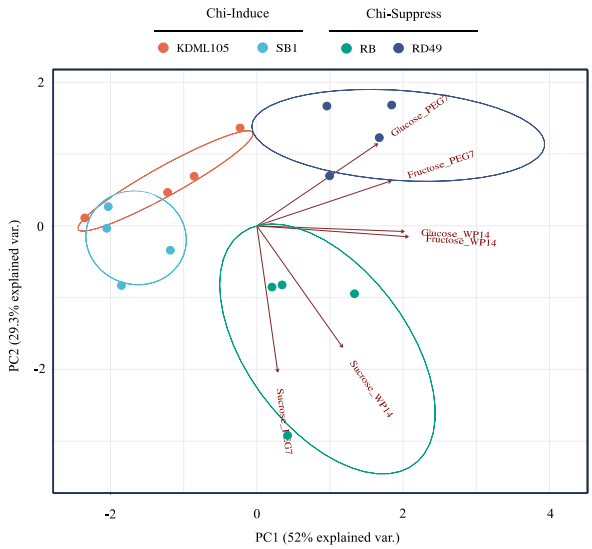

Fig. 6: Effect of chitosan treatment on the plant stress response in relation to soluble sugar. Principal component analysis (PCA) was performed. Each spot represents leaf-soluble sugar from each replicate. Arrows symbolize the original variables, with the direction of the arrows indicating the correlation between the original variable and principal components. The lengths of the arrows indicate the devotion of original data to principal components. Ellipses representing a 68% confidence interval for PCA was performed using the ggbiplot2 package in R

However, for the Chi-Induce group, KDML105, chitosan treatment at 10 ppm exhibits a high level of ILE, while 20 ppm shows a high level of THR, SER, ALA, PRO, VAL, LEU, GLY, and ABA compared to the control. For the SB1 cultivar, chitosan treatment at 10 and 40 ppm significantly increased the contents of some amino acids, such as ASP, GLN, SER, ALA, PRO, VAL, and LEU and compared to the control (Fig. 7). Taken together, these findings highlight the role of amino acids as the major differentially accumulated metabolites in chitosan-treated plants during drought stress.

DOI: 10.3844/ojbsci.2025.172.185

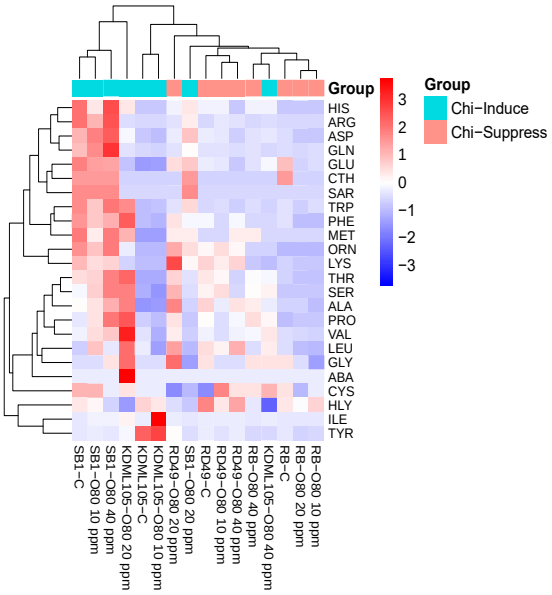

Fig. 7: Effect of chitosan treatment on the plant stress response in relation to free amino acid content. The red color was used to represent the higher value for each free amino acid, while the lower value was represented in blue. Heatmap was constructed using SRplot (Tang *et al*., 2023). The amino acids, listed alphabetically, are as follows: ALA; Alanine, ARG; Arginine, ASP; Aspartic acid, ABA; α-Aminobutyric acid, CTH; Cystathionine, CYS; Cysteine, GLN; Glutamine, GLU; Glutamic acid, GLY; Glycine, HIS; Histidine, HLY; Hydroxylysine, ILE; Isoleucine, LEU; Leucine, LYS; Lysine, MET; Methionine, ORN; Ornithine, PHE; Phenylalanine, PRO; Proline, SAR; Sarcosine, SER; Serine, THR; Threonine, TRP; Tryptophan, TYR; Tyrosine, and VAL; Valine

Discussion

Water deficit stress has emerged as a significant impediment to global rice cultivation due to climate change, particularly affecting dry and semi-arid regions with limited water resources. Plants coping with drought conditions adapt by reducing their size, leaf production, and stomatal activity. They also increase stress-related compounds, leading to stomatal closure and reduced carbon dioxide intake and water transpiration, thereby inhibiting photosynthesis. This limited photosynthetic capacity negatively impacts overall biological processes, resulting in reduced growth and productivity (Hammad and Ali, 2014; Makhlouf *et al*., 2022). To reduce the negative impacts of severe drought stress on plant yield and quality in water-scarce places, appropriate technical solutions must be developed (Takahashi *et al*., 2020). Several strategies have been explored to enhance plants' drought tolerance (Sohag *et al*., 2020; Aissa *et al*., 2018), with one solution involving the use of compounds that promote tolerance to abiotic stress. Exogenous application of natural compounds, such as biostimulants like chitosan, has garnered significant interest in improving a plant's drought resistance (Hidangmayum *et al*., 2019). The use of exogenously applied chitosan to improve drought tolerance in various crops is supported by a robust body of research evidence (Pichyangkura and Chadchawan, 2015; Roychoudhury *et al*., 2022).

In our study, we explored the effect of chitosan treatment at different concentrations (10, 20, and 40 ppm) on rice seedling drought stress response. We included four different rice cultivars of different genetic backgrounds to investigate whether the stress responses would vary among the cultivars or not. Notably, the stress responses of the four cultivars varied under chitosan treatment under simulated drought stress conditions as we observed the shoot and root weights, which are critical parameters in plant physiology, representing the water content and the actual biomass, respectively (Roberts *et al*., 1993). Fresh weight is a plant water status indicator that can be very important during droughts since it shows how well the plant can hold onto water and keep its turgor. Conversely, dry weight represents the plant's development and biomass accumulation independent of water content. Through a comparison of fresh and dry weight, we can speculate on the effects of treatments including chitosan on biomass accumulation and water retention, hence offering insights into the mechanisms of drought tolerance of the plant (Füzy *et al*., 2019; Hu and Schmidhalter, 2005). Among the four cultivars, KDML105 was the only one with enhanced shoots fresh and dry weights under chitosan treatment at 10 ppm (Fig. 2). The differential response to chitosan concentrations can be attributed to the dose-dependent nature of chitosan's effects. For KDML105, chitosan at 10 ppm significantly enhanced SFW and SDW, indicating an optimal concentration for promoting growth and drought tolerance. The lack of further enhancement at 40 ppm could be due to a saturation effect, where the beneficial impact of chitosan is maximized at 10 ppm and higher concentrations do not provide additional benefits (Hidangmayum *et al*., 2019) Alternatively, higher concentrations may lead to suppression effects, where excessive chitosan induces stress responses or inhibits growth, counteracting the benefits observed at lower concentrations (Pichyangkura and Chadchawan, 2015). For the RFW and RDW, SB1 was the only cultivar for which chitosan treatment positively affected the measured values under drought stress (Fig. 3C-D). The positive effect of chitosan treatment on root dry weight under drought stress conditions in SB1 suggests that chitosan induces root growth, making the roots larger and possibly deeper. This enhanced root growth can improve the plant's ability to access water and nutrients from deeper soil layers, which is critical for survival under drought

conditions (Hayat *et al*., 2012; Panda *et al*., 2021). The increase in root biomass indicates that chitosan stimulates the allocation of resources to root development, thus enhancing the plant's drought resilience by improving its water uptake capacity (Ghosh *et al*., 2021). Therefore, we consider these two cultivars as chitosan-induced ones. Fig. (3A) showed that under WP14 conditions, chitosan at 40 ppm enhanced the root fresh weight but not the root dry weight in RD49. This suggests increased water retention without a corresponding increase in biomass. The enhanced fresh weight indicates improved hydration status, potentially due to chitosan's role in modulating water uptake and retention (Makhlouf *et al*., 2022). However, the lack of increase in dry weight suggests that while chitosan enhances water retention, it does not necessarily promote dry matter accumulation, highlighting the complexity of its effects on plant physiology (Füzy *et al*., 2019). Under the PEG7 condition, for the RB and RD49, chitosan treatment at 20 and 40 ppm negatively affected the SFW and SDW as the measured parameters significantly declined compared to the control (Fig. 2C-D). Accordingly, we assume these two cultivars as the chitosan-suppressed ones. We then measured the H_2O_2 contents of the four cultivars. We observed no significant difference in the H_2O_2 contents of chitosan-treated KDML105 and SB1 seedlings compared to the control (Fig. 3). However, for RB and RD49, a significant increase in the H_2O_2 contents was observed when treated with chitosan (Fig. 4).

Drought stress inevitably leads to heightened reactive oxygen species (ROS) production within various cellular compartments, including chloroplasts, peroxisomes, and mitochondria. Despite this, a robust antioxidant system effectively regulates this increased ROS production, controlling the intracellular ROS levels and maintaining the cell's redox status. Moreover, under stress conditions, the elevated ROS serves as an alarm, initiating acclamatory and defense responses through specific signal transduction pathways, wherein H_2O_2 acts as a secondary messenger. As highlighted by Dat *et al*. (2000), the impact of ROS under abiotic stress appears dual, contingent upon their overall cellular quantity. At lower levels, they participate in stress signaling pathways, prompting stress defense and acclimation. However, once ROS surpass a certain threshold, they become highly destructive, provoking uncontrolled oxidative reactions that harm cellular structures, leading to oxidative stress and, ultimately, cell death (Camejo *et al*., 2020). Observing the significant rise in H_2O_2 levels in chitosan-treated RB and RD49 seedlings under drought stress, suggests oxidative stress conditions, indicating that the chitosan treatment did not impart stress tolerance. Conversely, for the chitosan-treated KDML105 and SB1, the stable H_2O_2 levels indicate induced stress tolerance (Fig. 4).

Figure (5) illustrates that chitosan treatment had a significant impact on the soluble sugar content in the leaves of different rice cultivars under drought stress conditions. PCA distinguished the KDML105 and SB1 from RB and RD49 (Fig. 6), indicating that the concentrations of fructose, glucose, and sucrose potentially influence the drought stress response among different rice cultivars under chitosan treatment (Dien *et al*., 2019; Wang *et al*., 2019). In particular, when KDML105 was exposed to 10 ppm of chitosan in the presence of PEG7, there was a significant rise in the levels of both fructose and glucose compared to the control (Fig. 5B-D). The increase in soluble sugar content is likely responsible for the observed improvement in SFW and SDW in KDML105 (Fig. 2C-D). This indicates a correlation between greater sugar levels and enhanced growth performance under drought stress. In contrast, both RB and RD49 exhibited a decrease in fructose and glucose levels when exposed to various doses of chitosan in the presence of PEG7. This reduction in sugar levels correlated with a decrease in SFW and SDW, suggesting that chitosan treatment had a detrimental effect on RB and RD49 under drought conditions. The distinct variations in sugar accumulation patterns among the different cultivars highlight the particular responses of each cultivar to chitosan. The regulation of sugar metabolism seems to be a key factor in determining the drought tolerance and growth results in rice seedlings (Dien *et al*., 2019; Vajrabhaya *et al*., 2001). However, for the SB1 cultivar, chitosan treatment led to a decrease in leaf sucrose levels across all concentrations under PEG7 conditions (Fig. 5D). This reduction in leaf sucrose may indicate enhanced translocation of sucrose to the roots, aiding in osmotic adjustment and root growth maintenance under drought stress (Fig. 3C-D) (Lemoine *et al*., 2013).

Examination of shoot amino acid profiles in chitosan-treated rice seedlings under drought stress conditions revealed a similar response in both KDML105 and SB1 cultivars. A notable increase in the levels of various free amino acids, specifically SER, ALA, PRO, VAL, and LEU was evident in both cultivars (Fig. 7). These findings indicate that amino acids play a crucial role in the metabolomic response to stress (Shim *et al*., 2023). Proline exhibits the greatest increase in accumulation in SB1 treated with 10 and 40 ppm, as compared to the control treatment. The control treatment involves the concentration of chitosan promoting RFW and RDW (Fig. 3C-D). Proline has a crucial role in improving plant resistance to stress. In addition to its osmolyte function, proline has three main roles during stress: As a metal chelator, an antioxidant defense molecule, and a signaling molecule. According to Wang *et al*. (2022), proline production in watermelon is primarily triggered by dry conditions and occurs mostly in the leaves. Subsequently, it is transferred to the roots. Consequently, proline

accumulation in leaves of many plant species, including SB1 rice, has been found to be positively correlated with resistance to abiotic stress in root growth (Hayat *et al*., 2012; Živanović *et al*., 2020).

Our findings show that chitosan has the ability to stimulate growth in specific KDML105 and SB1 rice varieties while inhibiting growth in others like RB and RD49 when exposed to drought conditions. The variety that was observed could be attributed to the distinct metabolic and physiological reactions of the cultivars to the application of chitosan. Chitosan improves drought tolerance in the chi-induce group (KDML105 and SB1) by increasing water retention and promoting the accumulation of stress-related metabolites, such as soluble sugars and some amino acids including proline in leaves. This improves osmotic adjustment and provides protection against oxidative stress (Moolphuerk *et al*., 2022). In RB and RD49, on the other hand, high levels of chitosan may cause too many reactive oxygen species (ROS), which can damage cells and stop them from growing (Román-Doval *et al*., 2023). Some cultivars show suppression, which means that there is a certain amount of chitosan that, when exceeded, weakens its beneficial effects because of damage caused by stress (Kazimi and Saxena, 2023). Our investigation was on the metabolic reactions in the leaves of different rice cultivars. While this offered useful information about how chitosan impacts soluble sugars and amino acids in leaves, it did not involve a thorough evaluation of root metabolites. In order to compare the responses of different parts of the rice plant, it is recommended that future research conduct measurements of metabolites in both the roots and leaves. This will provide a comprehensive understanding of how chitosan affects the overall physiology of the entire plant during periods of drought.

Conclusion

Our study provides important findings on how chitosan treatment affects different rice cultivars when they are exposed to drought stress. Our findings indicate that the presence of 10 ppm of chitosan has a considerable positive effect on the growth of shoots in KDML105. This reaction is associated with higher levels of soluble sugars, specifically fructose and glucose, in the leaves. Conversely, the use of chitosan in SB1 mostly promoted the development of roots. The observed effect is probably caused by the movement of sucrose from the above-ground part of the plant to the roots, which helps the plant adapt to drought conditions by adjusting its osmotic balance. Furthermore, SB1 demonstrated a greater accumulation of amino acids, including proline, which is crucial for maintaining root growth under drought. Nevertheless, the cultivars RB and RD49 did not exhibit substantial alterations in metabolite profiles when subjected to chitosan treatment. Additionally, when these cultivars were subjected to drought, the chitosan treatment inhibited their growth, suggesting that it had a negative or indifferent impact. This highlights the significance of adopting a cultivar-specific strategy when utilizing chitosan to augment drought resistance in rice.

Acknowledgment

The authors are grateful to Suphan Buri Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand) for providing rice seeds.

Funding Information

We would like to thank the Faculty of Science, Burapha University, and the Faculty of Science and Technology, Rajabhat Rajanagarindra University, for funding support and lab facilities.

Author's Contributions

Wasinee Pongprayoon: Conceptualized and acquired funding for the study.

Sataporn Deeying: Coordinated the rice seed propagation for the experiments.

Sakchai Hongthong: Analyzed the plant biochemical aspects of rice as part of the study.

Gholamreza Khaksar: Supervised the analysis of the results, reviewed and edited the manuscript.

Nichaphat Kanoksinwuttipong: Investigated the part of rice growth in this study.

Chakkree Lekklar: Designed the research plan, analyzed the results, and created the original draft, as well as edited the manuscript.

Ethics

This study is entirely original and has not been submitted or published anywhere. The datasets generated and/or examined in this investigation are accessible from the corresponding author upon a reasonable request.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ahmad, M., Zaffer, G., Razvi, S. M., Dar, Z. A., Mir, S. D., Bukhari, S. A., & Habib, M. (2014). Resilience of Cereal Crops to Abiotic Stress: A Review. *African Journal of Biotechnology, 13*(29).

Aissa, N., Malagoli, M., & Radhouane, L. (2018). An Approach to Alleviate the Impact of Drought Stress with Selenium Amendment. *Iranian Journal of Science and Technology, Transactions A: Science*, *42*(1), 283–288. https://doi.org/10.1007/s40995-018-0511-2

Bittelli, M., Flury, M., Campbell, G. S., & Nichols, E. J. (2001). Reduction of Transpiration Through Foliar Application of Chitosan. *Agricultural and Forest Meteorology*, *107*(3), 167–175. https://doi.org/10.1016/s0168-1923(00)00242-2

Boonlertnirun, S., Sarobol, E., Meechoui, S., & Sooksathan, I. (2007). Drought recovery and grain yield potential of rice after chitosan application. *Agriculture and Natural Resources*, *41*(1), 1–6.

Camejo, D., Frutos, A., Mestre, T. C., del Carmen Piñero, M., Rivero, R. M., & Martínez, V. (2020). Artificial Light Impacts the Physical and Nutritional Quality of Lettuce Plants. *Horticulture, Environment and Biotechnology*, *61*(1), 69–82. https://doi.org/10.1007/s13580-019-00191-z

Chamnanmanoontham, N., Pongprayoon, W., Pichayangkura, R., Roytrakul, S., & Chadchawan, S. (2015). Chitosan Enhances Rice Seedling Growth Via Gene Expression Network Between Nucleus and Chloroplast. *Plant Growth Regulation*, *75*(1), 101–114. https://doi.org/10.1007/s10725-014-9935-7

Dat, J., Vandenabeele, S., Vranov, E., Van Montagu, M., Inz*, D., & Van Breusegem, F. (2000). Dual Action of the Active Oxygen Species During Plant Stress Responses. *Cellular and Molecular Life Sciences (CMLS)*, *57*(5), 779–795. https://doi.org/10.1007/s000180050041

Davies, H. V. (1988). Rapid Determination of Glucose, Fructose and Sucrose in Potato Tubers by Capillary Gas Chromatography. *Potato Research*, *31*(4), 569–572. https://doi.org/10.1007/bf02361846

Dien, D. C., Mochizuki, T., & Yamakawa, T. (2019). Effect of Various Drought Stresses and Subsequent Recovery on Proline, Total Soluble Sugar and Starch Metabolisms in Rice (*Oryza sativa* L.) Varieties. *Plant Production Science*, *22*(4), 530–545. https://doi.org/10.1080/1343943x.2019.1647787

Dolatkhah Dashtmian, A., Hosseini Mazinani, S. M., & Pazoki, A. (2023). Exogenous Chitosan Nanoparticles Modulated Drought Stress Through Changing Yield, Biochemical Attributes and Fatty Acid Profile of Common Bean (*Phaseolus vulgaris* L.) Cultivars. *Gesunde Pflanzen*, *75*(6), 2463–2476. https://doi.org/10.1007/s10343-023-00912-6

Emami Bistgani, Z., Siadat, S. A., Bakhshandeh, A., Ghasemi Pirbalouti, A., & Hashemi, M. (2017). Morpho-Physiological and Phytochemical Traits of (*Thymus daenensis* Celak.) in Response to Deficit Irrigation and Chitosan Application. *Acta Physiologiae Plantarum*, *39*(10), 1–13. https://doi.org/10.1007/s11738-017-2526-2

Farouk, S., & Amany, A. (2012). Improving Growth and Yield of Cowpea by Foliar Application of Chitosan Under Water Stress. *Egyptian Journal of Biology*, *14*(1), 14–16. https://doi.org/10.4314/ejb.v14i1.2

Füzy, A., Kovács, R., Cseresnyés, I., Parádi, I., Szili-Kovács, T., Kelemen, B., Rajkai, K., & Takács, T. (2019). Selection of Plant Physiological Parameters to Detect Stress Effects in Pot Experiments Using Principal Component Analysis. *Acta Physiologiae Plantarum*, *41*(5), 1–10. https://doi.org/10.1007/s11738-019-2842-9

Ghosh, U. K., Islam, Md. N., Siddiqui, Md. N., & Khan, Md. A. R. (2021). Understanding the Roles of Osmolytes for Acclimatizing Plants to Changing Environment: A Review of Potential Mechanism. *Plant Signaling & Behavior*, *16*(8), 1913306. https://doi.org/10.1080/15592324.2021.1913306

Giraldo, J. D., Garrido-Miranda, K. A., & Schoebitz, M. (2023). Chitin and its Derivatives: Functional Biopolymers for Developing Bioproducts for Sustainable Agriculture—A Reality? *Carbohydrate Polymers*, *299*, 120196. https://doi.org/10.1016/j.carbpol.2022.120196

Górnik K., Grzesik M., & Romanowska-Duda B. (2008). The Effect of Chitosan on Rooting of Grapevine Cuttings and on Subsequent Plant Growth Under Drought and Temperature Stress. *Journal of Fruit and Ornamental Plant Research 16*, 333–343.

Guru, A., Dwivedi, P., Kaur, P., & Pandey, D. K. (2022). Exploring the Role of Elicitors in Enhancing Medicinal Values of Plants Under in Vitro Condition. *South African Journal of Botany*, *149*, 1029–1043. https://doi.org/10.1016/j.sajb.2021.10.014

Hammad, S. A. R., & Ali, O. A. M. (2014). Physiological and Biochemical Studies on Drought Tolerance of Wheat Plants by Application of Amino Acids and Yeast Extract. *Annals of Agricultural Sciences*, *59*(1), 133–145. https://doi.org/10.1016/j.aoas.2014.06.018

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of Proline Under Changing Environments. *Plant Signaling & Behavior*, *7*(11), 1456–1466. https://doi.org/10.4161/psb.21949

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*(2), 313–326. https://doi.org/10.1007/s12298-018-0633-1

Hu, Y., & Schmidhalter, U. (2005). Drought and Salinity: A Comparison of Their Effects on Mineral Nutrition of Plants. *Journal of Plant Nutrition and Soil Science*, *168*(4), 541–549. https://doi.org/10.1002/jpln.200420516

Jana, S., & Choudhuri, M. A. (1982). Glycolate Metabolism of Three Submersed Aquatic Angiosperms During Ageing. *Aquatic Botany*, *12*, 345–354. https://doi.org/10.1016/0304-3770(82)90026-2

Jiménez-Martín, E., Ruiz, J., Pérez-Palacios, T., Silva, A., & Antequera, T. (2012). Gas Chromatography–Mass Spectrometry Method for the Determination of Free Amino Acids as Their Dimethyl-*tert*-butylsilyl (TBDMS) Derivatives in Animal Source Food. *Journal of Agricultural and Food Chemistry*, *60*(10), 2456–2463. https://doi.org/10.1021/jf2052338

Kazimi, R., & Saxena, D. (2023). Significance of Chitosan Foliar Spraying on the Growth and yield of Vegetable Crop Under Protected Cultivation: A Review. *Plant Science Today*, *10*(3), 140–148. https://doi.org/10.14719/pst.2204

Khaleduzzaman, M., Hossain, M., Bhuiyan, M., Mahmud, S., Arif, M., & Murata, Y. (2021). Chitosan-Mediated Salt Stress Mitigation in Rice by Enhancing Antioxidant Defense System. *Fundamental and Applied Agriculture*, *6*(4), 336–348. https://doi.org/10.5455/faa.128175

Khush, G. S. (2005). What it Will Take to Feed 5.0 Billion Rice Consumers in 2030. *Plant Molecular Biology*, *59*(1), 1–6. https://doi.org/10.1007/s11103-005-2159-5

Konopianov, K., Anikina, I., Omarova, K., Arystangulov, S., Omarov, M., Tuganova, B., & Kabykenov, T. (2024). Enhancing the Effectiveness of Feed Production in Arid Areas, Northern Kazakhstan, Considering the Utilization of Soil Moisture Resources. *OnLine Journal of Biological Sciences*, *24*(4), 515–523. https://doi.org/10.3844/ojbsci.2024.515.523

Lekklar, C., Chadchawan, S., Boon-Long, P., Pfeiffer, W., & Chaidee, A. (2019). Salt Stress in Rice: Multivariate Analysis Separates Four Components of Beneficial Silicon Action. *Protoplasma*, *256*(2), 331–347. https://doi.org/10.1007/s00709-018-1293-2

Lemoine, R., Camera, S. L., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J.-L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J., & Durand, M. (2013). Source-to-Sink Transport of Sugar and Regulation by Environmental Factors. *Frontiers in Plant Science*, *4*, 272. https://doi.org/10.3389/fpls.2013.00272

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. https://doi.org/10.1021/acs.jproteome.7b00334

Limpanavech, P., Chaiyasuta, S., Vongpromek, R., Pichyangkura, R., Khunwasi, C., Chadchawan, S., Lotrakul, P., Bunjongrat, R., Chaidee, A., & Bangyeekhun, T. (2008). Chitosan Effects on Floral Production, Gene Expression and Anatomical Changes in the Dendrobium Orchid. *Scientia Horticulturae*, *116*(1), 65–72. https://doi.org/10.1016/j.scienta.2007.10.034

Makhlouf, B. S. I., Khalil, S. R. A. E., & Saudy, H. S. (2022). Efficacy of Humic Acids and Chitosan for Enhancing Yield and Sugar Quality of Sugar Beet Under Moderate and Severe Drought. *Journal of Soil Science and Plant Nutrition*, *22*(2), 1676–1691. https://doi.org/10.1007/s42729-022-00762-7

Malerba, M., & Cerana, R. (2015). Reactive Oxygen and Nitrogen Species in Defense/Stress Responses Activated by Chitosan in Sycamore Cultured Cells. *International Journal of Molecular Sciences*, *16*(2), 3019–3034. https://doi.org/10.3390/ijms16023019

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. https://doi.org/10.1080/15427528.2021.2000544

Moolphuerk, N., & Pattanagul, W. (2020). Pretreatment with Different Molecular Weight Chitosans Encourages Drought Tolerance in Rice (*Oryza sativa* L.) Seedling. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(4), 2072–2084. https://doi.org/10.15835/nbha48412018

Mukarram, M., Ali, J., Dadkhah-Aghdash, H., Kurjak, D., Kačík, F., & Ďurkovič, J. (2023). Chitosan-Induced Biotic Stress Tolerance and Crosstalk with Phytohormones, Antioxidants and Other Signalling Molecules. *Frontiers in Plant Science*, *14*, 1217822. https://doi.org/10.3389/fpls.2023.1217822

Panda, D., Mishra, S. S., & Behera, P. K. (2021). Drought Tolerance in Rice: Focus on Recent Mechanisms and Approaches. *Rice Science*, *28*(2), 119–132. https://doi.org/10.1016/j.rsci.2021.01.002

Phothi, R., & Theerakarunwong, C. D. (2017). Effect of Chitosan on Physiology, Photosynthesis and Biomass of Rice (*Oryza sativa* L.) Under Elevated Ozone. *Australian Journal of Crop Science*, *11*(05), 624–630. https://doi.org/10.21475/ajcs.17.11.05.p578

Pichyangkura, R., & Chadchawan, S. (2015). Biostimulant Activity of Chitosan in Horticulture. *Scientia Horticulturae*, *196*, 49–65. https://doi.org/10.1016/j.scienta.2015.09.031

Pongprayoon, W., Maksup, S., Phaonakrop, N., Jaresitthikunchai, J., Uawisetwathana, U., Panya, A., & Roytrakul, S. (2022). Phosphoproteome Analysis Reveals Chitosan-Induced Resistance to Osmotic Stress in Rice (*Oryza sativa* L.) Seedlings. *Journal of Plant Interactions*, *17*(1), 894–910. https://doi.org/10.1080/17429145.2022.2114556

Roberts, M. J., Long, S. P., Tieszen, L. L., & Beadle, C. L. (1993). *Measurement of plant biomass and net primary production of herbaceous vegetation* (pp. 1–21). Springer Netherlands. https://doi.org/10.1007/978-94-011-1566-7_1

Román-Doval, R., Torres-Arellanes, S. P., Tenorio-Barajas, A. Y., Gómez-Sánchez, A., & Valencia-Lazcano, A. A. (2023). Chitosan: Properties and Its Application in Agriculture in Context of Molecular Weight. *Polymers*, *15*(13), 2867. https://doi.org/10.3390/polym15132867

Roychoudhury, A., Datta, K., & Tagore, R. (2022). *Influence of Chitosan and Chitosan Based Nanoparticles Against Abiotic Stress in Plants* (Dr. S. Kumar & S. V. Madihally, Eds.; pp. 297–320). Elsevier. https://doi.org/10.1016/b978-0-323-85391-0.00001-0

Samal, R., Roy, P. S., Sahoo, A., Kar, M. K., Patra, B. C., Marndi, B. C., & Gundimeda, J. N. R. (2018). Morphological and Molecular Dissection of Wild Rices from Eastern India Suggests Distinct Speciation Between *O. Rufipogon* and *O. Nivara* Populations. *Scientific Reports*, *8*(1), 2773. https://doi.org/10.1038/s41598-018-20693-7

Shim, J. S., Jeong, H. I., Bang, S. W., Jung, S. E., Kim, G., Kim, Y. S., Redillas, M. C. F. R., Oh, S.-J., Seo, J. S., & Kim, J.-K. (2023). Drought-Induced Branched-Chain Amino A AmonoTransferase Enhances Drought Tolerance in Rice. *Plant Physiology*, *191*(2), 1435–1447. https://doi.org/10.1093/plphys/kiac560

Singh, T., Pun, K. B., Saikia, K., Bs, S., Bhagat, K., Das, A., & La, B. (2015). Abiotic Stress Management in Rice. *In*, 219–258.

Sohag, A. A. M., Tahjib-Ul-Arif, Md., Polash, M. A. S., Belal Chowdhury, Md., Afrin, S., Burritt, D. J., Murata, Y., Hossain, M. A., & Afzal Hossain, M. (2020). Exogenous Glutathione-Mediated Drought Stress Tolerance in Rice (*Oryza sativa* L.) is Associated with Lower Oxidative Damage and Favorable Ionic Homeostasis. *Iranian Journal of Science and Technology, Transactions A: Science*, *44*(4), 955–971. https://doi.org/10.1007/s40995-020-00917-0

Takahashi, F., Kuromori, T., Urano, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2020). Drought Stress Responses and Resistance in Plants: From Cellular Responses to Long-Distance Intercellular Communication. *Frontiers in Plant Science*, *11*, 556972. https://doi.org/10.3389/fpls.2020.556972

Tang, D., Chen, M., Huang, X., Zhang, G., Zeng, L., Zhang, G., Wu, S., & Wang, Y. (2023). SRplot: A Free Online Platform for Data Visualization and Graphing. *PLOS ONE*, *18*(11), e0294236. https://doi.org/10.1371/journal.pone.0294236

Turral, H., Burke, J., & Faurès, J. M. (2011). *Climate Change, Water and Food Security* (Vol. 31). Roma [ITA] : FAO Food and Agriculture Organization of the United Nations. ISBN-10: 978-92-5-106795-6.

Vajrabhaya, M., Kumpun, W., & Chadchawan, S. (2001). The Solute Accumulation: The Mechanism for Drought Tolerance in RD23 Rice (*Oryza sativa* L) Lines. *ScienceAsia*, *27*(2), 93–97. https://doi.org/10.2306/scienceasia1513-1874.2001.27.093

Vajrabhaya, M., & Vajrabhaya, T. (1991). *Somaclonal Variation for Salt Tolerance in Rice* (B. Y. P. S., Ed.; Vol. 14, pp. 368–382). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-83986-3_24

Wang, X., Liu, H., Yu, F., Hu, B., Jia, Y., Sha, H., & Zhao, H. (2019). Differential Activity of the Antioxidant Defence System and Alterations in the Accumulation of Osmolyte and Reactive Oxygen Species Under Drought Stress and Recovery in Rice (*Oryza sativa* L.) Tillering. *Scientific Reports*, *9*(1), 8543. https://doi.org/10.1038/s41598-019-44958-x

Wang, Z., Yang, Y., Yadav, V., Zhao, W., He, Y., Zhang, X., & Wei, C. (2022). Drought-Induced Proline is Mainly Synthesized in Leaves and Transported to Roots in Watermelon Under Water Deficit. *Horticultural Plant Journal*, *8*(5), 615–626. https://doi.org/10.1016/j.hpj.2022.06.009

Wassmann, R., Jagadish, S. V. K., Heuer, S., Ismail, A., Redona, E., Serraj, R., Singh, R. K., Howell, G., Pathak, H., & Sumfleth, K. (2009). *Chapter 2 Climate Change Affecting Rice Production: The Physiological and Agronomic Basis for Possible Adaptation Strategies* (D. L. Sparks, Ed.; Vol. 101, pp. 59–122). Elsevier. https://doi.org/10.1016/s0065-2113(08)00802-x

Živanović, B., Milić Komić, S., Tosti, T., Vidović, M., Prokić, L., & Veljović Jovanović, S. (2020). Leaf Soluble Sugars and Free Amino Acids as Important Components of Abscisic Acid—Mediated Drought Response in Tomato. *Plants*, *9*(9), 1147. https://doi.org/10.3390/plants9091147