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POTENTIAL EFFECTS OF NANOCHITOSAN ON RICE UNDER SALINITY

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Abstract

Chitosan has been widely used in agriculture to reduce harmful effects on plants during unfavorable conditions and promote plant growth. Nanotechnology is one of the most potent tools in modern agriculture for improving crop productivity. The study was conducted to (i) determine the salt tolerance of rice cultivars including ST24, ST25, and OM18, (ii) determine the effects of nanochitosan in reducing the harmful effects of salinity on rice cultivars, and (iii) analyze the influences of various nanochitosan concentrations on the photosynthetic activity and antioxidant enzymes of the three rice cultivars at the seedling stage. The results of screening the salt tolerance in a medium of NaCl (2‰, 4‰, 6‰, 8‰, 10‰) showed that salinity stress affected the development of the three rice cultivars, ST24, ST25, and OM18. The salt concentrations and rate reductions for plant height and root length were positively correlated. The results showed that all forms of chitosan nanoparticle treatment enhanced the photosynthesis of rice cultivars by increasing the chlorophyll a, chlorophyll b, and proline content. The addition of 0.5% nanochitosan in the 6‰ NaCl treatment increased the activity of antioxidant enzymes compared with the control treatment. The catalase activity reached the highest value in rice cultivar OM18, with 0.5% nanochitosan and an enzyme content of 0.049 U/min/g. The peroxidase activity was highest in rice cultivar ST24, with 0.5% nanochitosan (0.098 U/min/g). The ascorbate peroxidase activity was the highest in rice cultivar ST25, with 0.5% nanochitosan (0.084 U/min/g). Treatment with nanochitosan 0.5% enhanced the gene expression level of all tested enzymes. This suggests that the chitosan nanoparticle treatment promoted the self-protection mechanism by raising the antioxidant enzyme activity to reduce the amount of H_2O_2 and O_2^- that accumulated in the leaf cells.

Key words: Salt stress, Nanochitosan, Catalase, Peroxidase, Ascorbate peroxidase.

Introduction

Rice is an essential crop in different regions around the world, such as Asia, Europe, and Africa. However, rice cultivation is vulnerable to various factors. Salinity is believed to be one of the leading causes of stress to the rice plant, disrupting its metabolic processes and thereby reducing its growth rate and productivity. Zayed *et al*., (2017) demonstrated that the rate of seed germination and shoot length dramatically decreased with increasing salt stress levels. Xu *et al*., (2018) also revealed that salt-alkali stresses inhibit plant growth by limiting the plant's potential water uptake. Moreover, under high salt levels, plants may suffer from osmotic stress and toxic ions, ultimately leading to the disruption of normal physiological processes.

The climate has been rapidly changing in recent years, and salinity intrusion has worsened in many countries. In Bangladesh, for example, large agricultural lands in coastal areas are seriously affected by saltwater intrusion. In addition, a higher salinity level was recorded in 2020 compared to 2010. High salinity in agricultural soil has caused a major abatement in rice production in these regions (Islam, 2021). According to one study conducted in the Mekong Delta of Vietnam, saline intrusion is a significant problem that Vietnamese farmers face, especially during the dry season due to less or even no rainfall. Human activities and the rise of sea level are also speeding up the process of saline intrusion, threatening rice production in this region (Tri, 2018). Many methods have been studied and developed to help plants deal with salinity stress such as genetic modification, breeding, and the use of biostimulants. Among common biostimulants, chitosan is one of the promising candidates for promoting plant growth under abiotic stress fettles.

Chitosan is created by the deacetylation of chitin, a natural component commonly found in fungal cell walls and crustacean shells. Because of its useful biological properties, chitosan is extensively used for fruit and vegetable coatings to prevent microbial development or as organic fertilizer or biostimulant to promote plant growth. In recent years, nanochitosan has attracted significant interest from scientists thanks to its promising application in many fields, especially agriculture. Under salt stress, nanochitosan has been demonstrated to stimulate the seed starting and primary length in bean plants. Further, it helps to significantly increase the leaf area, plant height, and fresh and dry weights of both the roots and shoots of beans (Zayed *et al*., 2017). In another research on the influences of nanochitosan on *Zea* growth and soil health, the parameters related to plant growth, including seed germination, plant height and leaf area, and soil health indicator enzymes, were significantly enhanced. Further, the treatment of nanochitosan stimulates the maize plant's coping mechanism against stress conditions (Khati *et al*., 2017). Although many investigations have been conducted to research the effects of nanochitosan on plants, little evidence has been found for rice.

Material and Methods

The experiment was carried out from March 202 to December 2021 at the net house and Molecular Biology Laboratory of Biotechnology Research and Development Institute, Can Tho University. Rice cultivars ST24, ST25, OM18, and Jasmine85 and chitosan nanoparticles were provided by the laboratory.

Screening for salt tolerance in rice: This experiment aimed to test the salt tolerance at the seedling phase of rice. Rice seeds of cultivars ST24, ST25, OM18, and Jasmine85 were incubated for seeding for several days. Rice seedlings were transplanted into pots filled with sand and Yoshida solution. After seven days, rice seedlings were treated with six concentrations of NaCl (0‰, 2‰, 4‰, 6‰, 8‰, 10‰). The nutrient and salt solution (rate 200:1) was changed every day.

The height, rate of survival, and salt tolerance level of rice were recorded when rice seedlings of Jamine85 died completely and 21 days after adding salt. The level of salt tolerance was analyzed through the standard evaluation score (SES) as described by Gregorio (1997) (Table 1).

Table 1. Standard Evaluation Score (Gregorio, 1997).

External description	Tolerance level	Evaluation score
Plants developed in a normal leaf had no symptoms	Highly tolerant	1
Plants almost grew normally; few leaves or leaf tips were rolled and whitish	Tolerant	3
Plants were severely retarded; almost all leaves were rolled; only a few leaves were elongating	Slightly tolerant	5
Plants ceased growing completely; almost all leaves were dry; some plants were dying	Touchy	7
Nearly all plants were dying or dead	Highly touchy	9

Chitosan nanoparticles combined with salt treatment: The concentration of NaCl, which revealed 50% survival of the rice seedlings, was chosen to be combined with five concentrations of chitosan nanoparticles (0, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6%).

After seven days with Yoshida solution, rice seedlings were treated with NaCl solution. Then, the chitosan nanoparticles were sprayed on the leaves three times (every three days). The height, rate of survival, and salt tolerance level of rice were recorded on day 14 from the salt and nanoparticle treatment.

Effects of chitosan nanoparticles on biochemical and physiological traits of rice: The rice seedling that gave the highest criteria results in the nanoparticle concentration treatment was chosen to test the plant tolerance to salinity stress and biochemical and physiological traits.

Chlorophyll and carotenoid content: The value of chlorophyll content was estimated by Quan *et al*.'s (2016) method. In 2 mL of acetone solution (85%), a mass of 0.1 g of rice leaves was ground and centrifuged at 5000 rpm for 5 minutes. After diluting the above solution with acetone, the absorbance of the sample was measured at 644 and 663 nm, respectively, to quantify the contents of chlorophyll a and chlorophyll b. The carotenoid content was determined by a 470 nm wavelength.

Proline content: The accumulation of proline was estimated based on the standard procedure (Bates *et al*., 1973). The concentration of proline was determined spectrophotometrically at a wavelength of 520 nm. The proline content was processed by the standard curve to µmoles proline/gram of fresh weight material.

[(µg proline/mL) x mL toluene)/115.5 µg/µmole] / [(g sample)/5]

Effects of nanochitosan on antioxidant enzymes activity: Rice leaves (100mg) were homogenized with1.8 mL of PB (0.1 M, pH 6.8) and 0.2 mL of ethylenediaminetetraacetic acid (1 mM EDTA) and then centrifuged at 15,000 rpm for 20 minutes. The obtained solution was stored at -20°C for the subsequent experiments. Enzyme activity was evaluated based on Nakano and Asada's (1981) method, which was modified by Xuan and Khang (2018). A mixture of 0.1 mL of enzyme extract, 0.6 mL of PB (0.05 M, pH 7.0), 0.1 mL of EDTA (1 mM), 0.1 mL of ascorbate (5 mM), and 0.1 mL of H_2O_2(1 mM) was immediately recorded at a wavelength of 290 nm for 3 minutes. Catalase activity was determined by calculating the decomposed H_2O_2 (Damanik *et al*., 2010). A mixture of 0.05mL enzyme extract, 1.5 mL of PB (pH 7.0), 0.5 mL of H_2O_2 (75 mM), and 0.95 mL distilled water was recorded at 240 nm for 3 minutes. Peroxidase activity was determined by the guaiacol method of Herzog & Fahimi, (1973), modified by Xuan and Khang (2018). The absorbance of a mixture of 0.04 mL of enzyme extract, 2.66 mL of phosphate buffer (pH 7.0), 0.15 mL of 4% guaiacol, and 0.15 mL of 1% H_2O_2 was measured at a wavelength of 465 nm for 3 minutes.

Gene expression of antioxidant enzymes: RNA of rice leaves was extracted using the TopPURE® plant RNA extraction kit. One nanogram of purified RNA was used for One Step PrimeScriptTM RT-PCR Kit (Takara, USA). The PCR plate was enveloped by a transparent film before running it in the StepOne real-time PCR system (Applied Biosystem, USA). The program was installed for enzyme activation (95°C, 30 seconds), followed by 40 cycles of denaturing (95°C, 5 seconds) and annealing (60°C, 60 seconds). The primers included CAT enzyme gene (Forward: GTCGATTGGTGTTGAACAGG; Reverse: AGGACGACAAGGATCAAACC), POD enzyme gene (Forward: TTAGGGAGCAGTTTCCCACT; Reverse: AGGGTGAAAGGGAACATCAG), APX enzyme gene (Forward: GACTCTTGGAGCCCATTAGG; Reverse: AGGGTGAAAGGGAACATCAG) and housekeeping gene actin (Forward: TGGTCGGAATGGGACAGAAG; Reverse: CTCAGTCAGGAGAACAGGGT). The expression level was evaluated through the ΔCT (delta threshold cycle) calculation.

Data analysis: Research data were statistically analyzed by one-way analysis of variance (ANOVA), and mean comparisons were based on Tukey's test (Minitab 16.0). A p-value of less than 0.05 was considered to be a significant difference.

Results and Discussion

Effects of salinity on the growth of rice cultivars: Overall, shoot length decreased as the salt level increased, demonstrating that salt affects cell division and expansion in rice shoots. Under 8% and 10% of salt, the average shoot length was reduced by more than 50% compared to the control group during data collection (Table 2). This illustrates that salt significantly suppressed rice growth and development. High salt concentration adversely affects rice shoot growth because excess salt leads to osmotic imbalance, which disturbs the rice plant's ability to take up water and nutrients (Gain *et al*., 2014).

Rice is quite sensitive to salt stress during the seedling phase; however, it becomes more tolerant to salinity during the vegetative growth phase. (Zayes *et al*., 2017). Pearson (1961) also noted that rice had extreme salinity tolerance during the germination stage until the 2–3 leaf stage, where it got sensitive. This level of salt tolerance increases again during the tillering and elongation stage, followed by a decrease during pollination. However, salt has almost no impact on rice during its flowering stage. When salt concentration in rice plants is high enough, it may cause the leaves to mature too early, reducing the leaf area for photosynthesis and making it unable to maintain its growth and development (Munns, 2002). According to Akita (1986), when rice is exposed to saline soil, it exhibits some common symptoms, starting with a reduction in leaf area. Under mild salt salinization, dry weight tends to increase for a while but then drops significantly. During the seedling stage, mature leaves are affected by salinity sooner than young ones are. Islam *et al*. (2007) recorded a negative correlation between rice height and salt concentration. In Akbar's (1975) study, salt damage can be seen as early as on the first leaves and lastly on mature leaves. Salinity prevents the elongation of leaves and new leaf growth. High salt levels may suppress rice growth parameters, with height reducing by 6% compared to rice under normal living conditions (Razzaq *et al*., 2019).

One typical sign of salt damage is the slower growth rate of shoots and leaves at the later stage of growth and development compared to the initial stage. Munns (2002) reported that roots play a vital role in preventing excess salt from passing through (up to 98% of salt from soil cannot get through roots). However, roots damaged by salinity become vulnerable and unhealthy and cannot prevent salt penetration well. Byrt *et al*., (2018) also had a similar observation. Therefore, prolonged salinization inhibits the growth and development of plants, induces plant senescence, and even leads to plant death (Jouyban, 2012).

Impact of salinity on the salt tolerance of rice cultivars: After 14 days of 6‰ salt treatment, no IR28 could survive; they had a salt tolerance score of 9, while Pokkali scored 3 (moderately tolerant). The figures for ST24, ST25, and OM18 were all the same at level 7 (sensitive). ST24, ST25, and OM18 cultivars were still tolerant if exposed to the same salt concentration for only two weeks, with a tolerance score of level 5 (Table 3).

Plants gain salt tolerance by multigene interaction and varying physiological mechanisms such as increased osmotic pressure in the cytosol and the secretion of excess salt through stomata or salt glands. The identification, evaluation, and classification of salt tolerance of plants are of paramount importance for researching the salt tolerance of rice. Because 50% of the studied rice survived under 6‰ of salt, this concentration was also applied for the following experiments.

Effects of nanochitosan on salt tolerance of rice: The experiment was conducted to evaluate the nanochitosan treatment on salt tolerance of 5 rice cultivars (ST24, ST25, OM18, Pokkali, IR28) under the same salt concentration (6‰ of NaCl). Results showed that the shoot length and survival rate of all rice cultivars increased with increased concentration of nanochitosan (Table 4, Fig. 1 and Fig. 2).

According to Lauchli & Shu (2007), rice is most likely to be sensitive to salt during its initial stage of development. Table 4 shows that the spraying of nanochitosan did not affect shoot height among rice cultivars, including Pokkali. The impact of stress on rice was reduced due to the presence of nanochitosan in both high and low molecular weight (Namphueng *et al*., 2021).

Effects of nanochitosan treatment on biochemical characteristics of rice

Effects of nanochitosan on proline accumulation: Proline is an important biochemical marker used to evaluate the salt tolerance of plants. In addition, it is a type of amino acid that serves as an energy source for plants, helping to stabilize enzymatic activities and protect the integrity of cells. Proline protects cell membranes against the consequences of high salt concentration (Ashraf & Shahbaz, 2012). The decreased growth and development of rice are caused by this serious abiotic factor (salinity stress). A relatively large amount of proline is accumulated in the rice cytoplasm, facilitating rice to fight against salt stress. The pretreatment of proline helps boost the tolerance level of rice, and the accumulation of proline is helpful in improving the salt tolerance of rice (Igarashi & Yoshiba, 2000).

Table 2. Rice height (cm) after 14 days of salt treatment.

Cultivar	Salt concentrations						Average
	0‰	2‰	4‰	6‰	8‰	10‰	
ST24	71.57^{ab}	58.73^{abcd}	57.67^{abcd}	28.50^{def}	18.00^{ef}	-	39.08^{bc}
ST25	71.17^{ab}	61.63^{abc}	52.13^{abcd}	46.40^{abcde}	17.57^{ef}	-	41.48^{b}
OM18	65.50^{abc}	56.53^{abcd}	50.00^{abcd}	43.73^{bcde}	-	-	35.96^{bc}
Pokkali	74.90^{a}	64.90^{abc}	58.17^{abcd}	56.90^{abcd}	56.10^{abcd}	53.03^{abcd}	60.67^{a}
IR28	54.87^{abcd}	47.53^{abcde}	43.63^{bcde}	38.10^{cde}	-	-	30.69^{c}
Average salinity	67.60^{a}	57.87^{ab}	52.32^{bc}	42.73^{c}	18.33^{d}	10.61^{d}	

Note: The table shows the means of 3 replicates. Data followed by the same letters represent no statistically significant differences (95%)

Table 3. Effects of salinity on salt tolerance.

Cultivar	NaCl concentration					
	0‰	2‰	4‰	6‰	8‰	10‰
ST24	1	1	3	7		9
ST25	1	1	3	7		9
OM18	1	1	5	7		9
Pokkali	1	1	3	3		5
IR28	1	5	7	9		9

Table 4. Effects of nanochitosan on height and survival rate of rice cultivars.

Nanochitosan concentration (%)	Cultivar	Height (cm)
0	ST24	35.27^{abcd}
	ST25	31.80^{cdefg}
	OM18	27.37^{ghi}
0.1	Pokkali	36.77^{ab}
	IR28	27.03^{ghi}
	ST24	34.90^{abcd}
0.2	ST25	33.40^{abcde}
	OM18	29.10^{efghi}
	Pokkali	36.90^{ab}
	IR28	25.27^{i}
	ST24	34.70^{abcd}
0.3	ST25	34.43^{abcd}
	OM18	29.27^{efghi}
	Pokkali	36.63^{ab}
	IR28	27.10^{ghi}
	ST24	35.87^{abc}
0.4	ST25	34.30^{abcd}
	OM18	30.90^{defgh}
	Pokkali	36.37^{abc}
	IR28	26.83^{hi}
	ST24	36.03^{abc}
0.5	ST25	36.03^{abc}
	OM18	28.00^{fghi}
	Pokkali	35.63^{abcd}
	IR28	25.80^{i}
	ST24	37.53^{a}
0.6	ST25	36.80^{ab}
	OM18	32.40^{bcdef}
	Pokkali	37.07^{ab}
	IR28	26.93^{hi}
	ST24	34.77^{abcd}
	ST25	37.19^{ab}
	OM18	33.63^{abcde}
	Pokkali	36.50^{abc}
	IR28	26.27^{hi}

Note: The table shows the means of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%)

Table 5. Effects of nanochitosan on chlorophyll a content (µg/g).

Cultivar	Nanochitosan concentration		
	0%	0.5%	0.6%
ST24	1.233d	2.957ab	2.682abcd
ST25	1.006cd	2.558abcd	3.468a
OM18	1.774bcd	2.857abc	3.337ab

Note: The table shows the means of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%)

Table 6. Effects of nanochitosan on chlorophyll b content (µg/g).

Cultivar	Nanochitosan concentration		
	0%	0.5%	0.6%
ST24	0.5260cd	1.1465abc	1.1919ab
ST25	0.5001d	1.5304a	1.7018a
OM18	0.7599bcd	1.2490ab	1.4890a

Note: The table shows the means of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%)

Table 7. Effects of nanochitosan on carotenoids content (µg/g).

Cultivar	Nanochitosan concentration		
	0%	0.5%	0.6%
ST24	0.3116c	0.8715b	1.0667ab
ST25	0.4410c	1.1295ab	1.3327a
OM18	0.3394c	0.9317b	1.3051a

Note: The table shows means of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%)

After 7 days under 6‰ NaCl conditions, all 3 rice cultivars treated with 0.6% of nanochitosan produced the highest amount of proline (Fig. 3). In contrast, cultivars in the control group had the lowest level. Shuma *et al*., (2016) demonstrated that spraying mung bean plants with nanochitosan boosts proline production. The increased proline accumulation under salt stress happens due to the activation of adaptive mechanism of plants (Aspinall & Paleg, 1981); it could also signify cell damage (Hanson & Nelson, 1978).

Nanochitosan effects on pigment contents: Table 5 and Table 6 shows an increase compared to the control in chlorophyll content in rice cultivars treated with different concentrations of nanochitosan, with the highest content (3.468 µg/g) recorded in ST25.

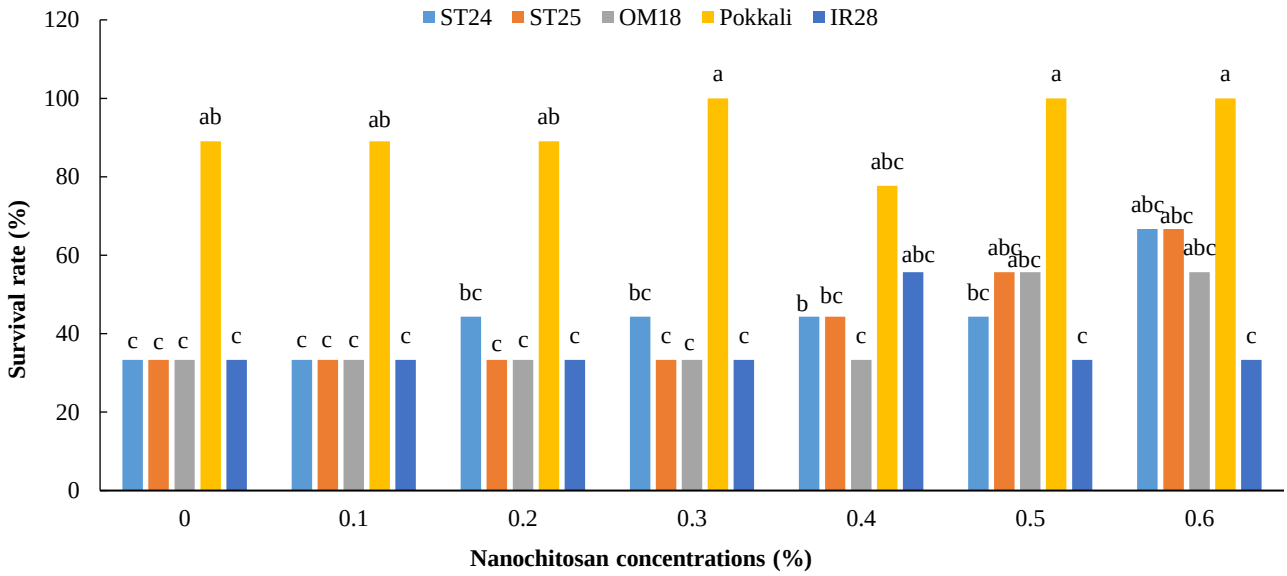

Fig. 1. Effects of nanochitosan concentration on survival rate.
Note: The figure shows the means of 3 replicates; means followed by the same letters represent no statistically significant differences (95%).

Fig. 2. OM18 cultivar after 7 days of nanochitosan spraying.

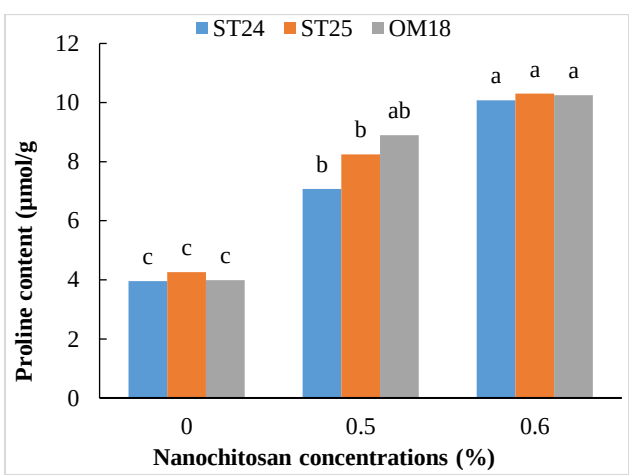

Fig. 3. Proline content in rice cultivars.
Note: The figure shows mean of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%).

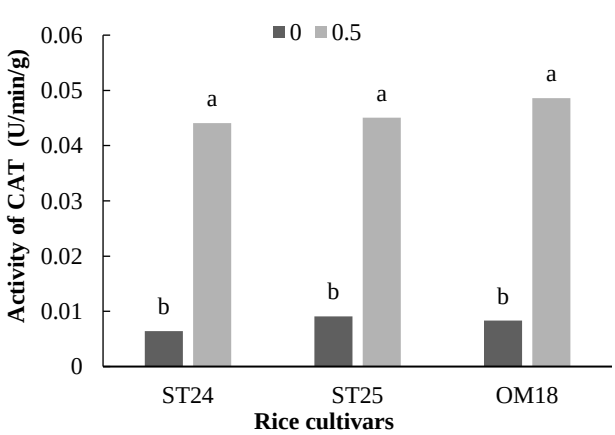

Fig. 4. Changes in catalase (CAT) enzyme activity under the influence of nanochitosan.
Note: The figure shows the means of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%).

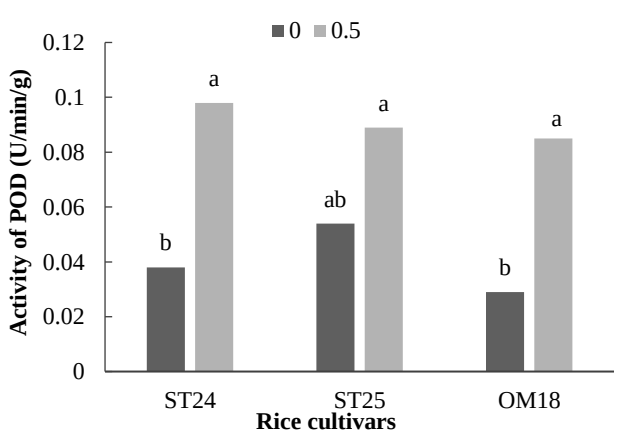

Fig. 5. Changes in peroxidase (POD) enzyme activity under the influence of nanochitosan.
Note: The figure shows mean of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%).

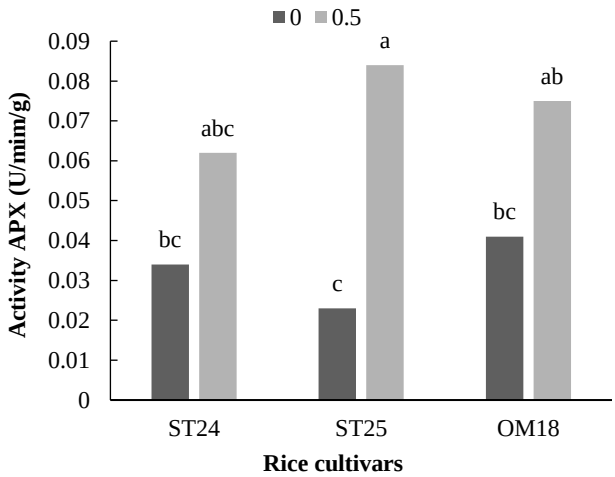

Fig. 6. Changes in ascorbate peroxidase (APX) enzyme activity under the influence of nanochitosan.
Note: The figure shows mean of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%).

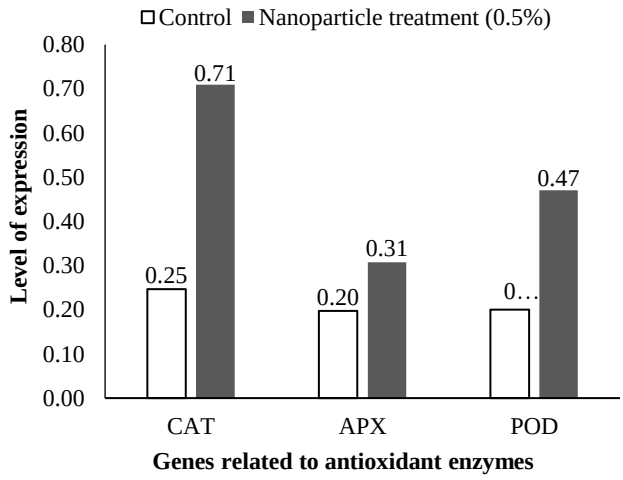

Fig. 7. Changes in gene expression of antioxidant enzyme related genes on ST25 variety.
Note: The figure shows mean of 3 replicates; data followed by the same lowercase letters represent no statistically significant differences (95%).

Nanoparticles been used globally since the early 21st century. Nanotechnology is also a powerful tool and technological basis for researching and transforming biological systems (Rufsni & Roberto, 2009). Among nanoparticles, nanochitosan has attracted great attention from scientists because of its bioactive activities. Many studies have demonstrated that nanoparticles promote photosynthesis and nitrogen metabolism, dramatically enhancing the growth of spinach plants (Hong *et al*., 2005). According to Javad *et al*. (2014), silica nanoparticles were found to markedly increase the growth of maize, significantly enhancing plant health through improving plant growth, leaf area, growth rate, and dry weight (Hasaneen *et al*., 2016). Zayed *et al*., (2017) studied the influences of nanochitosan on physiology, chemistry, and the growth of the *Phaseolus vulgaris* legume under salinity stress fettles. All treatments between NaCl solution (100 mM) and the various nanochitosan concentrations (0.1%, 0.2%, and 0.3%) had significant effects on seed germination and root length, and the highest germination rate was at the concentration of 0.3% nanochitosan. In Tantawy (2009), application of chitosan increased the growth and development of tomato plants. The investigation concluded that chitosan is a potential candidate that could be used to decrease the negative impact of salinity in the future. In addition, Rahman *et al*., (2018) explored the effects of nanochitosan on salt tolerance and the growth and development of maize. The complement of nanochitosan improved the maize growth under salinity conditions, and transpiration rates were not affected by salinity and the use of chitosan. A study by Lee *et al*., (2005) presented that the favorable effects of chitosan on the soybean seedling's growth and the stimulant activities of chitosan were proportional to the molecular weight of the compounds used in the experiment.

According to Siringam *et al*., (2009), photosynthetic pigments in leaves reduced under salinity conditions and decreased sharply with increasing salt concentration. Tables 7 indicate that, in the salinity experiment of 6‰, the content of photosynthetic pigments in the treatments with 0.5% and 0.6% nanochitosan concentrations increased compared to that of the control group. According to research by Piotr and Agnieszka (2014), nanochitosan lifted the chlorophyll concentration in green bean leaves; another study presented that spraying nanochitosan on coffee plants raised the content of chlorophyll and carotenoids (from 15.36% in the field to 6.38% in the greenhouse) (Dzung *et al*., 2011). Spraying coffee plants with nanochitosan also increased chlorophyll and carotenoids content (38.2%–72.2% higher than the control) in Sang *et al*., (2013).

Effects of nanochitosan on antioxidant enzyme activities of rice cultivars: Regarding catalase activity, the hydrolysis of H_2O_2 to H_2O and O_2, an increase in catalase activity contributes to reducing H_2O_2 in leaves. Catalase activity increased in treatments with nanochitosan (Fig. 4).

Catalase (CAT): Due to breaking down H_2O_2 to H_2O and O_2, CAT activity rose to contribute to the reduced content of H_2O_2 in leaves. Figure 4 shows that catalase activity increased in the experimental group treated with nanochitosan. CAT activity reached the highest value in rice cultivar OM18, especially in 0.5% nanochitosan, as enzyme activity reached 0.049 U/min/g. However, there was no statistically significant difference when compared with the ST24 (0.044 U/min/g) and ST25 (0.045 U/min/g) rice cultivars. This shows that the demand for catalase synthesis to detoxify rice cells was very high. Meanwhile, in the control treatment, this enzyme activity was low.

Peroxidase (POD): Figure 5 shows that nanochitosan increased peroxidase activity compared with that in the control treatment. In the treatment with 0.5% nanochitosan, the enzyme activity of three rice cultivars, ST24, ST25, and OM18, had a statistically insignificant difference. However, peroxidase activity had significant differences between the treatment of 0.5% nanochitosan and the control treatment.

Statistically, peroxidase enzyme activity reached the highest value in the ST24 rice cultivar, especially in 0.5% nanochitosan (0.098 U/min/g) without difference when compared with the ST25 rice cultivar treated with 0.5% nanochitosan (0.090 U/min/g) and with the OM18 rice cultivar (0.085 U/min/g). In the meantime, this enzyme activity was low in the control treatment. The activity of peroxidase had the highest value in the ST24 cultivar.

Ascorbate peroxidase (APX): APX is the enzyme catalyzing the detoxification of peroxide compounds in the cytoplasm. Therefore, the change in APX activity has a direct effect on the endogenous H_2O_2 transformation. The presence of nanochitosan increased ascorbate peroxidase activity compared with that in the control treatment (Fig. 6). Statistically, the ascorbate peroxidase enzyme activity was the highest in the ST25 rice cultivar, especially in 0.5% nanochitosan (0.084 U/min/g), which was not statistically significant when compared with the ST24 (0.062 U/min/g) and OM18 (0.075 U/min/g) rice cultivars. Meanwhile, in the control treatment, this enzyme had low activity.

To limit damage caused by salinity stress, plants have appropriate control and regulation mechanisms for ROS levels, including the participation of antioxidant enzymes such as peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and nonenzymatic antioxidants including ascorbate peroxidase (APX), proline, glutathione peroxidase (GPX) and peroxiredoxin (Wang, 2015). These enzymes are present in all small compartments of the cell. Frequently, an organ may have more than one enzyme involved in eliminating a single ROS (Mittler *et al*., 2004).

According to Raisa *et al*., (2021), the antioxidant activity, hydrogen peroxide, and malondialdehyde of the M-19 rice cultivar showed lower content, suggesting a potent defensive mechanism against osmotic and oxidative stress. In addition, in the study of Namphueng *et al*., (2021), the antioxidant enzyme activities consisting of ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) were enhanced upon reaction with chitosan. The study of Sujoy *et al.* (2020) showed that when mung beans were treated with nanochitosan under salinity stress conditions, the activities of various antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), increased significantly.

The antioxidative activities and content of hydrogen peroxide and malondialdehyde in the M-19 cultivar are lower, demonstrating that a strong mechanism in rice is activated to fight oxidative and osmotic stress under high salt conditions (Raisa *et al*., 2021).

Effects of nanochitosan on gene expression related to antioxidant enzymes: The antioxidant enzymes, including APX, CAT, and POD, increased after treatment with nanochitosan. To ensure the result, the gene expression of these enzymes was determined on the ST25 variety. The result similarly showed that treatment with nanochitosan 0.5% enhanced the level of gene expression of all tested enzymes (Fig. 7). The highest expression was found on the CAT enzyme-related gene with nearly three times after treatment.

Conclusions

The appropriate concentration of nanochitosan for salt purification in the 6% NaCl treatment was 0.5%. To adapt to saline conditions, three rice cultivars, ST24, ST25, and OM18, were found to have a high accumulation of chlorophyll and proline content in leaves. Compared with the two rice cultivars ST24 and OM18, the ST25 rice cultivar had the highest content of proline and chlorophyll a and b. Nanochitosan plays a substantial role in helping plants have salt tolerance. Nanochitosan rose the antioxidant enzymes activity and its gene expression seen as a defense mechanism by the body to minimize the negative impact on the environment.

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