RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 58 Special Issue: 1035-1041 (November 2024)

The Tolerance of Saline Conditions of Rice Seedlings in the Treatment of Oligochitosan

N.T.P. Thanh[1], L.T.T. Tien[1]

10.18805/IJARe.AF-814

ABSTRACT

Background: Rice is one of the most important crops and is sensitive to salinity stress. Salt stress is a major abiotic stress that causes inhibition in plant growth or even plant death. Looking for a solution to enhance the salt tolerance of rice is very necessary. Methods: Rice sprouts with 2-3 mm of radicles were treated in four treatments: distilled water, 0.6% NaCl, oligochitosan 5994 Da (75 ppm) and 0.6% NaCl supplemented with 75 ppm of oligochitosan 5994 Da. The physiological and biochemical parameters and gene expression of rice seedlings were evaluated after seven days of treatment.

Result: In the treatment of 0.6% NaCl, the development of rice seedlings was inhibited, but the salt-resistant systems were activated. The addition of oligochitosan maintained the growth of rice seedlings through the improvement of morphology, physiological parameters *and* the concentration of total sugar, proline and total protein. Oligochitosan raised the expression of genes related to proline biosynthesis (P5CS and P5CR) or genes related to antioxidant enzymes in salinity stress (cAPX, tAPX and sAPX).

Key words: Oligochitosan, Rice seedlings, Salinity stress, Salt-resistant systems.

INTRODUCTION

Rice is sensitive to salinity stress, especially at the seedling, early vegetative and reproductive stages, reducing the yield (Sajid *et al*., 2017). To reduce salt absorption, plants limit water uptake by producing abscisic acid to the close of stomata. This reduces photosynthesis and promotes other metabolic processes that produce harmful plant products, such as reactive oxygen species (ROS) (Zhao *et al.,* 2021). To minimise the harmful effects of ROS, plants induce antioxidant mechanisms to protect cells from ROS (Parvaiz *et al*., 2010). APX (ascorbate peroxidase) is the primary agent to remove H_2O_2 in many organelles in the cells (Teixeira *et al*., 2006). In rice, the APX gene family has eight genes in cytosol, peroxisomes, chloroplasts and mitochondria (Kibria *et al*., 2017). Proline often accumulates in large quantities when plants are exposed to adverse conditions (drought or salinity). Proline is biosynthesised in the cytosol and chloroplast with the catalysis of P5CS (pyrroline-5-carboxylate synthase) and P5CR (pyrroline-5-carboxylase reductase). Proline regulates cytoplasmic permeability, contributes to the stabilisation of subcellular structures under adverse conditions (Mirza and Masayuki, 2022).

Chitosan is a natural compound that is not harmful to plants and animals. Chitosan activates the defence system in plants against harmful factors, especially abiotic factors (Zhang *et al.,* 2021). Oligochitosan is smaller than chitosan and easily soluble in water. Oligochitosan improved the growth of *Salvia abrotanoides* (Kar.) under drought stress (Attaran *et al*., 2022), improved the growth, physiological and biochemical parameters of *Phaseolus vulgaris* under salinity stress (Zayed *et al*., 2017) or regulated the metabolisms of banana plants in cold stress (Anbang *et al*., 2021). To investigate oligochitosan's effects on rice's salinity tolerance, this research focused on oligochitosan's effects on the characteristics, physiology, biochemistry and gene expression level at the transcriptional level of rice seedlings.

[1]Department of Biotechnology, Hochiminh City University of Technology, VNU-HCM Hochiminh City, Vietnam.

Corresponding Author: L.T.T., Tien, Department of Biotechnology, Hochiminh City University of Technology, VNU-HCM Hochiminh City, Vietnam. Email: ltttien@hcmut.edu.vn

How to cite this article: Thanh, N.T.P. and Tien, L.T.T. (2024). The Tolerance of Saline Conditions of Rice Seedlings in the Treatment of Oligochitosan. Indian Journal of Agricultural Research. 58 (Special Issue): 1035-1041. doi: 10.18805/IJARe.AF-814.

Submitted: 17-08-2023 **Accepted:** 20-11-2023 **Online:** 29-11-2023

MATERIALS AND METHODS

Rice (*Oryza sativa* L.) seeds from variety IR64 from Dien Bien Seed Company, Vietnam. Oligochitosan 5994 Da was irradiated by gamma rays of Co-60 at 150 kGy from 5% chitosan (573170 Da) at 80% of deacetylation, which was provided by Hochiminh Biotechnology Center, Vietnam.

Seedling preparation and oligochitosan treatment

Rice sprouts with 2-3 mm of radicles were grown in glass pots on Whatman filter paper (20 sprouts per pot) in four treatments: distilled water (control), 0.6% NaCl solution (saline stress), 75 ppm of oligochitosan 5994 Da (oligochitosan) and 0.6% NaCl solution supplemented with 75 ppm of oligochitosan 5994 Da (oligochitosan in saline stress) (Lam *et al*., 2022). Each pot contained 20 mL of solution with pH 5.5. Pots were placed in a growth chamber

under a light intensity of 34 mmol $m^{-2}s^{-1}$, 12-hour photoperiod at 25±2°C.

Analysis of plant growth parameters

The seedling length, the number, the length and width of leaves and the number and length of roots were determined.

The surface of the leaves was observed and photographed under a scanning electron microscope (Hitachi S4000 FESEM) at room temperature.

The photosynthesis or respiration of leaves (mmol O_2/g FW/min) was determined by Leaflab 2+ System (Hansatech) with an oxygen electrode based on the oxygen evolution under a light intensity of 135 mmol $m^{-2}s^{-1}$ or the oxygen decrease in the darkness respectively at 27°C.

The photosynthetic pigments of leaves were extracted using ethanol and measured in a spectrophotometer at 470, 649 and 665 nm, as described by Lichtenthaler (1987).

Proline concentration in rice seedlings was determined *via* reaction with ninhydrin as described by Carillo and Gibon (2011). The products were measured spectrophotometrically at 520 nm.

The Bradford method (1976) was used to determine the total protein concentration in rice seedlings. The products were measured spectrophotometrically at 595 nm. Carbohydrates were measured spectrophotometrically at 490 nm (Dubois *et al*., 1956). APX was extracted and determined by measuring the decrease in absorbance at 290 nm in a spectrophotometer (Nakano and Asada, 1981).

The transcriptional level of P5CS and P5CR, cAPX, tAPX and sAPX were analysed by qRT-PCR. PCR thermal cycle steps in cDNA synthesis from RNA were 25°C in 5 minutes, 42°C in 5 minutes and 70°C in 15 minutes. The relative quantification method was used to calculate the gene expression level through threshold cycle index (CT) with the primers, as in Table 1 (Çelik *et al*., 2018).

Statistical analysis

The parameters were determined at 9 a.m. in 20 sprouts after seven days of treatments. All of the treatments were repeated three times with similar performance. One-way analysis of variance (ANOVA) was used to process the resulting data using Statistical Package for the Social Sciences (SPSS) version 20 software for Mac. Duncan's multiple range test demonstrated a statistically significant difference between treatments at $p \leq 0.05$.

RESULTS AND DISCUSSION

The growth of rice seedlings

Under salinity stress, the leaf width and root number were lower than control, but the other parameters were unchanged. In the treatment of oligochitosan under salinity stress, the leaf width and the number of roots were higher than in saline stress and the same as the control, while seedling length and leaf number were higher. The leaf length was unaffected in all treatments. There was no difference in seedling length, leaf number and leaf length between salt and oligochitosan treatments (Table 2). In the treatment of NaCl, the roots were elongated and dark brown, the leaves were slightly yellowish. The seedlings in the control and oligochitosan treatments had green leaves. The seedlings were healthy in the treatment of oligochitosan in saline stress, with green opening leaves and many long roots (Fig 1).

The stomata and silica bodies in leaf abaxial surfaces differed in all treatments. In saline conditions, the stomata closed and the silica bodies swelled. The supplement of oligochitosan under saline conditions reduced the swelling of the silica bodies, but the stomata were still closed. The opened stomata and the normal-sized silica bodies were observed on the surface of leaves under oligochitosan treatments and control (Fig 2).

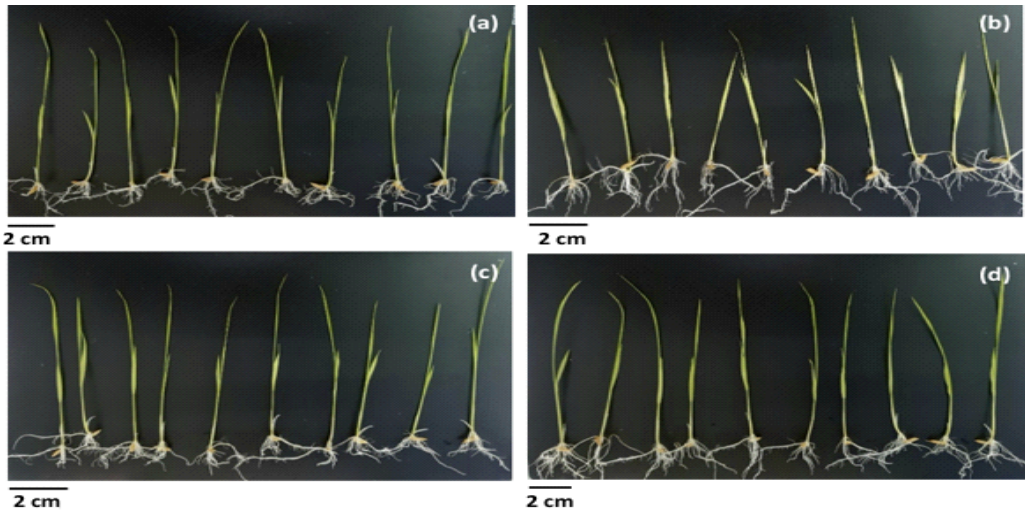
Table 1: The primer of the relative genes.

	Forward primer	Reverse primer
P5CS	CAAATGCTCCTTTTAGCCTGTT	GCGTTGGTACACAAGTTCTCAG
P5CR	AATAGAGGCCATGGCTGATG	AATGCACCCTTCTCAAGCTC
cAPX	GACAAGAAACCCTCTGCAGTTT	GTAGTCTGCTGGTTCACACTGG
tAPX	ATTTTCACTGGACGATGAACCA	GGAAGTAGTTGGACTGCAGAGG
sAPX	GTCTGGAGCACATACACTTGGA	TTAACCGTCCAACGTGAATCCC

Table 2: The growth parameters of rice seedlings under different conditions.

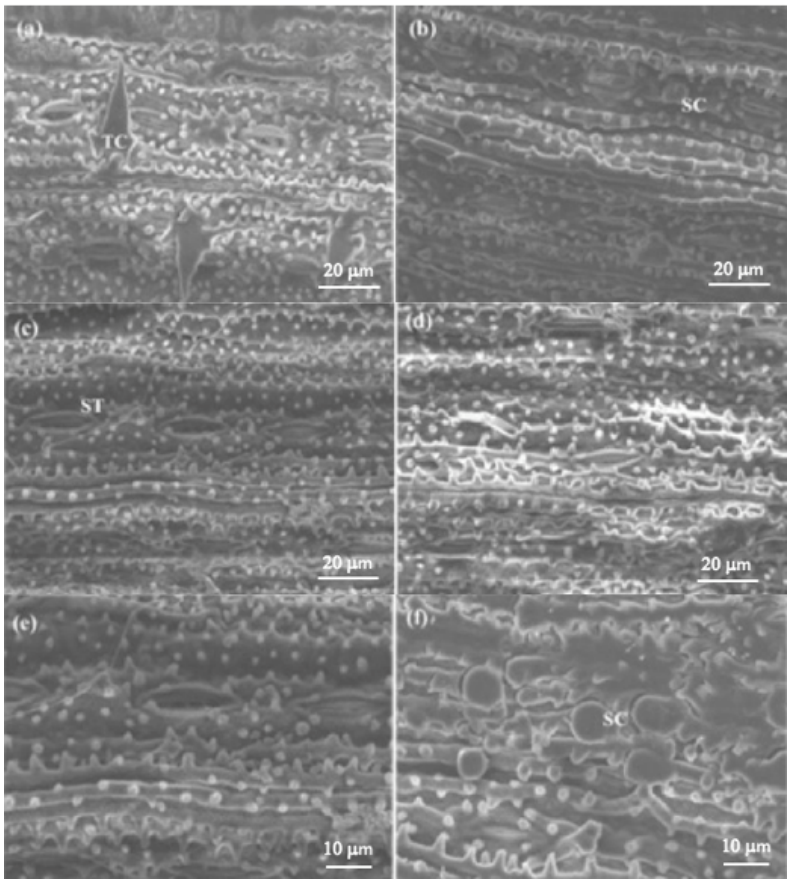
Treatments	Seedling length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Root number
H_2O	7.51^{b}	1.17^{bc}	4.04^{a}	0.30^{a}	7.23^{b}
NaCl	7.28^{b}	1.07^{c}	3.89^{a}	0.26^{b}	6.35^{c}
Oligochitosan	7.58^{b}	1.22^{b}	3.81^{a}	0.26^{b}	7.75^{a}
NaCl and oligochitosan	10.05^{a}	1.57^{a}	4.39^{a}	0.29^{a}	7.53^{ab}

Different alphabet characters indicate significant differences ($P<0.05$) between all treatments.



(a) Control; (b) saline condition; (c) oligochitosan; (d) oligochitosan in saline condition.

Fig 1: The morphology of rice seedlings in different treatments.



(a, e) Leaf in the control; (b, f) Leaf in saline condition; (c) Leaf in oligochitosan treatment; (d) Leaf in oligochitosan treatment under salinity conditions. TC: trichomes; ST: stomata, SC: silica bodies.

Fig 2: The underside leaf surfaces of rice seedlings.

Table 3: The photosynthetic pigment content in rice seedlings.

Treatments	Photosynthetic pigment content (µg/g)		
	Chlorophyll a	Chlorophyll b	Carotenoids
H_2O	189.95[b]	148.58[b]	50.06[b]
NaCl	165.88[b]	143.05[b]	41.73[b]
Oligochitosan	253.20[a]	147.42[b]	92.22[a]
NaCl and oligochitosan	267.19[a]	197.30[a]	98.72[a]

Different alphabet characters indicate significant differences (P<0.05) between all treatments.

Table 4: The photosynthesis and respiration of rice seedlings.

Treatments	O_2 concentration (µmol/g/min)	
	Photosynthesis	Respiration
H_2O	46.93[a]	31.26[c]
NaCl	18.72[c]	56.99[a]
Oligochitosan	31.96[b]	32.8[c]
NaCl and oligochitosan	32.79[b]	45.13[b]

Different alphabet characters indicate significant differences (P<0.05) between all treatments.

The photosynthetic pigments

Under saline stress, all photosynthetic pigments were not different from the control. However, in the treatment with oligochitosan and oligochitosan under saline stress, the content of photosynthetic pigments increased sharply except chlorophyll b (Table 3).

The photosynthesis and respiration

The photosynthesis decreased *and* the respiration increased in saline conditions in comparison to the control. In the treatments with oligochitosan and oligochitosan under salty stress, the photosynthesis was higher than the NaCl treatment but lower than the control. The respiration of rice seedlings in the treatment with oligochitosan was equivalent to the control but increased in saline stress with oligochitosan (Table 4).

The proline content

The proline content in the leaves and roots increased in saline conditions, but the supplement of oligochitosan reduced it. In the roots, there was no difference in proline content in all treatments except the NaCl. In the leaves, the proline content in the oligochitosan treatment was the same as the control (Table 5). Proline content in the leaves was significantly higher than in roots in control and salinity conditions.

The carbohydrates

The carbohydrate content increased under saline conditions (especially in the leaves) and decreased in the presence of oligochitosan (Table 5).

The total protein

The total protein in leaves decreased in salt stress but increased in all the treatments with oligochitosan. Protein was not detected in roots (Table 5).

Ascorbate peroxidase activity

In salinity conditions, the activity of ascorbate peroxidase increased in both leaves and roots. In the treatment with NaCl and oligochitosan, the enzyme activity increased in leaves but decreased in roots compared to the NaCl treatment. In the oligochitosan treatment, the enzyme activity in both leaves and roots was similar to that in the control (Table 6).

Gene expression

Under saline conditions, the expression of the P5CS gene was lower than the control in both roots and leaves, but the P5CR gene expression was higher than the control in the roots. In oligochitosan treatment, the increase in the expression of the P5CS gene in roots and P5CR in both leaves and roots was observed (Fig 3).

The expression of cAPX and sAPX increased in both roots and leaves in salt stress (especially sAPX in roots); tAPX increased in roots but decreased in leaves. In the presence of oligochitosan, the expression of all three genes remained in leaves and roots except for a decrease in sAPX in roots. In the treatment of oligochitosan, the expression of cAPX and tAPX was little changed, except for a sharp decrease in leaves of sAPX but an increase in roots (Fig 4).

Effects of salinity stress on the growth of rice seedlings

Under salinity stress, the photosynthetic pigment content in rice seedlings was unchanged (Table 3), but the stomata closed *and* the silica cells increased in size (Fig 2) to reduce transpiration, which decreased the photosynthesis (Table 4). The increase in the size of the silica cells on the rice leaves in salinity stress emphasised by Yang et al. (2015) to maintain water for plants. The decrease in photosynthesis might reduce the growth (Table 2) to contribute to the maintenance of energy to the stress tolerance of rice seedlings (Zhang *et al.*, 2021). The respiration increases sharply (Table 4), which might provide energy for the biosynthesis of osmolites or the antioxidant enzyme to protect the plants (Zhao *et al.*, 2021). Proline and soluble sugars are essential compounds that stabilise intracellular osmotic pressure at high salt concentrations (Kibria, 2017). In addition, excessive Na^+ absorption increased the Na^+/K^+ ratio, which inhibited protein synthesis (Assaha *et al.*, 2017). This might be the reason that the total protein decreased sharply, but the proline and carbohydrates increased in rice seedlings (Table 5).

Table 5: The contents of proline, carbohydrates and total protein in rice seedlings.

Treatments	Proline (nmol/g)		Carbohydrates (mg/g)		Total protein (mg/g)	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
H_2O	0.13^{c}	0.20^{b*}	3.95^{b}	8.50^{c}	37.08^{b}	-
NaCl	0.43^{a*}	0.31^{a}	11.62^{a}	18.63^{a}	26.12^{c}	-
Oligochitosan	0.14^{cNS}	0.20^{b}	3.90^{b}	13.10^{b}	37.02^{b}	-
NaCl and oligochitosan	0.27^{b}	0.22^{bNS}	5.14^{b}	10.23^{c}	45.83^{a}	-

Different alphabet characters indicate significant differences (P<0.05) between all treatments. In the same rows: *= Significant at 0.05; NS = Non-significant, - Not detected.

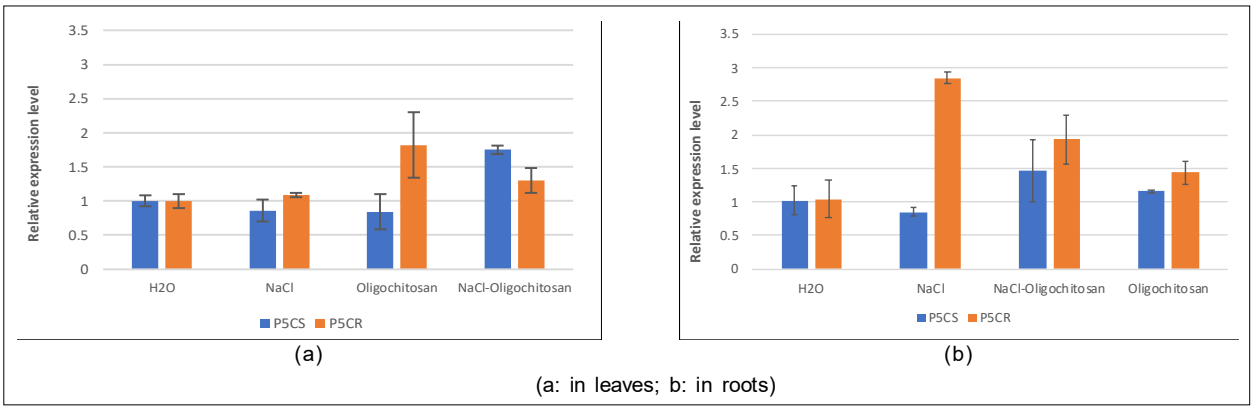


Fig 3: The expression of two genes involved in proline biosynthesis in rice seedlings in different treatments.

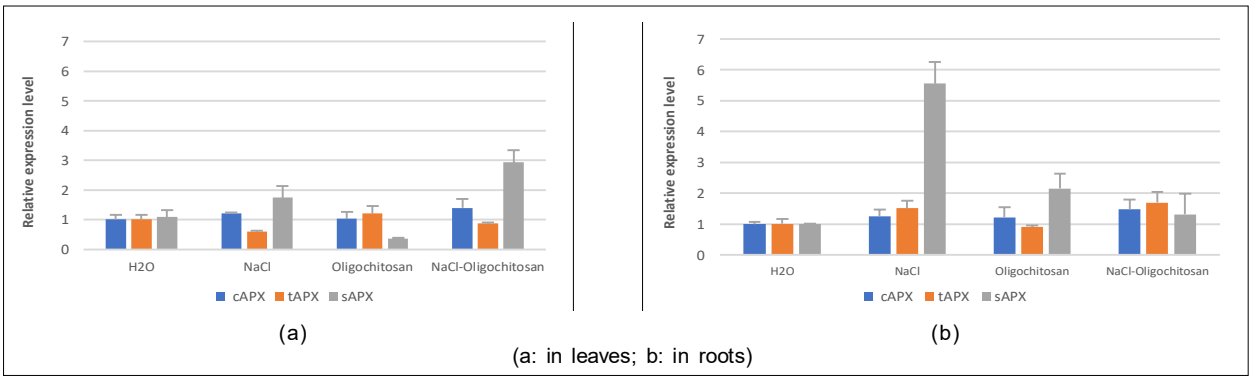


Fig 4: The expression levels of three genes related to ascorbate peroxidase activity in rice seedlings in different treatments.

Table 6: Ascorbate peroxidase activity of rice seedlings.

Treatments	Ascorbate peroxidase activity (U/g)	
	Leaves	Roots
H_2O	0.019^{c}	0.030^{c}
NaCl	0.052^{b}	0.134^{a}
Oliogochitosan	0.026^{c}	0.035^{c}
NaCl and oligochitosan	0.074^{a}	0.057^{b}

Different alphabet characters indicate significant differences (P<0.05) between all treatments.

Ascorbate peroxidase is one of the necessary antioxidant enzymes that breaks down H_2O_2 produced when plants are exposed to salinity stress. Therefore, when encountering salt, APX enzyme activity increased strongly (Table 6). This result is similar to the study of Mohammad *et al.* (2011) on salt resistance in rice; APX enzyme activity increased with salt concentration.

Effects of oligochitosan on the growth of rice seedlings

The treatment of oligochitosan improved the growth of rice seedlings under salinity stress. The photosynthetic intensity increased (Table 3) due to the increase of pigment contents, especially the chlorophyll a. This was similar to the study of Ma *et al.* (2012) on the treatment of wheat seeds with oligochitosan under salinity stress. The carotenoid content also increased sharply (Table 3), which is crucial in protecting chloroplasts from abiotic stress. The increase in photosynthesis will provide energy and materials for the growth of plants, thereby stimulating protein synthesis (Table 6). The decrease of proline and carbohydrate concentrations in the oligochitosan treatments (both in salinity stress and

normal conditions) might be the balance of physiological and biochemical state in rice seedlings, which decreased the concentration of ROS (Table 6) and improved the morphological parameters (Table 2). Peykani *et al.* (2019) showed decreased antioxidant enzyme activity in salt stress when chitosan was treated in *Triticum aestivum* L. and *Zea mays* L.

Effects of oligochitosan on the stress-related genes in rice seedlings

In salinity stress, the proline content increased (Table 5) with the increase of P5CR gene expression (especially in roots) and the decrease of P5CS (Fig 3). P5CS is an enzyme that initiates proline synthesis from glutamate (Glutamate pathway), while P5CR is a terminal metabolic enzyme. Besides, the Ornithine pathway is another way to proline biosynthesis (Hosseinifard *et al.*, 2022). The increase in proline content (Table 5) might depend on the Ornithine pathway, which was stronger than the Glutamate pathway in *Arabidopsis thaliana* in salinity conditions (Roosens *et al.*, 1998). Oligochitosan might create a balance in plant regulatory processes in salt stress by increasing P5CS and P5CR genes in leaves and roots and decreasing the P5CR in roots, leading to the decrease of proline. Furthermore, increasing the carbohydrate content in the treatment of NaCl and oligochitosan (Table 5) might stabilise the osmosis, thus reducing the need for excess proline accumulation (Khaleduzzaman *et al.*, 2021).

The APX activity increased in both leaves and roots under salinity stress (Table 6), along with the increase in expression of cAPX tAPX *and* sAPX genes, except the tAPX gene in leaves (Fig 4). Koo *et al.* (2010) concluded that the expression level of APX genes contributes to increasing the tolerance of rice under salt stress. These results in our study were the same as the research of Kim *et al.* (2007) in the conclusion that cAPX and sAPX genes in rice leaves increased, but tAPX decreased under saline conditions. In the presence of oligochitosan, APX activity increased in leaves but decreased in roots (Table 6) and the expression of all three genes increased in leaves, but the sAPX gene decreased in roots. Kibria *et al.* (2017) identified that the expression of the APX genes might depend on the characteristics of the tissues and organs of the plants and the intensity and duration of stress.

CONCLUSION

Oligochitosan 5994 Da at the concentration of 75 ppm improved the growth of rice seedlings under salinity conditions by increasing the intracellular osmolyte contents (proline, total carbohydrates) and antioxidant enzyme (APX) activity through the increase of gene expression in proline sõnthesis (P5CR) and APX synthesis (sAPX).

ACKNOWLEDGEMENT

This research is funded by Vietnam National University Hochiminh City under grant number 562-2020-20-02. We acknowledge Hochiminh City University of Technology and VNU-HCM for supporting this study.

Conflict of interest

All authors declare that they have no conflict of interest.

REFERENCES

Anbang, W., Jingyang, L., Arwa, A.A.H., Mohammad, S.A.H., Esmat, F.A., Jiashui, W., Zheli, D., Saudi, A.R., Adel, M.G. and Mamdouh, A.E. (2021). Mechanisms of chitosan nanoparticles in regulating cold stress resistance in banana plants. Nanomaterials. 11: 2670.

Assaha, D.V.M., Ueda, A., Saneoka, H., Rashid, A.Y., Mahmoud, W.Y. (2017). The role of Na^+ and K^+ transporters in salt stress adaptation in glycophytes. Frontier in Physiology. 8: 509.

Attaran, D.S., Zahra, K., Mahboubeh, M.D. and Leila, S. (2022). Chitosan nanoparticles improve physiological and biochemical responses of *Salvia abrotanoides* (Kar.) under drought stress. BMC Plant Biology. 22: 364.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Analytic Biochemistry. 72: 248-254.

Carillo, P. and Gibon, Y. (2011). Protocol: Extraction and determination of proline. Available online: http://prometheuswiki.publish.csiro.au.

Çelik, Ö., Çakır, B.C., Atak, Ç. (2018). Identiûcation of the antioxidant defense genes which may provide enhanced salt tolerance in *Oryza sativa* L. Physiology and Molecular Biology of Plants. 25: 85-99.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28: 350-356.

Hosseinifard, M., Stefaniak, S., Javid, M.G., Soltani, E., Wojtyla, L. and Garnczarska, M. (2022). Contribution of exogenous proline to abiotic stresses tolerance in plants: A review. International Journal of Molecular Science. 23: 5186.

Khaleduzzaman, M., Mohammad, A.H., Muhammad, J.H.B., Sakil, M., Tahjib-Ul-Arif, M. and Yoshiyuki, M. (2021). Chitosan mitigates salt stress in rice by enhancing antioxidant defense system. Fundamental and Applied Agriculture. 6(4): 336-348.

Kibria, M.G., Hossain, M., Hoque, M.A. (2017). Antioxidant defense mechanisms of salinity tolerance in rice genotypes. Rice Science. 24: 155-162.

Kim, D.W., Shibato, J., Agrawal, G.K., Fujihara, S., Iwahashi, H., Kim, D.H., Shim, I.S. and Rakwal, R. (2007). Gene transcription in the leaves of rice undergoing salt-induced morphological changes (*Oryza sativa* L.). Molecules and Cells. 24: 45-59.

Koo, J.S., Im, K.N., Chun, H.S. and Lee, C.B. (2010). Responses of photosynthetic efficiency and ascorbate peroxidase induced by salt stress in rice (*Oryza sativa* L.). Journal of Life Science. 20: 1173-1180.

Lam, V.T., Lan, N.T., Giang, P.H. and Tien, L.T.T. (2022). The effect of oligochitosans on the growth of *in vitro* rice seedlings on salinity stress. Indian Journal of Agricultural Research. doi: 10.18805/IJARe. AF-752.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology. 148: 350-382.

Ma, L., Li, Y., Yu, C., Wang, Y., Li, X., Li, N., Chen, Q. and Bu, N. (2012). Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. Protoplasma. 249: 393-399.

Mirza, H. and Masayuki, F. (2022). Plant responses and tolerance to salt stress: Physiological and Molecular interventions. International Journal of Molecular Science. 23: 4810.

Mohammad, R.A. (2011). Effect of salinity stress on growth, sugar content, pigment and enzyme activity of rice. International Journal of Botany. 7: 73-81.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology. 22(5): 867-880.

Parvaiz, A., Cheruth, A.J., Mohamed, A.S., Gowher, N. and Satyawati, S. (2010). Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Critical Reviews in Biotechnology. 30(3): 161-175.

Peykani, L.S. and Sepehr, M.F. (2019). Effect of chitosan on antioxidant enzyme activity, proline *and* malondialdehyde content in *Triticum aestivum* L. and *Zea maize* L. under salt stress condition. Plant Physiology 99. 2661-2670.

Roosens, N.H., Thu, T.T., Iskandar, H.M. and Jacobs, M. (1998). Isolation of the ornithine-δ-aminotransferase cDNA and effect of salt stress on its expression in *Arabidopsis thaliana*. Plant Physiol. 117: 263-271.

Sajid, H., Zhang, J.H., Zhong, C., Zhu, L.F., Cao, X.C., Yu, S.M., Allen, B.J., Hu, J.J. and Jin, Q.Y. (2017). Effects of salt stress on rice growth, development characteristics and the regulating ways: A review. Journal of Integrative Agriculture. 16(11): 2357-2374.

Teixeira, F.K., Menezes-Benavente, L., Galvão, V.C., Margis, R. and Margis-Pinheiro, M. (2006). Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. Planta. 224: 300-314.

Yang, C., Ma, S., Lee, I., Kim, J., Liu, S. (2015). Saline-induced changes of epicuticular waxy layer on the *Puccinellia tenuiflora* and *Oryza sativa* leaves surfaces. Biological Research. 48: 33.

Zayed, M.M., Elkafafi, S.H., Amina, M.G.Z. and Sherifa, F.M.D. (2017). Effect of nano chitosan on growth, physiological and biochemical parameters of *Phaseolus vulgaris* under salt stress. Journal Plant Production, Mansoura Univ. 8: 577-585.

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

Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C. and Wang, P. (2021). Regulation of plant responses to salt stress. International Journal Molecular Science. 22: 4609.