Vietnam Journal of Biotechnology **21**(3): 491-503, 2023

SUPPORTING FUNCTIONS OF OLIGOCHITOSAN ON SOYBEAN SEED GERMINATION UNDER NORMAL AND SALINITY STRESS CONDITIONS

Pham Nguyen Ngoc Lam[1,2], Tran Duong Tam Nghi[1,2], Do Quang Phat[1,2], Nguyen Thi Bang Tuyen[1,2], Nguyen Ngoc Huyen Tram[1,2], Xuan Lan Thi Hoang[1,2], Nguyen Phuong Thao[1,2,3,✉]

[1]Applied Biotechnology for Crop Development Research Unit, School of Biotechnology, International University, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam
[2]Vietnam National University, Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam
[3]Research Center for Infectious Diseases, International University, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

✉To whom correspondence should be addressed. E-mail: npthao@hcmiu.edu.vn

Received: 02.6.2023
Accepted: 25.8.2023

SUMMARY

Soybean is considered one of the important sources of oil and proteins worldwide. Nevertheless, the cultivation of soybeans is challenged by the threats of saline intrusion. Chitosan, a product derived from the shells of crustaceans such as shrimp, has been reported as a non-toxic biodegradable biopolymer that can minimize adverse impacts of salinity stress on plant growth. This study was to investigate the supportive activities of chitosan on germination under salinity stress of the local soybean cultivar MTD885-3. The assessment was conducted by pre-soaking soybean seeds in either distilled water, a commercial solution for seed germination, gibberellic acid 30 ppm, or oligochitosan solutions (500 ppm, 1000 ppm, and 2000 ppm), before allowing them to germinate in NaCl solution at different concentrations (0, 100, and 200 mM). As expected, soybean sprout emergence and growth were more adversely affected following the application of higher NaCl concentration. The pre-treatment of soybean seeds in oligochitosan solutions of 500 and/or 1000 ppm could enhance hypocotyl and radicle growth under both non-stressed and salinity conditions. Furthermore, seed pre-soaking with 1000 ppm oligochitosan could confer certain benefits for the soybean sprouts exposed to 200 mM NaCl treatment, which were also evidenced by shorter Mean Germination Time, higher Germination Index, and Coefficient of Velocity of Germination values. These findings indicate the practical application of chitosan in supporting the early growth of germinated seeds under osmotic stress conditions such as salinity.

Keywords: Chitosan, oligosaccharide, bio-stimulant, soybean germination, salinity stress

INTRODUCTION

Soybean, *Glycine max* L. Merrill, is one of the legume species that reliably offers high economic and nutritional values and is a major source of vegetable oil and proteins in the human food industry (Zhang *et al*., 2010). Soybean protein is seen as an excellent alternative to animal protein because it includes all the essential amino acids such as lysine, which is particularly lacking in other staple crops (Agyenim-Boateng *et al*., 2022). Many essential unsaturated fatty acids such as omega-3, omega-6, and omega-9 are highly concentrated in soybean seeds (Nikolić *et al*., 2009). Soybeans contain nutritional properties that lower blood serum cholesterol levels in humans and minimize the risk of cardiovascular diseases (Omoni, Aluko, 2005). Additionally, soybeans are utilized in agriculture for land reclamation and animal feed (Jegadeesan *et al*., 2020), as well as for the production of biodegradable materials (Tian *et al*., 2018). However, there are difficulties and barriers in the way of growing soybeans, such as declines in crop quality and yield due to biotic and abiotic pressures. Soybean is almost grown on semi-arid or dry soil that is frequently stressed by salinity and drought (FAO, 2023). Therefore, enhancement of the plant tolerance to stresses to maintain soybean profit is essential.

Salinity is one of the most common abiotic stresses worldwide that plants have to face with, and it is being made worse by climate change. As a result of this, more severe drought incidences and saline intrusion have occurred in Vietnam's Mekong River Delta, causing various degrees of damage to agriculture, fisheries, and people's livelihoods (CGIAR, 2016). Serious salinity stress can cause plant death, whereas mild salt stress negatively affects plant growth, agronomic attributes, and thus agricultural output as well as farmers' income. Practically, salinity can interfere with every stage of plant growth, including germination, seedling growth, vegetative development, and reproductive duration in soybeans (Mansouri *et al*., 2019). According to Ghassemi-Golezani and colleagues (2009), the large reductions not only in average grain yield under saline conditions but also in the oil and protein contents of the harvested soybean seeds.

To lessen the negative effects of salinity on plants, various strategies have been deployed and other novel ideas have been under investigation. Among the proposals, one option for mitigating the negative consequences of abiotic stress is to use chitosan, which is a natural non-toxic, and bio-degradable product (Morais *et al.*, 2008). Chitosan is a linear polysaccharide consisting of D-glucosamine and N-acetyl-D-glucosamine that is produced by treating the chitin shells of shrimp and other crustaceans with different methods for deproteinization, demineralization, decolorization, and deacetylation (Harugade *et al*., 2023). Oligochitosan has a lower molecular weight, a greater degree of deacetylation (DD $\geq$ 80%), and a higher degree of polymerization (DP). Oligochitosan is less viscous ($\leq$ 5.0 cPs) and entirely water-soluble, making it more appropriate for various industrial uses. Numerous studies have revealed that water-soluble chitosan could activate a variety of plant defense-related cellular responses (Akash *et al*., 2019, Yang *et al*., 2019; Ling *et al*., 2022).

Owing to limitless free amino and hydroxyl bunches on its spine, chitosan is

regarded as the ideal adsorbent. The unique features of chitosan and its derivatives, including hydrophilicity, biocompatibility, biodegradability, and antibacterial capabilities, make them promising for use in biotechnology, biomedicine, and cosmetics (Yuvaraja *et al.*, 2020). The report by Mahdavi and Rahimi (2013) indicates that chitosan increases the activity of antioxidant molecules (e.g., phenolic acid and ascorbic acid) and antioxidant enzymes (e.g. peroxidase and superoxide dismutase) to assist plants in combating cellular free radicals during stressful conditions such as heat stress, salt, and drought stress. In addition, chitosan promotes the synthesis of chlorophyll, amino acids (such as proline), phytohormones (abscisic acid), and soluble carbohydrate metabolisms to help control osmotic adjustment (Hidangmayum *et al.*, 2019). In addition to the eco-friendly properties of chitosan/oligochitosan, the abundant resources of chitin released from the seafood industry as wastes/by-products for chitosan production also display the practicability from the economic point of view. Therefore, in this study, the effects of oligochitosan on improving soybean germination and early growth under salt stress conditions were investigated.

MATERIALS AND METHODS

Chemicals and plant materials

The seeds of local soybean cultivar MTD885-3 in the present study were kindly provided by Can Tho University, Can Tho Province, Vietnam. These seeds had been recently harvested. Oligochitosan was extracted from shrimp by-products at a concentration of 40,000 ppm, which was produced by the Vietnam Food Joint Stock Company (VNF), Vietnam. Atonik (a commercial soaking solution for seed germination) manufactured by ADC Limited Company, Can Tho, Vietnam, and gibberellic acid (GA3) (Biobasic, Ontario, Canada) were used as the positive controls in the germination experiment, according to previous studies (Przybysz *et al*., 2014; Du *et al*., 2022).

Determine the effects of oligochitosan on the germination of soybean sprouts

Soybean seeds were surface-sterilized with 0.5% sodium hypochlorite solution for 5 min, then rinsed at least 3 times with water to remove chemical residues (Sauer, 1986). Next, the washed soybean seeds were soaked with various solutions, including oligochitosan solutions (500, 1000, or 2000 ppm), Atonik (according to the manufacturer's instruction), and GA3 (30 ppm) (Przybysz *et al*., 2014; Kurubar *et al*., 2017). The seeds were soaked with a ratio of 1:4 (weight of seeds/volume of solution) at 37 ºC for 30 min (Yang *et al.*, 2019). Following this, the seeds were placed in Petri dishes (10 seeds/plate) between 2 layers of filter papers, and different saline (NaCl) solutions (0, 100, 200 mM) were added to the Petri dishes (Islam *et al*., 2019). These plates were then incubated at 25-30 °C under dark conditions.

Data were collected each day from the second to the fifth day of the assay to analyze the final germination percentage (FGP), average germination time (MGT), speed of germination, seedling vigor index (VI), radicle, hypocotyl length, and fresh weight, using below formula:

Final germination percentage (FGP):

$$FGP = \frac{\text{Number of toal germinated seeds}}{\text{Total number of seed tested}} \times 100$$

(Hai *et al*., 2019)

Mean Germination Time (MGT):

$$MGT = \frac{\sum_{i=1}^{k}(N_i T_i)}{\sum_{i=1}^{k} N_i}$$

(Ellis, Roberts, 1981)

N_i =the number of germinated seeds on the examined day.

T_i =the number of days from the start of the assay.

Coefficient of Velocity of Germination (CVG):

$$CVG = \frac{1}{MGT} \times 100$$

(Jones, Sanders, 1987)

Germination Index (GI):

$$GI = \frac{\text{Number of germinated seeds on first count}}{\text{Days of first count}} + \cdots + \frac{\text{Number of germinated seeds on final count}}{\text{Days of final count}}$$

(Arnold *et al*., 1991)

Seedling vigor index (VI):

$$VI = \frac{\text{Final Germination \%} \times \text{Seedling length}}{100}$$

(Abdul-Baki, Anderson, 1973)

Seeds were considered germinated if their radicles were at least 2 mm in length (ISTA, 1985). Three replications were performed for each treatment, and each Petri dish was used as a replicate.

Data analysis

The data were analyzed using one-way ANOVA by IBM SPSS Statistics (version 20, US). Differences between means of all germination parameters were compared by using Duncan ‘s Multiple Test Range. Graphs were prepared using GraphPad (Version 9.1, Inc., La Jolla, CA, US). The *p*-value < 0.05 implies the difference between treatments according to statistical analysis. Data on graphs and tables were presented as means ± standard errors.

RESULTS

As salinity stress can inhibit seed germination and growth of the young seedlings, the effect of using oligochitosan as the seed pre-soaking solution prior to sowing was explored. Table 1 summarized the analyses of five parameters relating to seed vigor assessment when they were germinated on normal or NaCl conditions. According to the findings, the rate of seed germination was high under 0 mM NaCl treatment condition, which was more than 90% regardless of what

solution was used for pre-soaking. The average FGP values were decreased under salinity conditions, ranging from 86.7% - 96.7% and 73.3% - 93.3% upon exposure to 100 mM and 200 mM NaCl treatments, respectively. In detail, when pre-soaking the seeds with distilled water (dH_2O), all seeds germinated under non-stressed conditions, yet the germination rate was reduced to 73.3% under 200 mM NaCl condition. Meanwhile, the application of 500 ppm oligochitosan could maintain a significantly higher level of germination under such high salinity (93.3%).

Table 1. Germination parameters of soybean under normal and salinity stress conditions (n=3 replicates, each replicate included 10 seeds).

Salinity (mM)	Treatment	FGP (%)	VI	MGT (day)	GI	CVG
0	Distilled water	100 ^{a} ± 0	328.33^{a} ± 20.85	2.53 ^{a} ± 0.28	4.22 ^{a} ± 0.36	0.404 ^{a} ± 0.041
	Atonik	100 ^{a} ± 0	318.67^{a} ± 56.3	2.43 ^{a} ± 0.19	4.42 ^{a} ± 0.25	0.415 ^{a} ± 0.03
	GA3	96.67 ^{a} ± 3.33	373.23^{a} ± 23.57	2.32 ^{a} ± 0.17	4.39 ^{a} ± 0.36	0.436 ^{a} ± 0.033
	Chitosan 500 ppm	93.33 ^{b} ± 3.33	405.31 ^{a} ± 72.91	2.41 ^{a} ± 0.21	4.21 ^{a} ± 0.4	0.422 ^{a} ± 0.039
	Chitosan 1000 ppm	100 ^{a} ± 0	444.83 ^{a} ± 50.45	2.23 ^{a} ± 0.12	4.67 ^{a} ± 0.17	0.45 ^{a} ± 0.025
	Chitosan 2000 ppm	100 ^{a} ± 0	431.07 ^{a} ± 42.79	2.37 ^{a} ± 0.09	4.42 ^{a} ± 0.14	0.424 ^{a} ± 0.016
100	Distilled water	93.3 ^{a} ± 3.33	237.38 ^{a} ± 5.69	2.91 ^{a} ± 0.35	3.58 ^{a} ± 0.3	0.355 ^{a} ± 0.048
	Atonik	86.7 ^{a} ± 3.33	218.25 ^{a} ± 8.83	2.34 ^{a} ± 0.23	3.96 ^{a} ± 0.27	0.435 ^{a} ± 0.041
	GA3	93.3 ^{a} ± 3.33	250.73 ^{a} ± 24.93	2.63 ^{a} ± 0.27	3.9 ^{a} ± 0.47	0.39 ^{a} ± 0.044
	Chitosan 500 ppm	96.7 ^{a} ± 3.33	290.97 ^{a} ± 57.75	2.74 ^{a} ± 0.39	3.89 ^{a} ± 0.63	0.382 ^{a} ± 0.061
	Chitosan 1000 ppm	93.3 ^{a} ± 3.33	244.55 ^{a} ± 42.75	2.6 ^{a} ± 0.25	3.92 ^{a} ± 0.22	0.393 ^{a} ± 0.041
	Chitosan 2000 ppm	90 ^{a} ± 0	208.68 ^{a} ± 16.8	2.26 ^{a} ± 0.07	4.17 ^{a} ± 0.08	0.444 ^{a} ± 0.015
200	Distilled water	73.3 ^{a} ± 6.67	121.33 ^{a} ± 33.65	3.71 ^{a} ± 0.51	2.22 ^{a} ± 0.52	0.28 ^{a} ± 0.04
	Atonik	86.7 ^{a} ± 3.33	126.03 ^{a} ± 14.18	3.29 ^{ab} ± 0.22	2.93 ^{ab} ± 0.05	0.307 ^{ab} ± 0.022
	GA3	80 ^{a} ± 5.78	140.4 ^{a} ± 23.66	2.84 ^{ab} ± 0.11	3.06 ^{ab} ± 0.56	0.35 ^{ab} ± 0.014
	Chitosan 500 ppm	93.3 ^{b} ± 3.33	195.53 ^{a} ± 36.87	3.07 ^{ab} ± 0.2	3.31 ^{ab} ± 0.15	0.329 ^{ab} ± 0.023
	Chitosan 1000 ppm	86.7 ^{a} ± 3.33	158.05 ^{a} ± 11.67	2.61 ^{b} ± 0.12	3.57 ^{b} ± 0.02	0.385 ^{b} ± 0.018
	Chitosan 2000 ppm	80 ^{a} ± 0	131.59 ^{a} ± 22.08	2.75 ^{b} ± 0.19	3.18 ^{ab} ± 0.15	0.367 ^{ab} ± 0.024

Final Germination Percentage (FGP) and Seedling Vigor Index (VI) were recorded on the fifth day of the assay. Mean Germination Time (MGT) and Germination Index (GI) were calculated following the measurement from the second to fifth days of the assay. Coefficient Velocity of Germination (CVG) was calculated based on the MGT value. Different letters denote significant differences among solutions applied for pre-soaking under the same condition of salt treatment, as analyzed by One-way ANOVA.

It has been noted that using 1000 ppm oligochitosan could also reveal positive effects on the seed germination under adverse salt stress conditions of 200 mM NaCl, with shorter MGT, and higher GI and CVG values. According to the results, under this stress condition, the seeds treated with 1000 ppm oligochitosan solution on average could germinate one day earlier than the ones soaked in water (i.e. MGT 2.61 days versus 3.71 days) (Table 1). Treated with oligochitosan at this concentration displayed

the highest germination index (3.57) and coefficient velocity of germination (0.385) under 200 mM salt stress application among all the tested pre-soaking solutions. However, there was no difference in VI values across all the methods for seed soaking when compared under the same salt stress conditions.

Next, we examined the growth of seedlings by measuring the lengths of radicle and hypocotyl of germinated seeds. On the course of germination of a seed, the radicle will emerge first followed by its hypocotyl. Therefore, radicle length could be measured since the second day of the assay (Figure 1) while the measurement of hypocotyl length started on day 4th (Figure 2).

As shown in Figure 1, using commercial seed-soaking solution (Atonik), GA3 (30 ppm) or oligochitosan solutions could induce radicle growth significantly in comparison to the soaking treatment by dH_2O under non-stressed condition by 1.6-fold (using Atonik) to 2.2-fold (using 500 ppm oligochitosan), recorded on the fifth day of the assay. Similar results were also observed under 100 mM NaCl condition, whereby the average radicle length using dH_2O for seed soaking was 1.1 cm and the corresponding lengths when using other solutions ranged from 1.6 to 1.9 cm on the last day of recording (Figure 1). Under 200 mM NaCl conditions, a reduction in root growth was observed more clearly compared to the normal germination condition. In addition, the application of oligochitosan 500 ppm and 1000 ppm could maintain a better radicle growth, which was comparable with the use of Atonik solution for seed soaking.

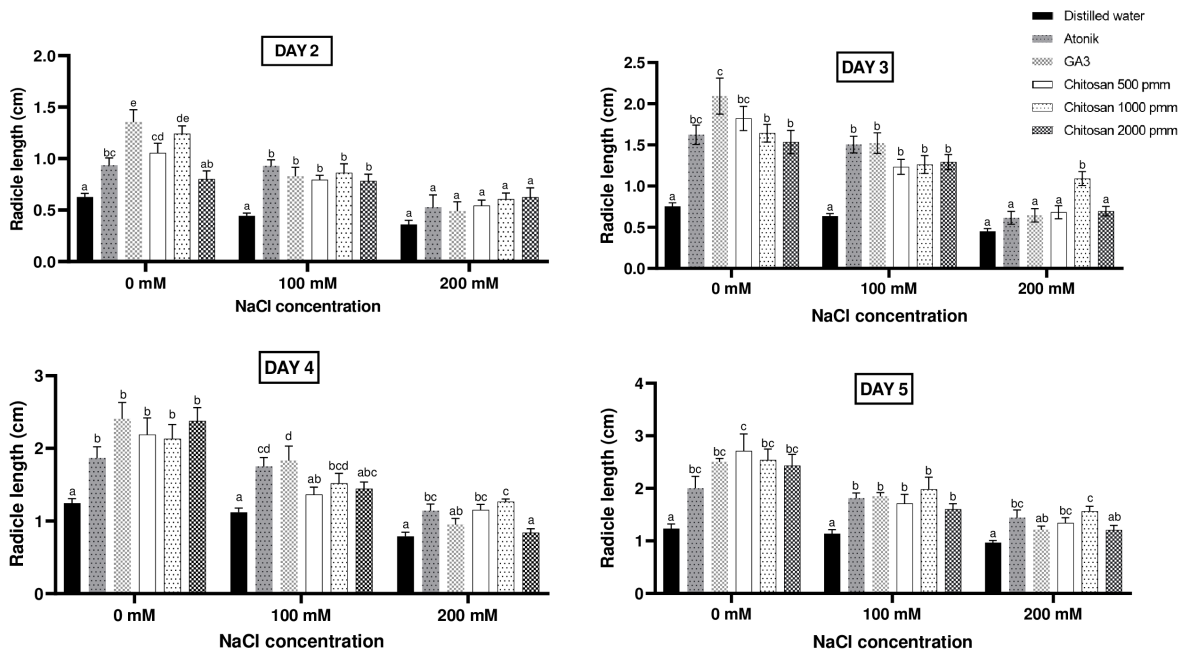

Figure 1. Measurement of average radicle lengths of soybean sprouts on the second, third, fourth and fifth days of the germination experiment. Atonik and GA3 were used as the positive controls while distilled water was used as the negative control. Different letters indicated significant differences among treatments.

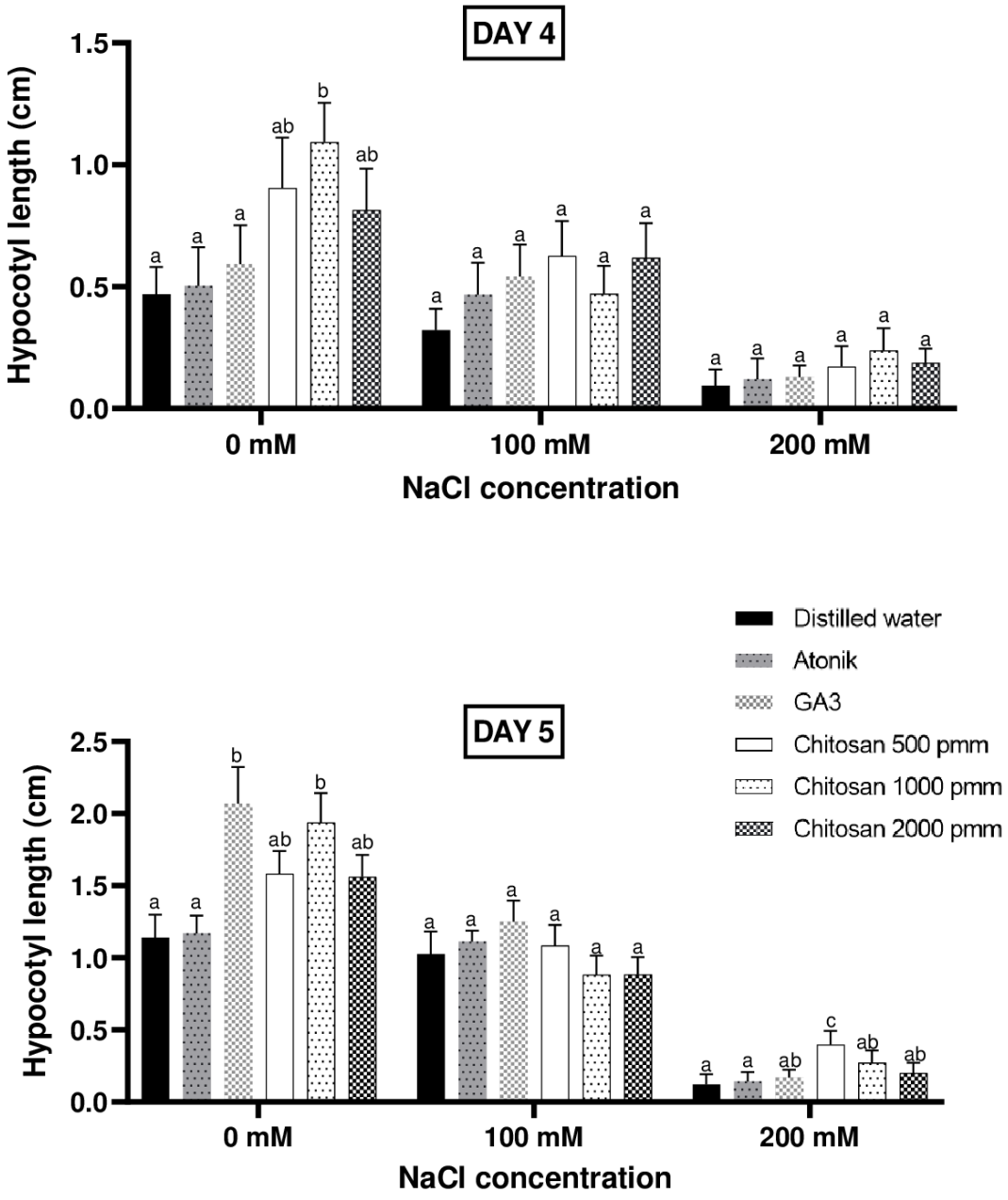

Figure 2. Measurement of average hypocotyl lengths of soybean sprouts on the fourth and fifth days of the germination experiment. Atonik and GA3 (30 ppm) were used as the positive controls while distilled water was used as the negative control. Different letters indicated significant differences among treatments.

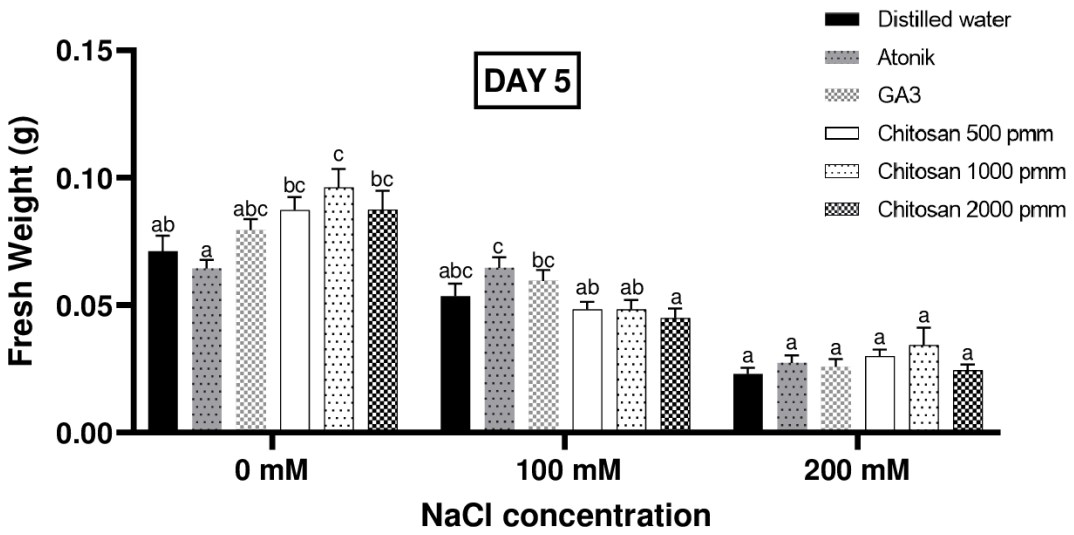

Figure 3. The average fresh weights of soybean sprouts measured on the fifth day of the germination experiment. Atonik and GA3 were used as the positive controls while distilled water was used as the negative control. Different letters indicated significant differences among treatments.

Regarding the hypocotyl length of germinated seeds, oligochitosan treatment could also yield positive outcomes, with better hypocotyl elongation. Among the examined concentrations, 1000 ppm resulted in significantly longer hypocotyl under normal conditions measured on both days 4th and 5th of the assay, which was around 2 times longer than the corresponding values using dH_2O for seed soaking and even better than the use of Atonik or GA3. Furthermore, although soybean seeds exposed to intermediate salinity stress (100 mM NaCl) showed no significant differences between oligochitosan treatments versus negative and positive controls, the use of 500 ppm oligochitosan could prevent the inhibition effects of salt stress on hypocotyl growth under high salt conditions (i.e. 200 mM NaCl). Under this condition, the average hypocotyl lengths of the seeds treated with the 500 ppm oligochitosan and dH_2O were 0.396 cm and 0.112 cm, respectively, meaning that the former group displayed three times longer for the hypocotyl compared to the latter.

The fresh weight of germinated soybean sprouts treated with oligochitosan solution at various concentrations is displayed in Figure 3. In general, oligochitosan treatments demonstrated a positive effect on increasing the fresh weights under non-stressed conditions, compared to the control (dH_2O). According to statistical analyses, intermediate oligochitosan concentration (1000 ppm) could significantly boost the fresh weights of soybean sprouts under normal (0 mM NaCl) conditions by 1.4-fold. Under high salt (200 mM NaCl) conditions, accumulated fresh weight of soybean sprouts treated with 1000 ppm oligochitosan was also noticed, of which the average weight was 0.034 g compared to 0.023 g of dH_20 pre-soaking group recorded on the fifth day of the assay.

DISCUSSION

Environmental stresses such as salt, drought, or severe temperatures, have posed threats on global agriculture activities and food security. For example, soybeans might lose up to 40% of their production under adverse conditions (Khan *et al*., 2015; Kang *et al*., 2023). It has been emphasized that at the stage of seed germination and post-germination, the young seedlings are more vulnerable to the detrimental effects caused by such abiotic factors (Khaleduzzaman *et al*., 2021). Saline stress leads to osmotic, ionic, and oxidative stress, resulting in the generation of cellular free radicals (Parida *et al*., 2004; Jacoby *et al*., 2011; Fu, Yang, 2023). Furthermore, the overdosed accumulation of sodium and chloride ions gives rise to serial disruptions in cellular function, signalling and metabolism (Jahromi *et al*., 2008; Daszkowska-Golec, 2011; Uçarlı, 2020). As a result, early growing events of soybean seeds such as imbibition, metabolism, seeds growth and establishment were decreased and delayed, followed by the significant changes in the germination rate and duration required by the seeds to germinate or other seed vigor characters.

According to our results, high seed germination rates under the non-stressed conditions even when using dH_2O for seed soaking indicated that the seeds with high quality were used for the assay (Table 1). Atonik is a commercial plant regulator that poses positive effects on the germination and vegetative growth of plants, whereas GA3 is a natural phytohormone promoting seed emergence, stem elongation and seedling development. Under adverse conditions, plants treated with these two biostimulants could enhance their ability to defend against various abiotic stresses such as extreme temperature, drought, heavy metals and salinity (Przybysz *et al*., 2014; Du *et al*., 2022).

When the seeds were challenged with high NaCl concentration such as 200 mM, differential FGPs were observed whereby oligochitosan treatments could minimize the reduction in germination compared to the negative control (Table 1). Significantly, utilization of 500 ppm oligochitosan could maintain an average FGP above 90% compared to the corresponding value of 73.3% for the dH_2O treatment. This indicated that the seeds treated with oligochitosan could encounter salt-induced adverse effects on the seeds in the process of germination. Furthermore, treatment with oligochitosan of 500-1000 ppm could support the seeds to germinate more rapidly. On average, it took around 2.61-2.84 days for the seeds treated by these oligochitosan solutions to germinate whereas the duration for the negative control group was 3.71 days. Values for CVG also consistently supported these findings, whereby the oligochitosan-treated groups had higher values (Table 1). With such treatments, higher values for overall germination index (GI) were also recorded for the treatments of 500 ppm or 1000 ppm oligochitosan, indicating supportive functions of these solutions for seed germination.

Following germination, salinity effects on early growth were further examined. The analyses also demonstrated the supportive effects of oligochitosan treated in the previous step on the radicle and hypocotyl growth of soybean sprouts. Under normal seed germination conditions, seeds that had been soaked with oligochitosan solutions with any tested concentrations could significantly boost the radicle elongation,

which were as good as the utilization of Atonik or GA3 (Figure 1). Regarding the effects on hypocotyl length, under normal conditions, all the tested oligochitosan concentrations displayed beneficial effects, although only the use of oligochitosan 1000 ppm could increase the growth according to statiscal analyses (Figure 2).

Similarly, all tested oligochitosan solutions could also maintain the radicle with an average length longer than the corresponding value in the dH_2O treatment for seed soaking under intermediate salt conditions (i.e. 100 mM NaCl). With higher salt concentration, distinct effects in maintaining better root growth were observed with the use of oligochitosan 500 or 1000 ppm (Figure 1). Meanwhile, 500 ppm oligochitosan was the only tested concentration that could support both root and shoot growth under the salt stress conditions of 200 mM NaCl (Figures 1, 2). In addition, the obtained data revealed that the young seedlings exhibited longer radicle and/or hypocotyl also had higher fresh weight. In particular, the use of 1000 ppm oligochitosan concentration could display the best effects in increasing the weight, not only under normal but also high salt stress conditions. Taken these findings together, the use of oligochitosan within the range 500-1000 ppm could enhance the growth of radicle and hypocotyl under normal and salt stress conditions, by either promoting the tissue elongation, tissue thickness or density.

The supportive effects of chitosan and its derivatives on seed germination and growth of various species have been reported including cucumber (Zohara *et al*., 2019), green pepper (Fard *et al*., 2010), and ajowan (Mahdavi, Rahimi, 2013). Chitosan has been suggested to act as a promoter, stimulating enzymes involved in various metabolic pathways such as nitrogen and carbon metabolism (Mukhtar *et al*., 2020). It has been shown that oligochitosan improved the content of bioactive compounds in germinated seeds, whereas phytic acid was largely eliminated, resulting in improved morphology of soybean sprouts (Yang *et al*., 2019). Furthermore, it has been evidenced for the stimulation effects by chitosan on hormone contents such as gibberellic acid and auxin that play vital role in seed germination, plant growth and development (Kuraishi, Muir, 1964; Safikhan *et al*., 2018).

Under salt stress conditions, it has been shown that chitosan treatment could increase the proline and sugar levels in plants, reducing the oxidative damage induced by the stress (Simaei *et al*., 2012; Safikhan *et al*., 2018). A previous study also pointed out that although chitosan could not limit the cellular accumulation of Na^+, it facilitated the absorption of K^+ ions, thus aiding the maintenance of the efflux and compartmentation of these K^+ and Na^+, leading to promoting salt tolerance in plants (Simaei *et al*., 2012). The increase in water and nutrient absorption, and enzyme activities along with the prevention of free radical accumulation in cells were also observed following chitosan application (Guan *et al*., 2009). In addition, the level of phenolic compounds such as flavonoids, phytoalexins and ascorbic acid content were boosted in the chitosan-treated plants, which might confer the plants better tolerance to salinity stress (Hamel, Beaudoin, 2010; Pichyangkura, Chadchawan 2015). Meanwhile, the stress indicators such as levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents were reduced following chitosan application (Iriti,

Varoni, 2015; Salah *et al*., 2021). Therefore, taken all of these data together, the results from this study demonstrated beneficial effects of using oligochitosan as the seed pre-soaking solution in supporting seed germination and early growth under salinity stress conditions.

CONCLUSION

In this study, we evaluated the chitosan effects on soybean seeds at the germination stage under salinity stress. The result indicates that pre-soaking the seeds with oligochitosan at concentrations of 500-1000 ppm might be relevant for improving the germination process and post-germinative growth of young seedlings, not only under normal but also salinity stress conditions. Therefore, the underlying mechanisms of oligochitosan effects on these seed germination and growth should be further investigated.

Acknowledgement: *This research is funded by the International University, VNU-HCM under grant number SV2022-BT-01.*

REFERENCES

Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria. *Crop Sci* 13(6): 630–633.

Agyenim-Boateng KG, Zhang S, Zhang S, Khattak AN, Shaibu A, Abdelghany AM, Qi J, Azam M, Ma C, Feng Y, Feng H, Liu Y, Li J, Li B, Sun J (2022) The nutritional composition of the vegetable soybean (maodou) and its potential in combatting malnutrition. *Front Nutr* 9: 1034115.

Akash H, Dwivedi P, Katiyar D, Hemantaranjan A (2019) Application of chitosan on plant responses with special reference to abiotic stress. *Physiol Mol Biol Plants* 25(2): 313–326.

Arnold RLB, Fenner M, Edwards PJ (1991) Changes in germinability, ABA content and ABA embryonic sensitivity in developing seeds of *Sorghum bicolor* (L.) Moench. Induced by water stress during grain filling. *New Phytol* 118(2): 339–347.

CGIAR (2016) *The drought and salinity intrusion in the Mekong River Delta of Vietnam: Assessment Report* [Report]. CGIAR Research Program on Climate Change, Agriculture and Food Security-Southeast Asia.

Daszkowska-Golec A (2011) *Arabidopsis* seed germination under abiotic stress as a concert of action of phytohormones. *OMICS J Integr Biol* 15(11): 763–774.

Du G, Zhang H, Yang Y, Zhao Y, Tang K, Liu F (2022) Effects of gibberellin pre-treatment on seed germination and seedling physiology characteristics in industrial hemp under drought stress condition. *Life* 12(11): 1907.

Ellis RH, Roberts EH (1981) The quantification of ageing and survival in orthodox seeds. *Seed Sci Technol* 9: 373–409.

FAO (2023) Soybean. Retrieved November 27, 2023, from https://www.fao.org/land-water/databases-and-software/crop-information/soybean/en/.

Fard K, Kamari S, Ghasemnezhad M, Fotouhi Ghazvini R (2010) Effect of chitosan coating on weight loss and postharvest quality of green pepper (*Capsicum annum* L.) fruits. *Acta Hortic* 877: 821–826.

Fu H, Yang Y (2023) How plants tolerate salt stress. *Curr Issues Mol Biol 45*(7): 5914–5934.

Ghassemi-Golezani K, Taifeh-Noori M, Oustan S, Moghaddam M (2009) Response of soybean cultivars to salinity stress. *JAFE* 7(2): 401–404.

Guan Y, Hu J, Wang X, Shao C (2009) Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J Zhejiang Univ Sci B* 10(6): 427–433.

Hai NTT, Thu LH, Nga NTT, Hoa TT, Tuan LNA, Van Phu D, Hien NQ (2019) Preparation of chitooligosaccharide by hydrogen peroxide degradation of chitosan and its effect on soybean seed germination. *J Polym Environ* 27(9): 2098–2104.

Hamel LP, Beaudoin N (2010) Chitooligosaccharide sensing and downstream signaling: Contrasted outcomes in pathogenic and beneficial plant-microbe interactions. *Planta* 232(4): 787–806.

Harugade A, Sherje AP, Pethe A (2023) Chitosan: A review on properties, biological activities and recent progress in biomedical applications. *React Funct Polym* 191: 105634.

Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A (2019) Application of chitosan on plant responses with special reference to abiotic stress. *Physiol Mol Biol Plants* 25(2): 313–326.

Iriti M, Varoni EM (2015) Chitosan-induced antiviral activity and innate immunity in plants. *Environ Sci Pollut Res* 22(4): 2935–2944.

Islam M, Ahmed R, Shila A, Jhan P, Nuruzzaman M, Fahim M, Fahim B (2019) Assessing salt tolerance level of soybean during germination and seedling stage. *JNSTU* 3(1,2): 8–14.

ISTA (1985) International rules for seed testing. *SST* 13: 307–520.

Jacoby R, Taylor N, Millar H (2011) The role of mitochondrial respiration in salinity tolerance. *Trends Plant Sci* 16(11): 614–623.

Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM (2008) Influence of salinity on the *in vitro* development of *Glomus intraradices* and on the *in vivo* physiological and molecular responses of mycorrhizal lettuce plants. *Microb Ecol* 55(1): 45–53.

Jegadeesan S, Yu K (2020) *Food grade soybean breeding, current status and future directions*. In Hassanuzzaman, ed. *Legume crops—prospects, production and uses*. *IntechOpen*: 51-77.

Jones KW, Sanders DC (1987) The influence of soaking pepper seed in water or potassium salt solutions on germination at three temperatures. *J Seed Technol* 11(1): 97–102.

Kang SM, Hoque MIU, Woo JI, Lee IJ (2023) Mitigation of salinity stress on soybean seedlings using Indole Acetic Acid-producing *Acinetobacter pittii* YNA40. *Agriculture* 13(5): 5.

Khan SA, Karim MA, Parveen S, Bazzaz M, Hossain A (2015) Plant water relations and proline accumulations in soybean under salt and water stress environment. *J Plant Sci* 3(5): 272–278.

Khaleduzzaman M, Hossain M, Bhuiyan MJ, Mahmud S, Tahjib-Ul-Arif M, Murata Y (2021) Chitosan-mediated salt stress mitigation in rice by enhancing antioxidant defense system. *Fundam Appl Agric* 6: 1.

Kuraishi S, Muir RM (1964) The relationship of Gibberellin and Auxin in plant growth. *Plant Cell Physiol* 5(1): 61–69.

Kurubar AR, Allolli TB, Naik MK, Angadi SG (2017) Effects of gibberellic acid on growth, yield and fruit quality of fig (*Ficus carica* L.). *Acta Hortic* 1173: 183–188.

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

Mahdavi B, Rahimi A (2013) Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum copticum*) under salt stress. *EurAsian J Biosci* 7: 69–76.

Mansouri LM, Heleili N, Boukhatem ZF, Kheloufi A (2019) Seed germination and radicle establishment related to type and level of salt in common bean (*Phaseolus vulgaris* L. var. Djedida). *ALSE* 52(3): 262–277.

Morais WA, de Almeida ALP, Pereira MR, Fonseca JLC (2008) Equilibrium and kinetic

analysis of methyl orange sorption on chitosan spheres. *Carbohydr Res* 343(14): 2489–2493.

Mukhtar AKB, Khan MMA, Siddiqui H, Jahan A (2020) Chitosan and its oligosaccharides, a promising option for sustainable crop production- a review. *Carbohydr Polym* 227: 115331.

Nikolić NČ, Cakić SM, Novaković SM, Cvetković MD, Stanković MZ (2009) Effect of extraction techniques on yield and composition of soybean oil. *Maced J Chem Chem Eng* 28(2): 2.

Omoni AO, Aluko RE (2005) Soybean foods and their benefits: Potential mechanisms of action. *Nutr Rev* 63(8): 272–283.

Parida AK, Das AB, Mohanty P (2004) Defense potentials to NaCl in a mangrove, Bruguiera parviflora: Differential changes of isoforms of some antioxidative enzymes. *J Plant Physiol* 161(5): 531–542.

Pichyangkura R, Chadchawan S (2015) Biostimulant activity of chitosan in horticulture. *Sci Hortic* 196: 49–65.

Przybysz A, Gawrońska H, Gajc-Wolska J (2014) Biological mode of action of a nitrophenolates-based biostimulant: Case study. *Front Plant Sci* 5: 713.

Safikhan S, Khoshbakht K, Chaichi MR, Amini A, Motesharezadeh B (2018) Role of chitosan on the growth, physiological parameters and enzymatic activity of milk thistle (*Silybum marianum* (L.) Gaertn.) in a pot experiment. *J Appl Res Med Aromat Plants* 10: 49–58.

Salah AM, Osman M, Mohamed A, Mahgoub H, Garada M, Abdelmouty E, Hamed A, Abdel Latef A (2021) Impact of foliar application of chitosan dissolved in different organic acids on isozymes, protein patterns and physio-biochemical characteristics of tomato grown under salinity stress. *Plants 10*: 388.

Sauer DB (1986) Disinfection of seed surfaces with sodium hypochlorite. *Phytopathology* 76(7): 745.

Simaei M, Khavari-Nejad R, Bernard F (2012) Exogenous application of salicylic acid and nitric oxide on the ionic contents and enzymatic activities in NaCl-stressed soybean plants. *Am J Plant Sci* 03: 1495–1503.

Tian H, Guo G, Fu X, Yao Y, Yuan L, Xiang A (2018) Fabrication, properties and applications of soy-protein-based materials: A review. *Int J Biol Macromol* 120: 475–490.

Uçarlı C (2020) *Effects of salinity on seed germination and early seedling stage*. In Fahad S, Saud S, Chen Y, Wu C, Wang D, eds. *Abiotic Stress in Plants*. *IntechOpen*: 211-232.

Yang R, Jiang Y, Xiu L, Huang J (2019) Effect of chitosan pre-soaking on the growth and quality of yellow soybean sprouts. *J Sci Food Agric* 99(4): 1596–1603.

Yuvaraja G, Su M, Chen DY, Pang Y, Kong LJ, Subbaiah MV, Wen JC, Reddy GM (2020) Impregnation of magnetic-*Momordica charantia* leaf powder into chitosan for the removal of U(VI) from aqueous and polluted wastewater. *Int J Biol Macromol* 149: 127–139.

Zhang B, Chen P, Florez-Palacios SL, Shi A, Hou A, Ishibashi T (2010) Seed quality attributes of food-grade soybeans from the U.S. and Asian. *Euphytica* 173: 387–396.

Zohara F, Surovy M, Khatun A, Prince M, Akanda M, Islam H, Rahman M, Islam T, Hossain M (2019) Chitosan biostimulant controls infection of cucumber by *Phytophthora capsici* through suppression of asexual reproduction of the pathogen. *Acta Agrobot* 72(1): 1763.