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# The Effects of Oligochitosans on the Growth of *in vitro* Rice Seedlings under Salinity Stress

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## ABSTRACT

**Background:** Seawater intrusion causes many unfavourable effects on crops, especially rice, which is very sensitive to salt. Salinity stress reduces the growth and development of rice, thereby reducing the yield. This is an important issue affecting the food security of many countries in the world. This study focused on using oligochitosans to increase salinity tolerance and improve rice growth at the seedling stage.

**Methods:** Dry and rice-sprouted seeds were treated with oligochitosan solutions of different molecular weights and concentrations and incubated in NaCl solution at a concentration of 0.6%. The salinity tolerance of rice seedlings was assessed through *in vitro* indices in the plants' shoot length, number of leaves, number of roots (apical and lateral roots) and root length, fresh and dry weight, photosynthetic pigments, proline, total sugar and total protein.

**Result:** Oligochitosan 5994 Da at a concentration of 75 ppm improved the growth of rice seedlings under salinity conditions. Oligochitosan 11126 Da at the concentration of 100 ppm improved the growth of rice seedlings under salinity stress in the investigation of dry seed treatment. The salinity tolerance of rice seedlings was observed through the increase of photosynthetic pigments, proline, and total sugar concentration and morphology.


**Key words:** Oligochitosans, Photosynthetic pigments, Proline, Rice seedlings, Salinity stress.

## INTRODUCTION

Salinity and drought seriously affect crop production in Vietnam- an agricultural country in Asia. Salinity stress causes adverse reactions in plants and inhibits nutrient and water absorption. This leads to an imbalance of metabolism in the cell and stimulates the production of ROS (reactive oxygen species), which is harmful to cells (Zhao *et al*., 2021). Plants tolerate NaCl by activating a series of adaptations, including morphological, physiological and biochemical changes. The accumulation of osmolytes to maintain water absorption, increase of endogenous abscisic acid (ABA) content, and changes in principle genetic expression in salt conditions are responses of plants to stress (Mirza and Masayuki, 2022).

As the rice is sensitive to salinity stress, especially at the seedling, early vegetative, and reproductive stages, its yield decreases with saltwater intrusion (Singh *et al*., 2009). To ensure sustainable agricultural production, many methods were suggested to overcome salinity; one of them is using exogenous factors to increase the resistance of plants to abiotic factors.

Chitosan is a natural compound that is widely used in agriculture. Chitosan is not harmful to plants and has a high biocompatibility with plants, which can activate the defence system in plants against abiotic stress. Application of chitosan under abiotic stress stimulates the production of many compounds that balance intracellular pressure (proline, glycine betaine, and simple sugars), reducing the effects of ROS by stimulating the production of antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and reduces MDA (malondialdehyde) accumulation. MDA is a product of lipid membrane peroxidation. The increase in MDA can cause cell membrane damage. Chitosan also activates the synthesis of jasmonic acid (JA) and abscisic acid. In particular, JA and ABA play an essential role in regulating the water in the cell to balance osmotic pressure. All of these signalling molecules contribute to the adaptive mechanisms of plants in response to abiotic stress (Peykani and Sepehr, 2019; Namphueng and Wattana, 2020).

Oligochitosans are smaller than chitosan in molecular weight, easily soluble in water and have been shown to affect the abiotic stress regulation in plants positively. Oligochitosans improved the growth of wheat, *Salvia abrotanoides* (Kar.) under drought stress (Faride *et al*., 2019; Attaran *et al*., 2022), improved growth, physiological and

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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). On the other hand, oligochitosans were used to enhance antioxidant activity and alkaloid biosynthesis in *Catharanthus roseus* under salinity stress (Hassan *et al*., 2021).

There have been studies on chitosan to increase the salt tolerance of rice and some other crops, but the studies using oligochitosans are very few. This study focused on the effects of oligochitosans on the growth of rice seedlings under salinity stress to look for new substances that can enhance the development of rice under adverse environmental conditions.

## MATERIALS AND METHODS

Rice (*Oryza sativa* L.) seeds from variety IR64 (supplied by Dien Bien Seed Joint Stock Company, Vietnam). Oligochitosans at several molecular weights 30143 Da (OC1), 11126 Da (OC2), 5994 Da (OC3), and 4592 Da (OC4) were provided by the Biotechnological Materials and Nanobiology laboratory of the Biotechnology Center of Ho Chi Minh City. Oligochitosans were made from 5% chitosan (573170 Da) (Funakoshi-Tokyo, Japan) at 80% of deacetylation by irradiation with gamma-rays of Co-60 at various doses (50, 100, 150 and 200 kGy).

### Seedling preparation and salinity stress assay

Rice seeds were soaked in distilled water for 24 hours after sterilising with 15% javel for 20 minutes. Subsequently, seeds were placed in Petri dishes with distilled water in the dark at 25±2°C for germination. After 96 hours, sprouted seeds with 2-3 mm of radicle were transferred to glass pots (20 seeds per pot) with 20 mL of NaCl solution of different concentrations (0 to 1%). Pots were placed in the growth chamber at 2500 lux of 12-hour photoperiod at 25±2°C.

### Oligochitosan sprouted seeds treatment

Twenty sprouted seeds with 2-3 mm of radicle were transferred to glass pots with 20 mL of 0.6% NaCl supplemented with oligochitosans of different molecular weights: 30143 Da; 11126 Da; 5994 Da and 4592 Da, respectively, at the concentrations of 25, 50, 75 and 100 ppm. After seven days, shoot length, root length and the number of leaves and roots were measured.

### Oligochitosan dry seeds treatment

Dry rice seeds were incubated in oligochitosan solutions of different molecular weights (30143 Da, 11126 Da, 5994 Da and 4592 Da, respectively) at 25, 50, 75 and 100 ppm in 24 hours. Rice seeds were then placed in Petri dishes with distilled water in the dark at 25 2°C for germination. After 96 hours, twenty sprouted seeds with 2-3 mm of radicle were transferred to glass pots with 20 mL of 0.6% NaCl. Seven days later, the length of shoots and roots and the number of leaves and roots were measured.

### Biomass determination

The fresh weight of the seedlings (shoots and roots) was determined by an analytical balance with an accuracy of 0.001 g. The seedlings were then placed in an oven at 80°C until the weight was constant. The dry weight of the seedlings was also determined by an analytical balance with an accuracy of 0.001 g.

### Photosynthetic pigments determination

Rice leaf photosynthetic pigments were extracted using ethanol (0.2 g of rice leaf fresh weight was incubated in 10 mL of 96% ethanol at room temperature for extraction). The extraction was measured in a spectrophotometer at 470, 649 and 665 nm, as described by Lichtenthaler (1987).

### Proline determination

Proline accumulation in the shoots and roots of rice seedlings was determined *via* reaction with ninhydrin (after the extraction with ethanol), as described by Carillo and Gibon (2011). The products were measured spectro photometrically at 520 nm. Standard proline (Merck) was used to build a standard curve for proline concentration quantification.

### Total protein determination

The Bradford method (1976) was used to determine the total protein concentration in rice seedlings' shoots and roots. The principle of this essay is that the binding of protein molecules (extracted by ethanol) to Coomassie dye under acidic conditions results in a colour change from brown to blue. The products were measured spectrophotometrically at 595 nm. Albumin (Sigma) was used to build a standard curve for protein concentration quantification.

### Total sugar (carbohydrates) determination

Carbohydrates in shoots and roots of rice seedlings (extracted with ethanol) reacted to generate furan derivatives in sulphuric acid and heat condensed with phenol to form stable yellow-gold compounds. The products were measured spectro photometrically at 490 nm. Standard D-glucose (Merck) was used to build a standard curve for carbohydrate concentration quantification (Dubois *et al*., 1956).

### Statistical analysis

The experiments 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

### Effects of NaCl on rice seedlings' growth

NaCl affected rice seedlings' development. In the treatments of 0.8 and 1% NaCl, some sprouted seeds could not develop.

Some seedlings had short, brown roots and pale leaves. In the treatment of 0.6% NaCl, the shoot length, root length, leaf number, and root number of rice seedlings were smaller than the control (distilled water) and 0.2 and 0.4% NaCl treatments (Table 1). 0.6% NaCl would be applicated in all following experiments to cause salinity stress because of the disadvantages on rice seedlings, such as chlorosis and dry leaves, white leaf tips and the roots being less branched (symptoms of plants under salinity stress).

Rice is more sensitive to salinity than other cereals, mainly at the seedling and reproduction stages. Salinity stress inhibits water and nutrient absorption from the roots, leading to an imbalance of metabolism in the cells and stimulating the production of reactive oxygen species, which are harmful to cells (Jing *et al*., 2019). The plants stop growing in case of prolonged salinity stress and even die.

**Effects of oligochitosans on the growth of rice seedlings in the treatment of sprouted seeds**

In the treatment with 0.6% NaCl, shoot length, the number of leaves, the number of roots and root length were decreased in comparison to the control (water). With the supplement of oligochitosans, the growth of seedlings was improved (Table 2). Under salinity stress, the treatments of sprouted seeds with oligochitosans at different molecular weights and concentrations were ineffective on root elongation. However, the fragments of oligochitosan at molecular weight 30143, 11126 and 5994 Da increased the root numbers at 75 and 100 ppm concentrations. Oligochitosans positively affected the shoot length and the number of leaves. The increase in leaf number was realised with an oligochitosan fragment of 30143 Da, while oligochitosan of 11126 Da elongated the shoots' length at 50, 75 and 100 ppm. However, the rice leaves were thin and pale. The oligochitosan fragment of 5994 Da at the concentration of 75 ppm positively affected the shoot length, and the leaves were dark green at the same time.

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were analysed in rice seedlings under salinity stress in the presence of oligochitosans. All four oligochitosan fragments effectively induced the biosynthesis of chlorophyll a but not chlorophyll b and carotenoids. The concentration of chlorophyll a in salinity stress in the presence of 5994 Da oligochitosan fragment was at par with

**Table 1:** The effects of NaCl concentration on the growth of rice seedlings after 7 days of treatment.

| Treatments | Shoot length (mm) | Number of leaves | Number of roots | Root length (mm) |
|---|---|---|---|---|
| Distilled water | $58.27^{e}$ | $1.93^{c}$ | $4.37^{d}$ | $21.17^{c}$ |
| NaCl 0.2% | $57.73^{de}$ | $1.87^{c}$ | $4.13^{cd}$ | $18.6^{c}$ |
| NaCl 0.4% | $54.57^{cd}$ | $1.93^{c}$ | $3.90^{cd}$ | $16.63^{bc}$ |
| NaCl 0.6% | $53.27^{c}$ | $1.73^{bc}$ | $3.40^{bc}$ | $11.37^{ab}$ |
| NaCl 0.8% | $46.40^{b}$ | $1.50^{ab}$ | $2.70^{ab}$ | $10.17^{a}$ |
| NaCl 1.0% | $42.20^{a}$ | $1.37^{a}$ | $2.47^{a}$ | $9.07^{a}$ |

Values with different letters in each column indicate a significant difference at the 5% level.

**Table 2:** The growth of rice seedlings in salinity stress at seven days of oligochitosan treatment on sprouted seeds.

| Treatments | Shoot length (mm) | Number of leaves | Number of roots | Root length (mm) |
|---|---|---|---|---|
| $H_2O$ | $51.08^{e}$ | $1.22^{abc}$ | $7.78^{g}$ | $35.24^{e}$ |
| 0.6% NaCl | $40.66^{a}$ | $1.07^{ab}$ | $4.65^{bc}$ | $23.05^{abcd}$ |
| 0.6% NaCl+25 ppm oligochitosan 30143 Da | $44.39^{abcd}$ | $1.37^{cd}$ | $5.25^{bcde}$ | $23.30^{abcd}$ |
| 0.6% NaCl+50 ppm oligochitosan 30143 Da | $42.94^{abc}$ | $1.47^{de}$ | $5.37^{bcdef}$ | $23.43^{abcd}$ |
| 0.6% NaCl+75 ppm oligochitosan 30143 Da | $44.63^{abcd}$ | $1.60^{e}$ | $6.03^{ef}$ | $26.72^{d}$ |
| 0.6% NaCl+100 ppm oligochitosan 30143 Da | $42.33^{abc}$ | $1.50^{de}$ | $4.77^{bc}$ | $26.20^{d}$ |
| 0.6% NaCl+25 ppm oligochitosan 11126 Da | $41.89^{ab}$ | $1.22^{abc}$ | $5.20^{bcde}$ | $25.44^{bcd}$ |
| 0.6% NaCl+50 ppm oligochitosan 11126 Da | $47.54^{de}$ | $1.08^{ab}$ | $5.60^{cdef}$ | $24.02^{abcd}$ |
| 0.6% NaCl+75 ppm oligochitosan 11126 Da | $47.35^{de}$ | $1.20^{abc}$ | $4.78^{bc}$ | $23.82^{abcd}$ |
| 0.6% NaCl+100 ppm oligochitosan 11126 Da | $45.63^{bcd}$ | $1.27^{bc}$ | $5.82^{def}$ | $23.01^{abcd}$ |
| 0.6% NaCl+25 ppm oligochitosan 5994 Da | $43.11^{abc}$ | $1.08^{ab}$ | $4.55^{b}$ | $20.77^{a}$ |
| 0.6% NaCl+50 ppm oligochitosan 5994 Da | $43.01^{abc}$ | $1.07^{ab}$ | $5.02^{bcd}$ | $21.69^{abc}$ |
| 0.6% NaCl+75 ppm oligochitosan 5994 Da | $46.24^{cd}$ | $1.27^{bc}$ | $6.22^{f}$ | $26.49^{d}$ |
| 0.6% NaCl+100 ppm oligochitosan 5994 Da | $41.57^{ab}$ | $1.08^{ab}$ | $3.58^{a}$ | $21.16^{ab}$ |
| 0.6% NaCl+25 ppm oligochitosan 4592 Da | $43.76^{abcd}$ | $1.08^{ab}$ | $5.12^{bcde}$ | $21.78^{abc}$ |
| 0.6% NaCl+50 ppm oligochitosan 4592 Da | $44.80^{abcd}$ | $1.05^{a}$ | $5.45^{bcdef}$ | $23.62^{abcd}$ |
| 0.6% NaCl+75 ppm oligochitosan 4592 Da | $42.10^{abc}$ | $1.10^{ab}$ | $5.60^{cdef}$ | $21.29^{abc}$ |
| 0.6% NaCl+100 ppm oligochitosan 4592 Da | $45.63^{bcd}$ | $1.07^{ab}$ | $4.88^{bcd}$ | $25.54^{cd}$ |

Values with different letters in each column indicate a significant difference at the 5% level.

chlorophyll a in control (Fig 1). The positive effects of oligochitosans on chlorophyll biosynthesis were demonstrated in *Catharanthus roseus* under salinity stress (Hassan *et al*., 2021). Oligochitosans retarded the reduction of chlorophyll contents and induced the activities of antioxidant enzymes such as catalase, ascorbate peroxidase and glutathione reductase in *Catharanthus roseus* in the application as a foliar spray at 1%.

**Effects of oligochitosan 5994 Da on rice seedlings in the treatments of sprouted seeds**

Under salinity stress, the fresh weight of shoots decreased (19.95 mg/plant) compared with the control (25.81 mg/plant). However, the treatment with oligochitosan 5994 Da could maintain the growth of rice seedlings, as evidenced by the fresh weight of shoots being similar to the control (23.51 mg/plant). On the other side, the dry weight of the shoots was identical to each other in all treatments. Like shoots, the fresh weight of roots under salinity stress was 28.95 mg/plant, lower than in control (34.27 mg/plant), but oligochitosans maintained and the value was at par with control (the fresh weight of roots was 33.01 mg/plant). The dry weight of roots remained the same in all treatments (Table 3). The growth of rice seedlings depended on the grain reserves (no nutrients were supplied throughout the experiments). This probably was why the seedlings could not increase their biomass in shoots and roots.

Under salinity stress, there were no fluctuations in proline content (1.16 nmol/g FW) and total protein content (1.47 mg/g FW) in the shoots of rice seedlings in comparison with the control (0.93 nmol/g FW of proline and 1.25 mg/g FW of total protein) (the difference were not statistically significant). The treatment of sprouted seeds with oligochitosan 5994 Da did not affect the total protein content of the shoots (1.24 nmol/g FW) but reduced proline concentration (0.77 nmol/g FW). Proline and protein contents were not detected in roots. The carbohydrate contents in shoots increased under stress and in the treatment with oligochitosan (13.55 mg/g FW and 14.31 mg/g FW respectively, but not in the roots (Table 4). The concentrations of proline and soluble sugar are enhanced in plant cells when affected by salinity stress to maintain membrane permeability, regulate osmotic pressure in cells, reduce free radicals, and help plant cells resist stress (Mirza and Masayuki, 2022). The content of these substances gradually decreases when the plants return to a steady state (Mosavikia *et al*., 2020). In this experiment, it seemed carbohydrates had a supporting role in rice seedlings under salinity stress that the proline did not. The proline content in the treatment of oligochitosan decreased, perhaps corresponding to a reduction in the number of harmful free radicals in the cell under the osmotic stabilising effect of carbohydrates. In salinity stress, the rice seedlings looked better in the treatments with oligochitosan (Fig 2).

**Effects of oligochitosans on the growth of rice seedlings in the dry seeds treatments**

The treatment of rice dry seeds with oligochitosans before incubating for germination improved the growth of seedlings under salinity stress. The fragment of oligochitosan 30143 Da stimulated root elongation at concentrations of 50, 75 and 100 ppm but did not increase the root numbers of rice seedlings. The oligochitosan 11126 Da fragment induced root elongation at all concentrations while increasing the length of shoots at the concentrations of 25 and 100 ppm. The oligochitosan 5994 Da fragment increased leaf numbers at concentrations of 75 and 100 ppm, the number of roots at 25 ppm and root elongation at concentrations of 75 and 100 ppm but did not increase the length of shoots. The oligochitosan 4592 Da fragment increased root elongation

**Table 3:** Effects of oligochitosan 5994 Da on the biomass of rice seedlings in salinity stress in the treatment of sprouted seeds.

| Treatments | Biomass (mg/plant) | | | |
|---|---|---|---|---|
| | Fresh weight | | Dry weight | |
| | Shoots | Roots | Shoots | Roots |
| $H_2O$ | 25.81$^{b}$ | 34.27$^{b}$ | 5.98$^{a}$ | 6.74$^{a}$ |
| 0.6% NaCl | 19.95$^{a}$ | 28.95$^{a}$ | 5.00$^{a}$ | 5.72$^{ab}$ |
| 0.6% NaCl and oligochitosan 9554 Da | 23.51$^{b}$ | 33.01$^{b}$ | 5.84$^{a}$ | 5.86$^{a}$ |

Values with different letters in each column indicate a significant difference at the 5% level.

**Table 4:** Effects of oligochitosan 5994 Da on the concentration of proline, protein and carbohydrates of rice seedlings under salinity stress in the treatments of sprouted seeds.

| Treatments | Proline (nmol/g FW) | | Protein (mg/g FW) | | Carbohydrates (mg/g FW) | |
|---|---|---|---|---|---|---|
| | Shoots | Roots | Shoots | Roots | Shoots | Roots |
| $H_2O$ | 0.93$^{ab}$ | Not detected | 1.25$^{a}$ | Not detected | 7.30$^{a}$ | 14.05$^{a}$ |
| 0.6% NaCl | 1.16$^{b}$ | Not detected | 1.47$^{a}$ | Not detected | 13.55$^{b}$ | 10.87$^{a}$ |
| 0.6% NaCl+75 ppm oligochitosan 5994 Da | 0.77$^{a}$ | Not detected | 1.24$^{a}$ | Not detected | 14.31$^{b}$ | 9.70$^{a}$ |

Values with different letters in each column indicate a significant difference at the 5% level.

at a concentration of 100 ppm and increased leaf numbers at a concentration of 25 ppm (Table 5). In the research on seed priming by Garude *et al*. (2019), rice seed priming with chitosan dissolved in 0.5% acetic acid enhanced the shoot length and root length more than the control in salinity stress (Garude *et al*. 2019). Chitosan can activate the defence system in plants against abiotic stress such as stimulating the production of many compounds that balance intracellular pressure, reducing the effects of ROS and enhancing the synthesis of jasmonic acid and abscisic acid to regulate the water in the cell to balance osmotic pressure. As the result, the treatment of chitosan on seeds increased the vigour and the growth of seedlings.

The treatment of rice dry seeds with the fragment of oligochitosans 30143 Da and 11126 Da before incubating for germination improved the biosynthesis of photosynthetic pigments of seedlings in salinity stress. In the treatment with oligochitosan 30143 Da at the concentration of 75 ppm, the contents of chlorophyll a and carotenoids were higher than in the salt treatment and even higher than in the control. The fragment of oligochitosan 11126 Da induced the biosynthesis of chlorophyll a and b but not with carotenoids (Fig 3).

**Effects of oligochitosan 11126 Da on rice seedlings in the treatments of dry seeds**

The fresh weight of the shoots and roots of rice seedlings was reduced under salinity stress. The treatment with oligochitosan 11124 Da did not improve the fresh weight of shoots and roots, but increased the dry weight of shoots and did not affect the dry weight of the roots (Table 6). There was some research on the use of chitosan in seed treatment to increase the germinated ratio and the growth of seedlings. In *Plantago ovata* Forsk, chitosan (0.2%) induced shoot and root lengths, as well as root dry mass under salt stress conditions (Mahdavi, 2013). In another research, salinity stress caused a significant reduction in germination percentage, shoot length, root length, shoot and root dry weight and relative water content of *Carum copticum* seedlings and 0.2% chitosan adjusted the salt toxicity (by increasing germination percentage, germination rate, seedling vigour index, length and dry weight of hypocotyls and radicles) (Mahdavi and Asgha, 2013).

**Table 5:** The growth of rice seedlings in salinity stress at 7 days of oligochitosan treatments on dry seeds.

| Treatments | Shoot length (mm) | Number of leaves | Number of roots | Root length (mm) |
|---|---|---|---|---|
| $H_2O$ | $44.02^{cd}$ | $1.30^{cdef}$ | $6.11^{bc}$ | $18.51^{e}$ |
| 0.6% NaCl | $37.57^{abc}$ | $1.16^{abcd}$ | $6.25^{bc}$ | $13.88^{a}$ |
| 0.6% NaCl+25 ppm oligochitosan 30143 Da | $37.71^{abc}$ | $1.20^{abcd}$ | $6.25^{bc}$ | $15.73^{acd}$ |
| 0.6% NaCl+50 ppm oligochitosan 30143 Da | $33.23^{a}$ | $1.12^{abc}$ | $5.62^{ab}$ | $16.30^{cd}$ |
| 0.6% NaCl+75 ppm oligochitosan 30143 Da | $40.80^{bcd}$ | $1.12^{abc}$ | $5.87^{ab}$ | $15.78^{bcd}$ |
| 0.6% NaCl+100 ppm oligochitosan 30143 Da | $37.08^{ab}$ | $1.05^{ab}$ | $5.60^{ab}$ | $16.58^{cd}$ |
| 0.6% NaCl+25 ppm oligochitosan 11126 Da | $44.50^{d}$ | $1.02^{a}$ | $5.35^{a}$ | $15.91^{bcd}$ |
| 0.6% NaCl+50 ppm oligochitosan 11126 Da | $40.94^{bcd}$ | $1.03^{a}$ | $5.78^{ab}$ | $16.28^{cd}$ |
| 0.6% NaCl+75 ppm oligochitosan 11126 Da | $40.30^{bcd}$ | $1.10^{abc}$ | $6.13^{bc}$ | $16.17^{cd}$ |
| 0.6% NaCl+100 ppm oligochitosan 11126 Da | $45.04^{d}$ | $1.15^{abcd}$ | $5.35^{a}$ | $17.21^{de}$ |
| 0.6% NaCl+25 ppm oligochitosan 5994 Da | $41.22^{bcd}$ | $1.22^{abcde}$ | $6.60^{c}$ | $13.63^{a}$ |
| 0.6% NaCl+50 ppm oligochitosan 5994 Da | $40.99^{bcd}$ | $1.35^{def}$ | $6.15^{bc}$ | $15.76^{bcd}$ |
| 0.6% NaCl+75 ppm oligochitosan 5994 Da | $41.93^{bcd}$ | $1.40^{ef}$ | $6.27^{bc}$ | $16.23^{cd}$ |
| 0.6% NaCl+100 ppm oligochitosan 5994 Da | $41.82^{bcd}$ | $1.43^{f}$ | $6.10^{bc}$ | $14.16^{ab}$ |
| 0.6% NaCl+25 ppm oligochitosan 4592 Da | $40.72^{bcd}$ | $1.62^{f}$ | $6.08^{bc}$ | $15.24^{abc}$ |
| 0.6% NaCl+50 ppm oligochitosan 4592 Da | $36.32^{ab}$ | $1.10^{abc}$ | $5.72^{ab}$ | $14.26^{ab}$ |
| 0.6% NaCl+75 ppm oligochitosan 4592 Da | $39.69^{bcd}$ | $1.25^{bcdef}$ | $6.08^{bc}$ | $15.09^{abc}$ |
| 0.6% NaCl+100 ppm oligochitosan 4592 Da | $41.31^{bcd}$ | $1.33^{def}$ | $6.13^{bc}$ | $15.74^{bcd}$ |

Values with different letters in each column indicate a significant difference at the 5% level.

**Table 6:** Effects of oligochitosan 11126 Da on the biomass of rice seedlings under salinity stress in the treatments of dry seeds.

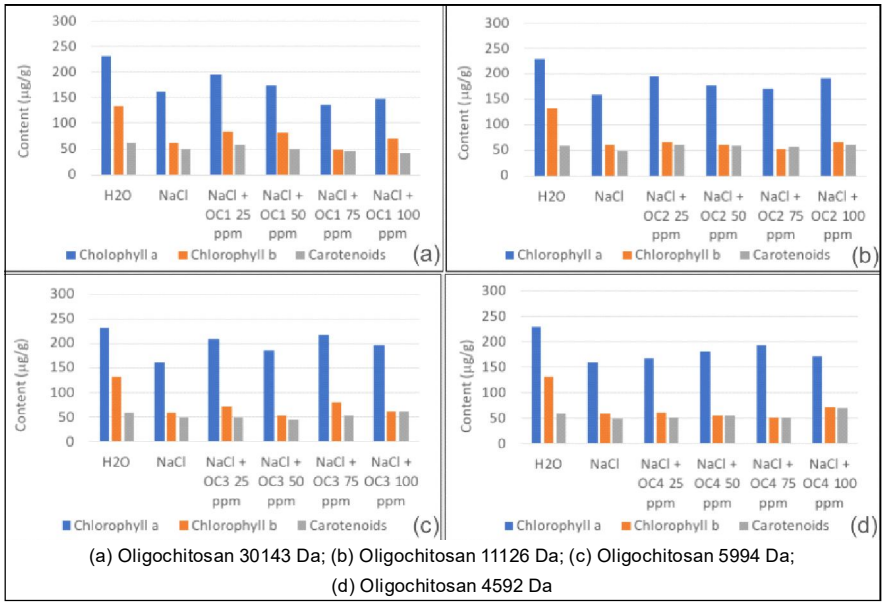
| Treatments | Biomass (mg/plant) | | | |
|---|---|---|---|---|
| | Fresh weight | | Dry weight | |
| | Shoots | Roots | Shoots | Roots |
| $H_2O$ | $23.81^{b}$ | $28.61^{b}$ | $4.67^{b}$ | $3.41^{a}$ |
| 0.6% NaCl | $18.95^{a}$ | $16.62^{a}$ | $3.98^{a}$ | $3.38^{a}$ |
| 0.6% NaCl+100 ppm oligochitosan 11126 Da | $18.56^{a}$ | $17.01^{a}$ | $4.84^{b}$ | $3.20^{a}$ |

Values with different letters in each column indicate a significant difference at the 5% level.

**Table 7:** Effects of oligochitosan 11126 Da on the concentrations of the proline, protein and carbohydrates of rice seedlings under salinity stress in the treatments of dry seeds.

| Treatments | Proline (nmol/g FW) | | Total protein (mg/g FW) | | Carbohydrates (mg/g FW) | |
|---|---|---|---|---|---|---|
| | Shoots | Roots | Shoots | Roots | Shoots | Roots |
| $H_2O$ | 0.70[a] | Not detected | 6.12[a] | 2.66[a] | 11.08[a] | 4.71[a] |
| 0.6% NaCl | 5.08[b] | Not detected | 7.83[b] | 3.16[ab] | 16.01[ab] | 15.67[b] |
| 0.6% NaCl+100 ppm oligochitosan 11126 Da | 3.08[b] | Not detected | 7.55[ab] | 3.93[b] | 17.26[b] | 17.86[b] |

Values with different letters in each column indicate a significant difference at the 5% level.



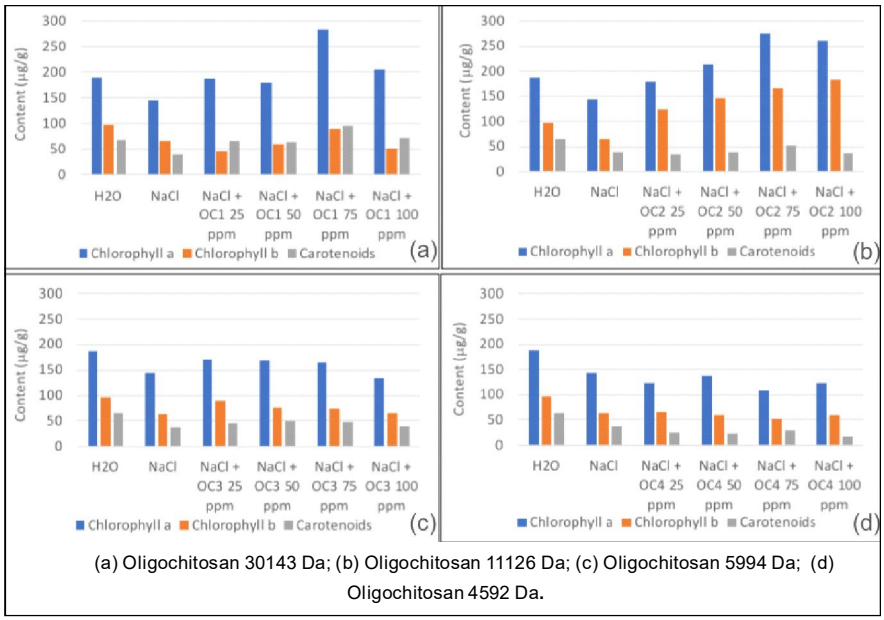

(a) Oligochitosan 30143 Da; (b) Oligochitosan 11126 Da; (c) Oligochitosan 5994 Da; (d) Oligochitosan 4592 Da

**Fig 1:** The photosynthetic pigments concentration of rice seedlings under salinity stress in the treatments of oligochitosans on germinated seeds.




(a) $H_2O$; (b) 0.6% NaCl; (c) 0.6% NaCl and oligochitosan 5994 Da.

**Fig 2:** Rice seedlings in different treatments.




(a) Oligochitosan 30143 Da; (b) Oligochitosan 11126 Da; (c) Oligochitosan 5994 Da; (d) Oligochitosan 4592 Da.

**Fig 3:** The photosynthetic pigment concentrations of rice seedlings under salinity stress in the treatments of oligochitosan 11126 Da on dry seeds.

The concentration of proline in the shoots increased in the treatment of NaCl and NaCl with oligochitosan 11126 Da (5.08 nmol/g FW and 3.08 nmol/g FW, respectively) while the content of proline was lower in the control (0.7 nmol/g FW). The proline in roots could not be detected. The total protein contents in shoots increased under salinity stress and were maintained in the treatment with oligochitosan 11126 Da (Table 7). In the roots, the total protein content increased when the seedlings were treated with oligochitosan. Carbohydrate contents in roots increased under stress and in oligochitosan treatment, while in the shoots, the carbohydrate content increased in the treatment with oligochitosan. The increase in proline and total carbohydrate concentration might maintain seedlings' water absorption, which supports the biosynthesis of chlorophyll a and b for photosynthesis.

## CONCLUSION

Oligochitosans could protect rice seedlings from salinity stress. The oligochitosan molecular weight of 5994 Da was suitable for treating germinated seeds, and the oligochitosan molecular weight of 11126 Da was ideal for treating dry seeds. Oligochitosans induced the total carbohydrates in shoots and maintained the biosynthesis of chlorophylls and the prolines in nodes.

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**Conflict of interest:** None.

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