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Research Article

Enabling Seawater Farming of Cabbage Using Indole-3-Acetic Acid Producing Bacteria as a Fungicide Alternative

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Abstract
Fungicides are crucial for enhancing agricultural production to meet global food demands. Seawater farming offers a sustainable alternative by utilizing seawater to control fungal growth, potentially reducing reliance on chemical fungicides and preventing water pollution. However, salt stress limits its application by compromising yields. We explored optimal conditions for crop growth by treating plants with *Rhodobacter capsulatus*, which produces indole-3-acetic acid (IAA) to mitigate plant stress, in seawater farming. We cultured *R. capsulatus* under 1% w/v NaCl and sea salt to assess IAA production and salt tolerance. Cabbage plants were treated with varying concentrations of *R. capsulatus*, NaCl, sea salt, and IAA, and growth metrics such as root and stem length, chlorophyll content, and fungal inhibition were measured. Our findings showed that 1% w/v of NaCl and sea salt supported *R. capsulatus* survival and IAA production, reducing salt stress and enhancing cabbage growth. Compared with *Fusarium graminearum* treatment, NaCl and sea salt solutions, combined with *R. capsulatus*, considerably enhanced cabbage growth. *R. capsulatus*'s IAA effectively reduced salt stress in cabbage, and 1% w/v sea salt served as a natural fungicide while promoting growth. This approach provides a sustainable solution to enhance food production and reduce environmental impacts.
Keywords: Seawater Farming, *Rhodobacter capsulatus*, Salt Stress, Indole-3-Acetic Acid (IAA), Sustainable Agriculture.

1. Introduction

Over three billion kilograms of pesticides are utilized in agriculture annually [1]. By controlling weeds, fungi, and insects, pesticides boost crop yields and support the global food supply [2]. Without pesticides, fruit production would decrease by 78%, vegetable production by 54%, and cereal production by 32% [3]. While only 1% of pesticides effectively reach their target pests [4], the remaining 99% contribute to environmental pollution, affect non-target plants, or undergo transfer and degradation [5, 6].

Pesticides can move from their target sites to other parts of the environment through adsorption, leaching, and volatilization [7]. In soil, pesticides may bind to particles, where they can persist, be absorbed by plants, cause crop damage, and leave residues [8–12]. Some pesticides also leach into groundwater, leading to water pollution [13]. Additionally, pesticide degradation can produce harmful chemicals, such as chlorpyrifos and its endocrine-disrupting byproducts, which are frequently detected in soils, sediments, and groundwater [14, 15].

One alternative to pesticide use is seawater farming, which uses seawater for irrigation either on land or over sea surfaces [16]. This method can improve poor soil conditions, supply essential minerals, and enhance crop, flower, and fruit production [17]. Seawater also reduces fungal activity. For instance, a farmer in Daegu, South Korea, used diluted seawater (20%) on 10 acres, considerably reducing powdery mildew [18]. Another farmer found seawater more effective than commercial disinfectants for downy mildew control [18]. Using seawater four times a year on 400 hectares could save approximately 160 million won (approximately $120,000 USD) annually, improve crop quality, and suppress young weeds [18].

Despite its benefits, seawater farming faces challenges due to salt stress, which can limit its applicability. Excessive sodium in the soil can cause toxicity, reduce nutrient uptake, and disrupt plant metabolism [19,

20], ultimately inhibiting crop growth [21]. To mitigate salt stress in seawater farming, supplementing crops with indole-3-acetic acid (IAA) or auxin can counteract growth reduction. Auxin, a crucial plant hormone, enhances tolerance to salt stress by regulating root growth and stress response [22, 23]. However, under salt stress, auxin levels decrease, hindering growth due to the tight control of its regulatory mechanisms by receptor gene expression [24–26].

Auxin biosynthesis involves the IAA pathway, including enzymes such as tryptophan aminotransferase and the Yucca family, which are crucial for auxin production in roots [27, 28]. Salt stress shifts auxin production from columella cells to the root epidermis, reducing primary and lateral root growth [29]. Despite salt stress's negative impact on auxin function, auxin remains critical for salt tolerance. The increased expression of YUC genes in response to salt stress highlights auxin's role in mediating stress responses. Additionally, GH3 enzymes regulate auxin homeostasis through negative feedback, generating auxin conjugates such as ILR and IAR to maintain balance under stress [30]. Thus, supplementing crops with auxin can help counteract the effects of salinity, maintaining growth and productivity while benefiting from the reduced fungicide use from seawater farming.

One potential method to supplement IAA is through photosynthetic bacteria that secrete auxin. These bacteria, commonly found in freshwater environments, are utilized in agriculture for promoting root and stem development, as well as increasing flower and fruit production [31]. Studies have shown that crops treated with photosynthetic bacteria exhibited a 7.2% increase in plant height, a 22.4% increase in female flowers, and a 25.7% boost in overall yield, compared with untreated crops [32].

In this study, we focused on *Rhodobacter capsulatus*, a photosynthetic bacterium known for producing IAA and promoting plant growth [33]. *R. capsulatus* has demonstrated high IAA productivity, which enhances plant growth and chlorophyll density [33]. This suggests that photosynthetic bacteria could help to mitigate the challenges of seawater farming by promoting crop growth. Additionally, these bacteria are also being explored for their potential as fertilizers and water quality enhancers [32]. However, whether these bacteria can survive in seawater remains unknown. While some photosynthetic bacteria such as *Rhodobacter sphaeroides* require sodium for growth and can adapt to NaCl concentrations up to approximately 0.4 M, higher levels can inhibit growth [34].

We aimed to cultivate *R. capsulatus* in seawater concentrations typically used for fungal control and applied this solution to cabbage (*Brassica campestris* L. ssp. *pekinensis*) to evaluate its ability to mitigate salinity stress and fungal infections. We hypothesize that IAA-producing bacteria will improve their tolerance to salt stress. We also explored whether the combination of salts and bacteria could serve as a natural pesticide, promoting plant health under environmental stresses.

The experimental design involved cultivating *R. capsulatus* under saline conditions and comparing crop growth under 1% NaCl and 1% sea salt, both alone and in combination with *R. capsulatus*, to determine the optimal conditions. We also evaluated the effects of these treatments on fungal growth and their agriculture applications, particularly for cabbage.

2. Materials and Methods

When using seawater for cultivation, factors such as crop type, disease control, pest management, and weed control are crucial. For cabbages, the seawater dilution ratio ranges from 1:10 to 1:100 to minimize damage, as lower ratios can cause harm [35]. We used a 1% w/v concentration of NaCl and sea salt. NaCl represents ocean water, while sea salt contains additional minerals. *R. capsulatus* can tolerate up to 0.4 M NaCl (equivalent to 2.35% w/v NaCl) [34]. The Rural Development Administration found that seawater diluted to 20% w/v was effective against powdery mildew, making 1% w/v a suitable starting point for experiments.

2.1. Cultivation and Growth of *R. capsulatus* in Saline Conditions

R. capsulatus (KACC 15928) was activated on nutrient agar for confirmation and cultured in nutrient broth with 1% w/v NaCl or sea salt. Growth was measured by absorbance at 600 nm after 1 d [36].

2.2. IAA Production by *R. capsulatus*

To test IAA production, *R. capsulatus* was treated with 5 mM L-Tryptophan, increasing IAA concentration fivefold [37]. L-Tryptophan was dissolved in methanol and formic acid to create a 0.005 M solution. Varying volumes (150, 200, 250 μL) were added to *R. capsulatus*, and after culturing for 1 d, the supernatant was

mixed with Salkowski reagent 1 (composed of 150 mL concentrated H_2SO_4, 250 mL distilled water, and 7.5 mL 0.5 M $FeCl_3 \cdot 6H_2O$). The resulting color intensity was measured at 530 nm.

If results were inconclusive, *R. capsulatus* was inoculated in tryptophan broth without formic acid. We also assessed IAA production in a media containing 1% w/v NaCl and 1% w/v sea salt. The Salkowski reagent method was used for IAA detection.

Salkowski reagents were validated by testing known IAA concentrations (e.g., 10 and 100 ppm IAA solutions), which turned pink, with higher concentrations showing more intense color, confirming the reagent's effectiveness (Figure 1).

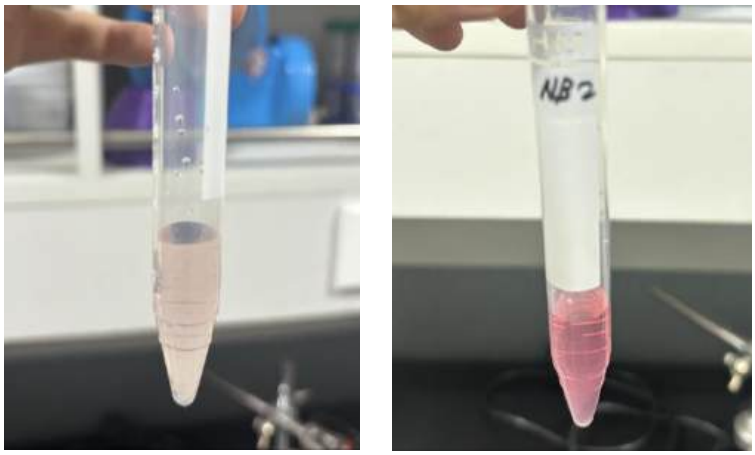

Figure 1. Color change in the Salkowski reagent reacted with 10 and 100 ppm of IAA.

Salkowski reagent 1, used in the first trial to assess IAA production, reacted vigorously with sulfuric acid and showed poor sensitivity to low IAA concentrations. Consequently, we switched to Salkowski reagent 2, which combines 1 mL of 0.1 M $FeCl_2$ with 50 mL of 35 % $HClO_4$. This reagent was mixed with the bacterial supernatant in a 2:1 ratio and left in the dark for 30 min before absorbance was measured [38]. With Salkowski reagent 2, color development was rapid and correlated well with the amount of tryptophan added.

2.3. Inhibition of *Fusarium graminearum* by Sea Salt, and NaCl

Pathogen *F. graminearum* (KACC 46437) was prepared, and 1% w/v solutions of NaCl and sea salt were applied in 200 µL volumes on PDA medium. *F. graminearum* was inoculated in the center of the medium, and fungal spread was measured using ImageJ software after 10 d.

2.4. Cabbage Growth with 1% w/v NaCl, 1% w/v Sea Salt, *R. capsulatus*, and IAA

A 1.5% agar solution (CAS No. 9002-18-0) was microwaved, and 2 mL was added to each well of a 24-well plate (Figure 2). Solutions of 1% w/v sea salt and NaCl were prepared, and 20 µL of each solution was added to the wells.

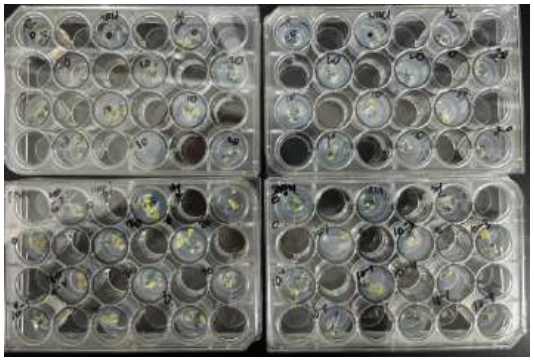

Figure 2. 24-well plate setup.

IAA solutions of 10^{-4}, 10^{-3}, and 10^{-1} ppm were prepared and added to two rows of the plate. We added 2.0 absorbance units (AU) *R. capsulatus* solutions of 0, 10, 20, and 30 µL to the other two rows. Cabbage growth was compared between the IAA and *R. capsulatus* treatments. Subsequently, 2.0 AU *R. capsulatus* was diluted

in ratios of 1/100, 1/50, 1/25 and 1/2, with 20 µL of each dilution added to the wells to observe cabbage growth depending on varying amounts of bacteria. After adding the solutions, five cabbage seeds were placed in each agar well and treated with different NaCl (1% w/v), sea salt (1% w/v), *R. capsulatus*, and IAA. After one week, cabbages were removed from the agar, and the stem and root lengths were measured. Chlorophyll was extracted from the leaves using 1.5 mL of 99% ethanol, and absorbance at 663 nm and 645 nm was measured to assess salt stress effects [39]. Solutions were sprayed 3 d after seed germination, following seawater farming practices. A vertical plate filled with 1.5% w/v agar and seeds was used to observe root growth.

2.5. Cabbage Growth When Treated with Combinations of *R. capsulatus*, Sea Salt, NaCl, and *F. graminearum* Solution

The same 24-well plate method was employed to evaluate cabbage growth when treated with combinations of *R. capsulatus* and either NaCl or sea salt (1% w/v). *R. capsulatus* was added post-germination to assess its effect on seed development under salt conditions. Additionally, a square plate method was employed to examine cabbage growth under combinations of *R. capsulatus,* sea salt, NaCl, and *F. graminearum.* The bacterial extract was applied to test the effects of IAA alone. *R. capsulatus* supernatant was collected after centrifugation (2.0 AU), filtered through a 0.2-µm syringe filter, and sprayed 3 d after seed germination. For all square plate methods, root and stem lengths were measured using ImageJ software, and chlorophyll absorbance was determined as previously described.

2.6. Impact of IAA Production by *R. capsulatus* on Salt Stress-related Gene Expression

We investigated whether IAA produced by *R. capsulatus* influences the expression of salt stress-related genes. For PCR primers, Bactin and the salt stress-related gene BrZHD10 were synthesized by Macrogen at 10 pmol (Table 1) [40]. After treating the plants with 2% w/v NaCl and 2% w/v sea salt, cabbage leaves were collected after 4 d and placed in microtubes for RNA extraction. RNA was extracted using the AccuPrep® Universal RNA Extraction Kit (Bioneer), following the kit's manual. After RNA extraction, 1 µL of primer and 5 µL of One Step RT-PCR Kit (Enzynomics) were added to the template (Total RNA), which was then reconstituted to a total volume of 20 µL using distilled water. This process was conducted on an ice pack to minimize RNA degradation. PCR was then performed using an MJ Mini 48-Well Personal Thermal Cycler (Bio-Rad) under the following conditions (Table 2). An electrophoresis unit (Genius Skbio) was operated at 100 W for 30 min for gel loading. Subsequently, the bands were visualized with a UV visualizer, and the gel density was analyzed using ImageJ. The band values were represented as a percentage of the total intensity.

Table 1. Bactin and BrZHD10 and corresponding forward and reverse primers.

Gene	Forward primer (F)	Reverse primer (R)
Batin	5′-TGGCATCACACTTTTCTACAA-3′	5-CAACGGAATCTCTCAGCTCC-3′
BrZHD10	5′-ATGGATATGGCGACTCATACAACAA-3′	5′-TCACGACGACGACGACCCGTTTACG-3′

Table 2. PCR amplification process.

Process	Condition
Reverse transcription	50℃ for 30 min
Initial denaturation	95℃ for 30 min
Denaturation	95℃ for 30 s
Annealing	60℃ for 40 s (Bactin)
	64℃ for 40 s (BrZHD10)
Elongation	72℃ for 1 min
Number of cycles	35 times

3. Results

3.1. Cultivation and Growth of *R. capsulatus* in Saline Conditions

The absorbance of *R. capsulatus* at 600 nm was recorded to assess its tolerance to varying concentrations of NaCl and sea salt. Higher bacterial density correlates with greater light absorption; therefore, higher absorbance values (600 nm) indicate higher bacterial concentrations. The results suggest that *R. capsulatus* can tolerate moderate salt concentrations up to 1 w/v%, with them even exhibiting better growth than the control group at these concentrations. For NaCl, the control group showed an absorbance of 1.351, while the 0.4% w/v and 1% w/v groups had higher values at 2.174 and 1.557, respectively. Growth differed based on the type of salt used. At 0.4 w/v%, 0.7 w/v%, and 1% w/v concentrations, *R. capsulatus* exhibited higher absorbance with sea salt treatment than with NaCl. Specifically, the absorbance measurements were 2.222,

2.138, and 1.587 AU for sea salt, compared with 2.174, 1.484, and 1.557 AU, respectively. These results indicate that the absorbance was the highest with 0.4% w/v sea salt and NaCl, compared with that of the control group, but decreased at 0.7% w/v and 1 w/v%. However, the overall results indicate that sea salt generally promotes better growth than NaCl at concentrations up to 1% w/v (Figure 3).

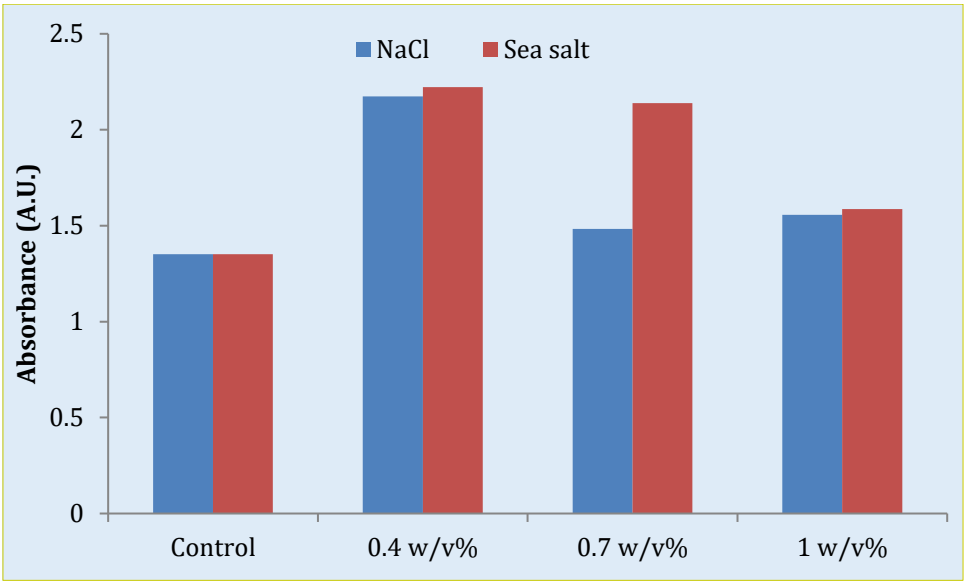

Figure 3. Growth of *R. capsulatus* under different salt conditions. Absorbance (600 nm) measured for control and varying concentrations of NaCl and sea salt.

3.2. IAA Production by *R. capsulatus*

3.2.1. IAA Production Under Standard Conditions

Rhizobacteria have been studied for their economic viability and environmental safety for mitigating plant stress through IAA production [40]. We conducted an experiment to assess the IAA production by *R. capsulatus*.

Formic acid was used to dissolve tryptophan, which enhances IAA production by *R. capsulatus*, but it may also inhibit bacterial growth. To assess this, absorbance at 660 nm was measured. As the tryptophan dissolved in formic acid increased from 0 to 50, 100, 150, and 200 µL, the *R. capsulatus* concentration decreased from 1.055, 0.848, 0.698, 0.584 to 0.531 AU, respectively, suggesting that formic acid inhibited bacterial growth (Figure 4.1).

Despite this inhibition, IAA production increased with higher tryptophan levels. Absorbance at 530 nm showed that IAA production rose steadily from 0.01 to 0.024 and 0.058 AU as tryptophan concentrations increased from 50 to 100 and 150 µL, respectively. Thus, while formic acid might reduce bacterial growth, the increase in IAA production with higher tryptophan concentrations confirmed that these photosynthetic bacteria produce IAA (Figure 4.1).

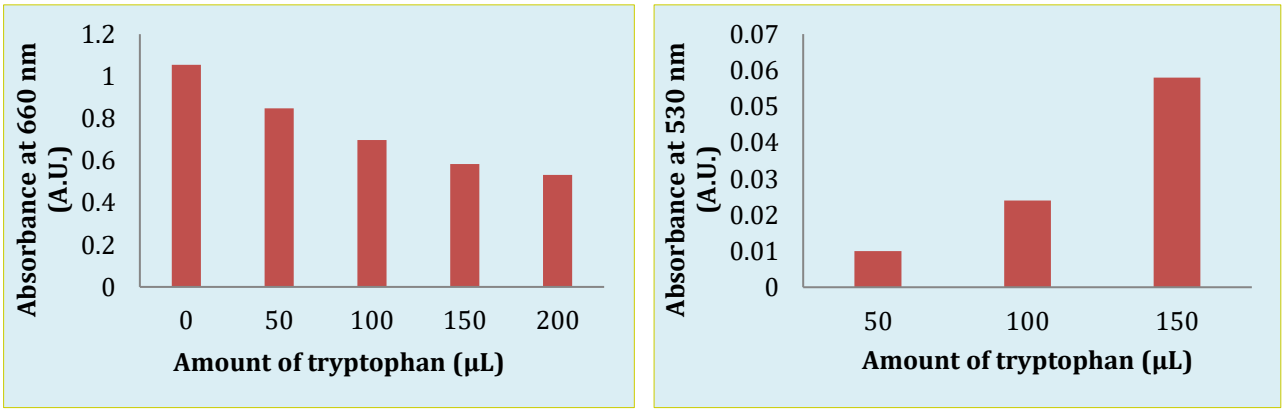

Figure 4.1. *R. capsulatus* growth inhibition by tryptophan (left), IAA production by *R. capsulatus* with tryptophan (right). The amount of tryptophan added includes 0, 50, 100, 150, and 200 µL. Absorbance (AU) at 660 nm and 530 nm was measured for each case, respectively.

Salkowski reagent 2 was used to verify IAA production, with color development correlating to the amount of tryptophan added to the bacterial supernatant. This confirmed that *R. capsulatus* produces IAA.

3.2.2. IAA Production Under 1% w/v NaCl and 1% w/v Sea Salt Conditions

To avoid the inhibitory effects of formic acid, tryptophan broth was used to assess *R. capsulatus* IAA production under saline conditions (1% w/v NaCl and 1% w/v sea salt). Results showed that, except for the 100 µL sample with NaCl, absorbance values were similar to the control group.

For the 100 µL sample, absorbance at 530 nm was 0.109 AU for the control group, 0.034 AU for NaCl (an exception), and 0.102 AU for sea salt. With 200 µL, the absorbance was 0.108 AU for the control group, 0.101 for NaCl, and 0.112 AU for sea salt. Finally, with 300 µL, which showed only a 0.001 AU difference between the groups, absorbance was 0.102 AU, 0.101 AU, and 0.102 AU for control, NaCl, and sea salt, respectively (Figure 4.2).

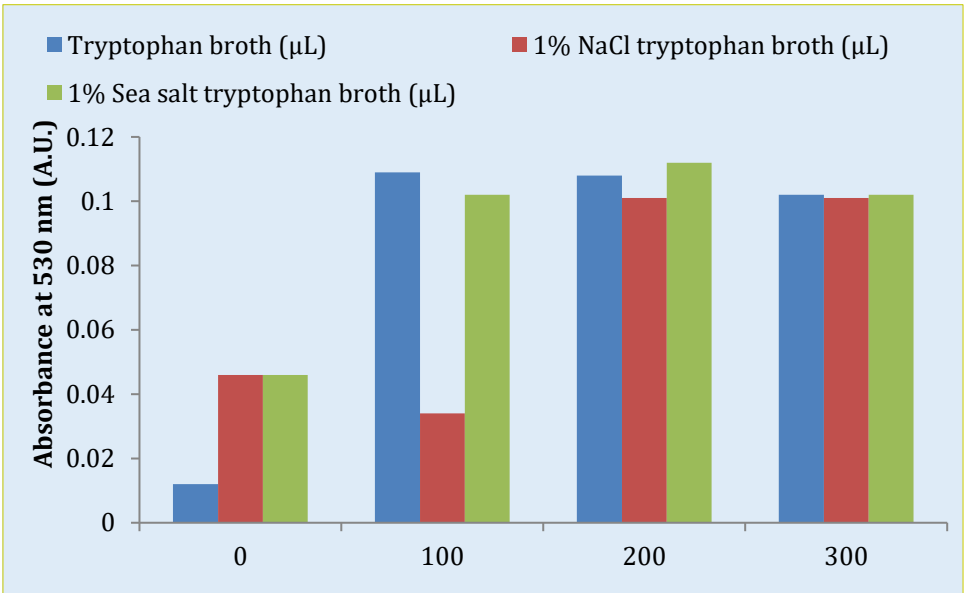

Figure 4.2. IAA production by *R. capsulatus* with tryptophan broth, 1% w/v NaCl tryptophan broth, and 1% w/v sea salt tryptophan broth, with amounts of 0, 100, 200, and 300 µL. Absorbance (AU) at 530 nm was measured.

3.3. Inhibition of *F. graminearum* by NaCl and Sea salt

Our results showed that fungal growth was reduced at 1% w/v NaCl (790 mm^2) and 1% w/v sea salt (1063 mm^2) compared to the control group (1407 mm^2). This indicates that seawater farming has the potential to inhibit fungal growth, with NaCl demonstrating greater effectiveness in prevention (Figures 5.1 and 5.2).

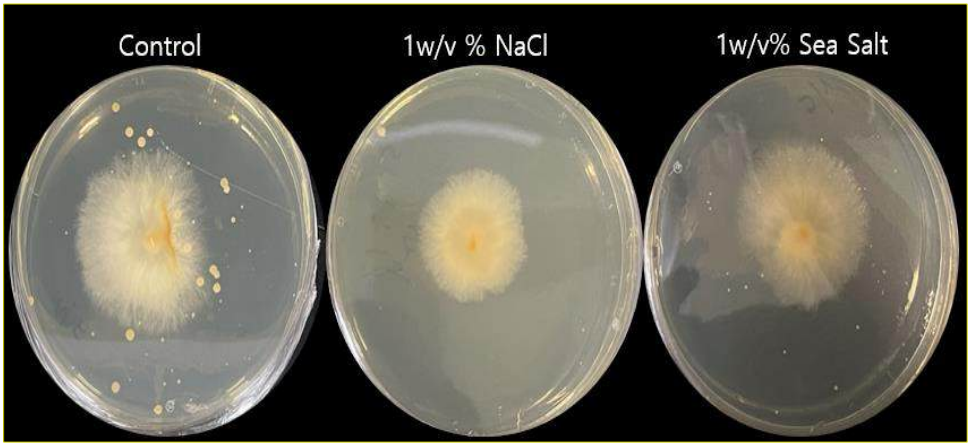

Figure 5.1. Inhibition of *F. graminearum* by *R. capsulatus*, 1% w/v NaCl, and 1% w/v sea salt to assess the effectiveness of *R. capsulatus* in preventing fungal growth in crops. Photos of the badge were taken, and circles were drawn on these images. The area of the circles was measured using ImageJ, representing the extent of fungal growth under each treatment condition.

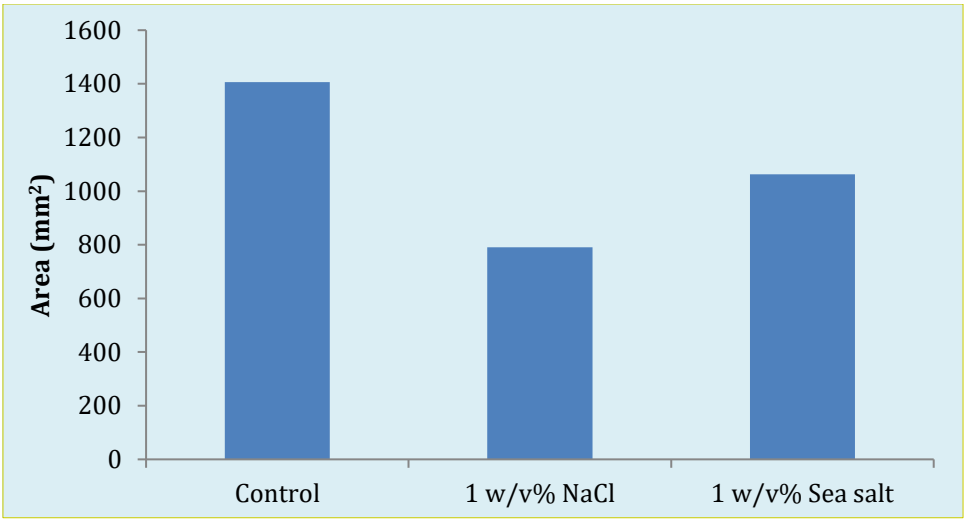

Figure 5.2. Inhibition of fungal growth, the area measured for each control, 1% w/v NaCl, and 1% w/v sea salt.

3.4. Cabbage Growth When Treated with *R. capsulatus*, 1% w/v NaCl, and 1% w/v Sea Salt

3.4.1. Cabbage Growth with 1% w/v NaCl and 1% w/v Sea Salt: Agar-based Method

Cabbage growth was assessed under three conditions: control, 1% w/v NaCl, and 1% w/v sea salt, by measuring the stem and root lengths of five seeds grown for each concentration. Our results indicated that 1% w/v concentrations of NaCl and sea salt did not substantially affect cabbage growth, compared with the control. For stem growth, the control group averaged 3.2 cm, the NaCl group was slightly lower at 3.02 cm, while the sea salt group was higher at 3.625 cm. Root growth followed similar trends, with the control group at 1.83 cm, the NaCl group slightly higher at 2.02 cm, and the sea salt group at 2.17 cm.

Total growth (stem + root lengths) was 5.2 cm for the control, 5 units for the NaCl group, and 6 units for sea salt. These observations indicate that up to a 1% w/v concentration, neither NaCl nor sea salt considerably inhibited cabbage growth, with sea salt potentially enhancing it slightly (Figure 6.1).

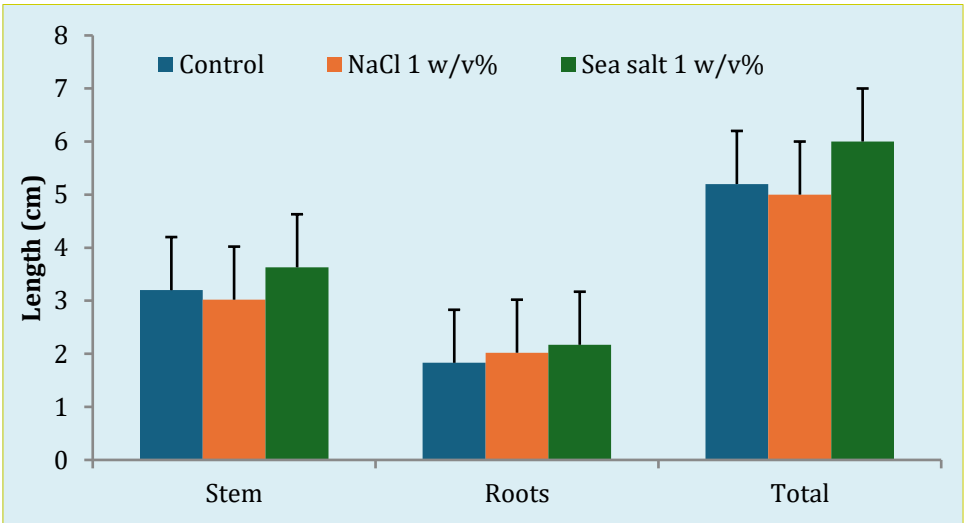

Figure 6.1. Growth of cabbages at control, 1% w/v NaCl, and 1% w/v sea salt. The length of the stem, roots, and five seeds were measured, and average values were recorded. The blue bar represents the control group, the orange bar represents the 1% NaCl group, and the green bar represents the 1% sea salt group.

The growth of cabbages under control, 1% w/v NaCl, and 1% w/v sea salt conditions was further assessed by measuring the absorbance of chlorophyll extracted from cabbage leaves at 663 nm (chlorophyll a) and 645 nm (chlorophyll b). The chlorophyll levels were higher in salt treatments than in the control, with minimal differences between the NaCl and sea salt treatments. The chlorophyll a to b ratio in healthy plants typically ranges from 2.5 to 3.0, reflecting a balance of photosynthetic pigments and overall photosynthetic capacity. In this experiment, the control group showed a ratio of approximately 2.82, indicating a normal chlorophyll composition. The NaCl-treated group had a slightly higher ratio of 2.89, and the sea salt-treated

group had a ratio of 2.87. These results indicate that 1% w/v salt stress does not considerably inhibit chlorophyll production and may even enhance chlorophyll a levels slightly (Figure 6.2).

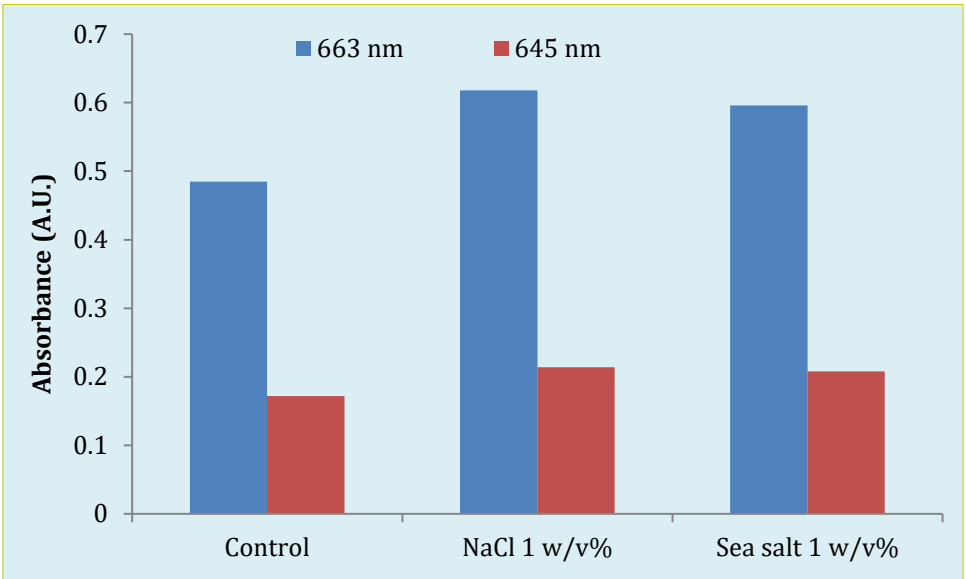

Figure 6.2. Absorbance of chlorophyll in leaves of cabbages treated with 1% w/v NaCl and 1% w/v sea salt. The absorbance of the leaves collected from the five cabbage plants at each concentration was measured at 663 nm and 645 nm, each representing chlorophyll a and b, respectively. The absorbance value was then divided into the number of cabbage seeds from which the leaves were collected. The blue bar represents absorbance at 663 nm, while the dark red bar represents 645 nm.

3.4.2. Cabbage Growth with *R. capsulatus*, 1% w/v NaCl, and 1% w/v Sea Salt: Spraying Method

After 3 d of treatment, the cabbages in the 1% w/v sea salt group showed the highest stem growth at 17.6 cm, compared with the control group at 16.8 cm. The groups treated with *R. capsulatus* and NaCl measured 16.9 and 15.7 cm, respectively. By day 6, all groups showed increased growth from the initial measurement, with the sea salt group showing the most significant growth at 6.2 cm. After 10 d, growth continued in all groups except for the sea salt and NaCl groups, which had stabilized. Notably, between 3 to 6 d, the sea salt group's stem growth was 6.23 cm, and root growth was 6.87 cm, whereas growth from 6 to 10 d was reduced to 0.786 cm for the stem and 1.69 cm for the root. Overall, the sea salt group achieved the most substantial cabbage growth with a total growth of 5.44 cm, while the NaCl group showed a decrease of 1.56 cm over 10 days. Both the control and *R. capsulatus* groups exhibited a slight increase in stem length (Figures 6.3 and 6.4).

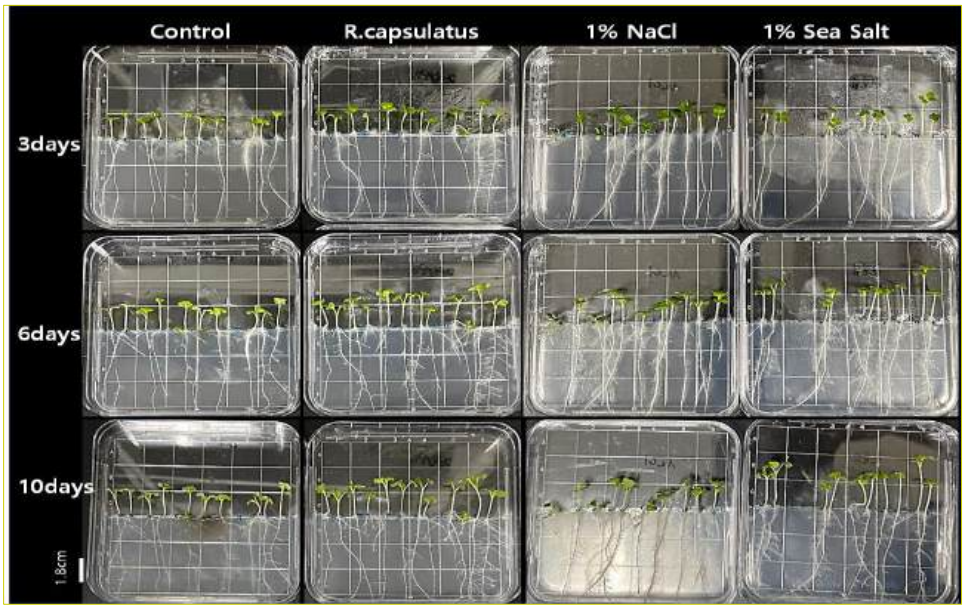

Figure 6.3. Cabbage growth when treated with *R. capsulatus*, 1% NaCl, and 1% sea salt separately, via spraying method. The changes in the growth over 3, 6, and 10 days were observed.

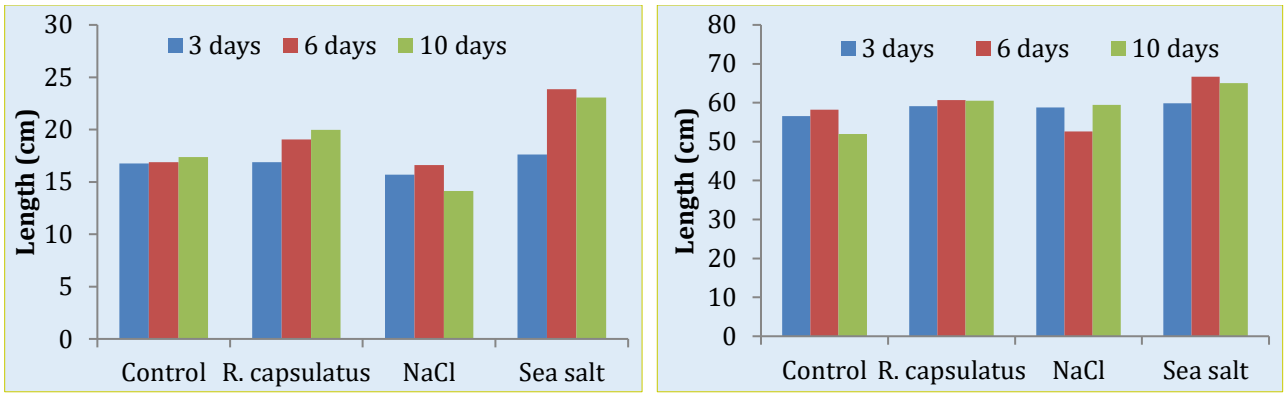

Figure 6.4. Stem (left) and root (right) growth of cabbages when treated with *R. capsulatus*, 1% NaCl, and 1% sea salt separately via spraying method. The changes in the growth over 3, 6, and 10 days were observed.

3.4.3. Cabbage Growth When Treated with Varying Dilutions of *R. capsulatus* and IAA Separately

3.4.3.1. Cabbage Growth When Treated with IAA

To assess the impact of *R. capsulatus* on cabbage growth, cabbages were treated with varying concentrations of IAA solutions and compared to plants treated with varying concentrations of *R. capsulatus*. We aimed to determine the optimal IAA concentration for plant growth and estimate the amount of IAA produced by photosynthetic bacteria, thereby identifying the optimal concentration of *R. capsulatus* for enhancing growth. Our results showed that an IAA concentration of 1×10^{-4} ppm resulted in shorter or poorly developed roots compared to concentrations lower than 1×10^{-4} ppm. However, seeds treated with photosynthetic bacteria exhibited well-embedded roots and strong growth. Furthermore, when comparing the effects of *R. capsulatus* and IAA, cabbage seeds treated with 2.0 AU of *R. capsulatus* diluted in ratios of 1/100, 1/50, 1/25 and 1/2 showed better growth than those treated with various IAA concentrations.

3.4.3.2. Cabbage Growth When Treated with *R. capsulatus*

The growth of cabbage treated with diluted concentrations of 2.0 AU *R. capsulatus,* as determined to be suitable in previous experiments, was assessed by measuring stem length, root length, and total plant growth to analyze the overall impact of photosynthetic bacteria.

The dilution ratios were as follows: 20X indicates a dilution of 1:500, and 10X corresponds to a dilution of 1:100. Cabbage growth was highest when *R. capsulatus* was applied undiluted (1X), with total growth measuring 5.11 cm. At dilution ratios of 5X and 2X, the growths were 5.05 cm and 4.69 cm, respectively, exceeding the control group's growth of 4.66 cm. Conversely, at higher dilution ratios of 20X and 10X, the growth was less than that of the control, measuring 3.02 cm and 4.34 cm, respectively, indicating a decrease in effectiveness with greater dilution (Figure 7).

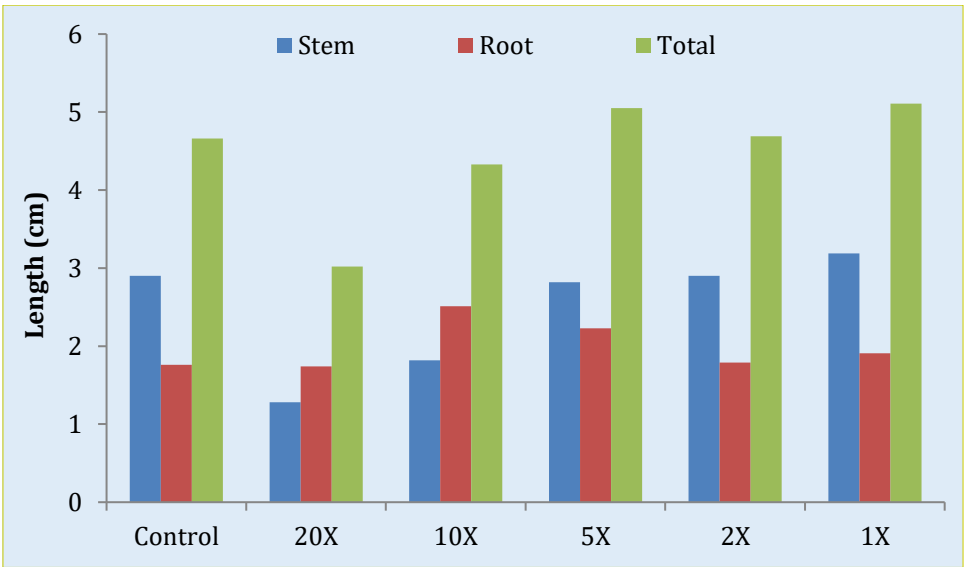

Figure 7. Growth of cabbages with *R. capsulatus*. The control group indicates no *R. capsulatus*. The number in front of X indicates the dilution ratio. The length of the stem, roots, and the total stem and roots of the five seeds were measured, and the average values were taken.

3.4.4. Cabbage Growth When Treated with Combinations of *R. capsulatus*, Sea Salt, and NaCl

The total growths of cabbage stem and root were investigated by adding varying volumes (0 µL, 10 µL, 20 µL, 30 µL) of a 2.0 AU concentration of *R. capsulatus* to agar plates under three conditions: no salt, NaCl, and sea salt (Figure 8.1). Stem and root lengths from five seeds were measured for each treatment to assess how different amounts of photosynthetic bacteria, when combined with salt conditions, affect growth.

In the no-salt condition, agar wells supplemented with 10, 20, and 30 µL of photosynthetic bacteria exhibited growths of 5.80 cm, 7.50 cm, and 6.38 cm, respectively, all of which surpassed that of the control (0 µL), which reached 4.66 cm. The highest growth in the group was observed at 20 µL, with a total length of 7.50 cm.

In the NaCl concentration, wells with 10 and 20 µL of *R. capsulatus* reached 7.10 cm and 7.17 cm, respectively, both higher than the control (5.325 cm). While the data for 30 µL treatment is missing, the well with 20 µL of *R. capsulatus* showed the maximum growth at 7.17 cm.

In the sea salt condition, wells with 10, 20, and 30 µL of *R. capsulatus* showed growths of 7.15 cm, 7.70 cm, and 8.10 cm, respectively, compared with the control's 6.54 cm. The 30 µL treatment yielded the highest growth at 8.10 cm (Figure 8.1).

Overall, the sea salt group generally showed higher total growth than other groups, except for the NaCl group, which exhibited higher growth than the sea salt group in some instances.

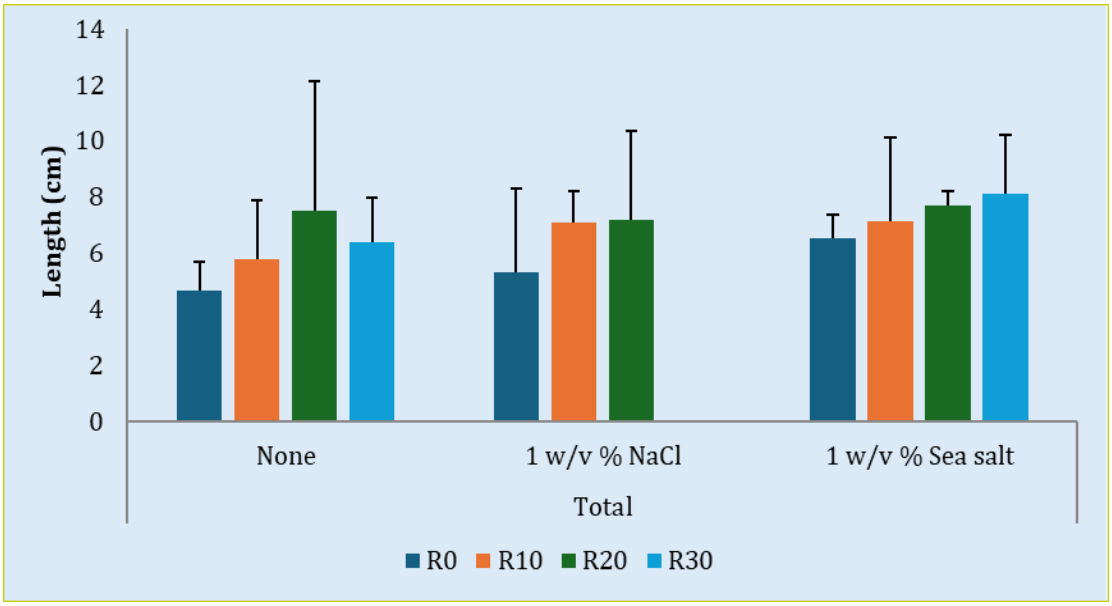

Figure 8.1. Total growth of cabbages with varying amounts of *R. capsulatus*, 0 µL, 10 µL, 20 µL, and 30 µL combined with no salt, NaCl, and sea salt. The length of stem, roots, and total stem and roots of five seeds were measured, and average values were recorded. The dark blue bar represents the R0 group, the orange bar represents the R10 group, the green bar represents the R20 group, and the light blue bar represents the R30 group.

Cabbage stem growth was investigated under varying *R. capsulatus* conditions, combined with no salt, NaCl, and sea salt. In the no-salt condition, agar wells supplied with 10 µL, 20 µL, and 30 µL of photosynthetic bacteria exhibited higher growths at 3.38 cm, 4.13 cm, and 3.50 cm, respectively, compared to the control's growth of 2.90 cm. The highest growth was observed in the well with 20 µL of photosynthetic bacteria.

In the presence of NaCl, the agar wells with 10 µL and 20 µL of photosynthetic bacteria exhibited stem growth of 4.24 cm and 3.93 cm, respectively, exceeding the control's growth at 3.43 cm. Data for 30 µL treatment was missing.

Under the sea salt conditions, wells with 10 µL and 30 µL of bacteria showed higher growth of 5.08 and 4.52 cm, respectively, surpassing the control's growth of 4.38 cm. Notably, the 20 µL treatment resulted in the lowest growth of 4.18 cm (Figure 8.2).

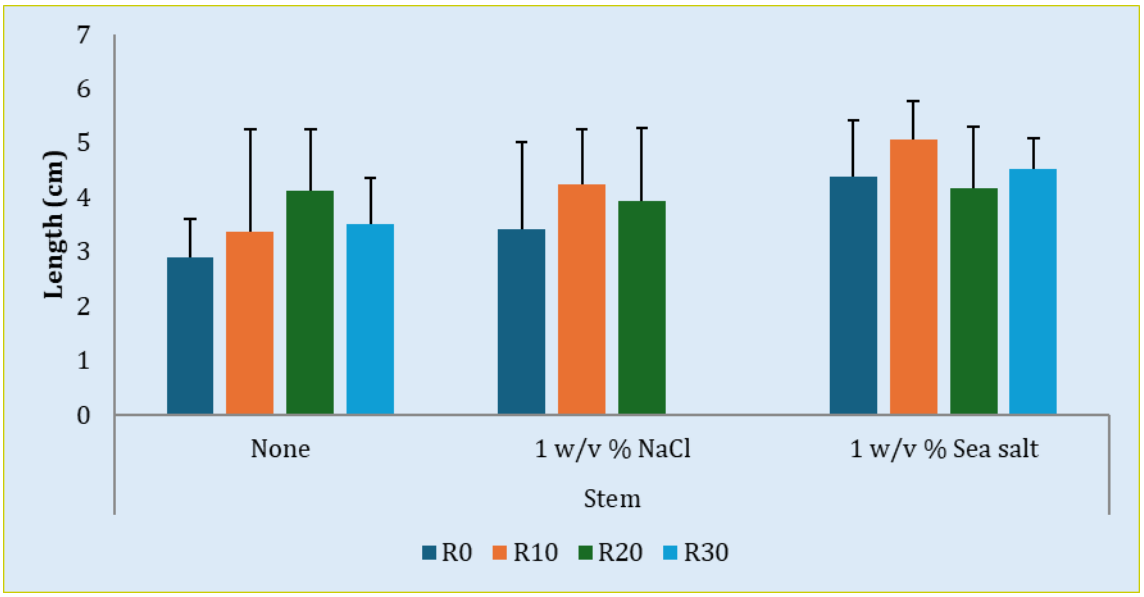

Figure 8.2. Stem growth of cabbages with varying amounts of *R. capsulatus*, 0 µL, 10 µL, 20 µL, and 30 µL combined with no salt, NaCl, and sea salt. The length of stem, roots, and total stem and roots of five seeds were measured, and average values were recorded. The dark blue bar represents the R0 group, the orange bar represents the R10 group, the green bar represents the R20 group, and the light blue bar represents the R30 group.

Root growth of cabbages was specifically investigated under varying conditions of *R. capsulatus* with no salt, NaCl, and sea salt. In the no-salt condition, agar wells supplied with 10 µL, 20 µL and 30 µL of photosynthetic bacteria exhibited higher growths, at 2.42 cm, 3.38 cm, and 2.88 cm, respectively, compared with the control's 1.76 cm. The highest growth was observed in the well with 20 µL of photosynthetic bacteria, aligning with the trend observed in stem and total growth.

In the NaCl condition, agar wells with 10 µL and 20 µL of *R. capsulatus* showed root growth of 2.86 cm and 3.24 cm, respectively, surpassing the control's 1.9 cm. The well with 30 µL of photosynthetic bacteria showed the highest root growth, although data for this concentration is not provided. Under sea salt conditions, wells with 20 µL and 30 µL of photosynthetic bacteria exhibited higher root growth at 3.52 cm and 3.58 cm, respectively, compared with the control's 2.16 cm. However, the well with 10 µL of photosynthetic bacteria exhibited slightly lower growth at 2.08 cm compared with the control (Figure 8.3).

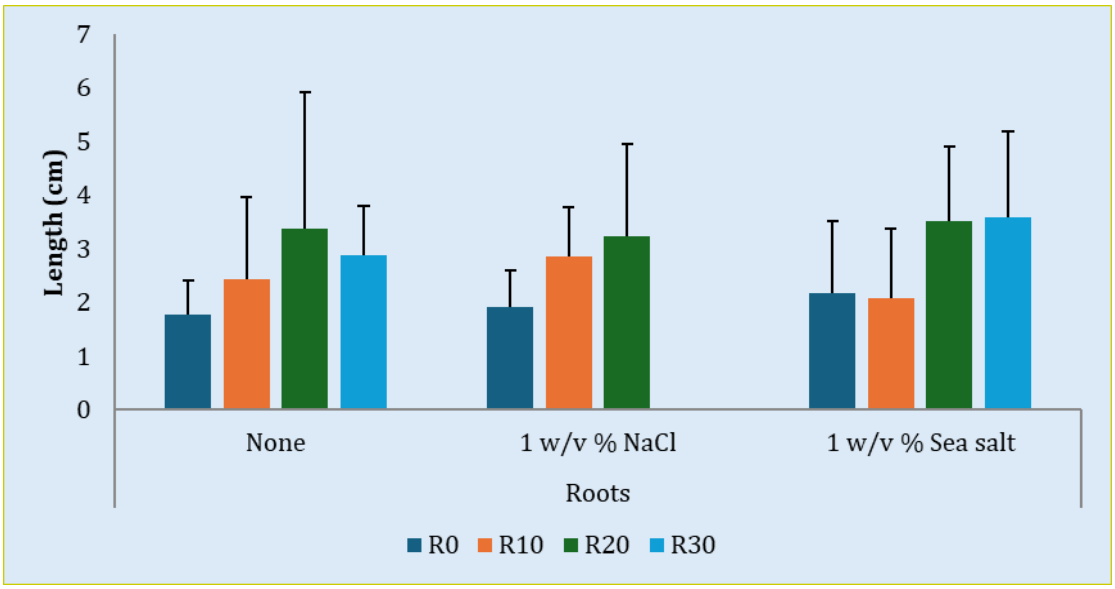

Figure 8.3. Root growth of cabbages with varying amounts of *R. capsulatus*, 0 µL, 10 µL, 20 µL, and 30 µL combined with no salt, NaCl, and sea salt. The length of stem, roots, and total stem and roots of five seeds were measured, and average values were recorded. The dark blue bar represents the R0 group, the orange bar represents the R10 group, the green bar represents the R20 group, and the light blue bar represents the R30 group.

From this experiment, adding 20 µL of *R. capsulatus* was found to be the most effective for enhancing cabbage growth under no-salt conditions across all metrics. Under saline conditions, a 10-µL concentration was particularly effective in promoting stem growth. Additionally, higher concentrations of photosynthetic bacteria generally enhanced overall growth in both stems and roots, with the effect being most pronounced in the total plant growth. Sea salt was the most effective in promoting growth, with higher total growth than other conditions (Figure 8.4).

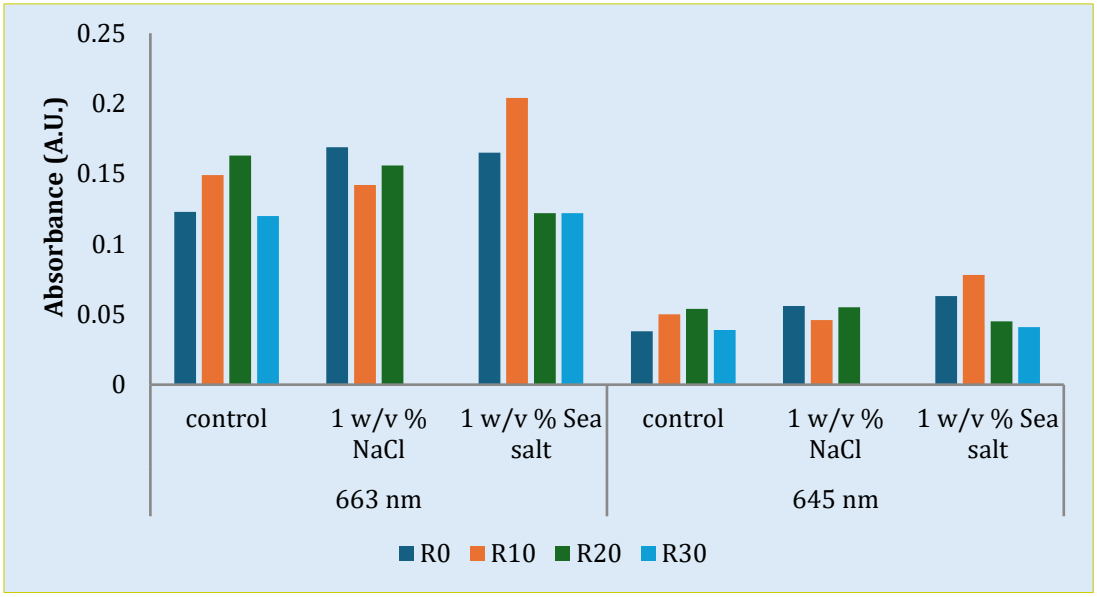

Figure 8.4. Absorbance of chlorophyll in leaves of cabbages treated with varying amounts of *R. capsulatus*, 0 µL, 10 µL, 20 µL, and 30 µL, each represented by dark blue, orange, green and light blue bars, combined with no salt, 1% w/v NaCl, and 1% w/v sea salt. The control group indicates no salt. The absorbance of the leaves collected from the five cabbage plants at each concentration was measured at 663 nm and 645 nm, each representing chlorophyll a and b, respectively. The absorbance value was then divided into the number of cabbage seeds from which the leaves were collected.

3.4.5. Growth When Treated with Combinations of *R. capsulatus*, Sea Salt, NaCl, and *F. graminearum* Solution

Compared to the control group, plants treated solely with *F. graminearum* exhibited substantially reduced growth, indicating the detrimental impact of this fungus on plant growth and highlighting the critical need for fungicides.

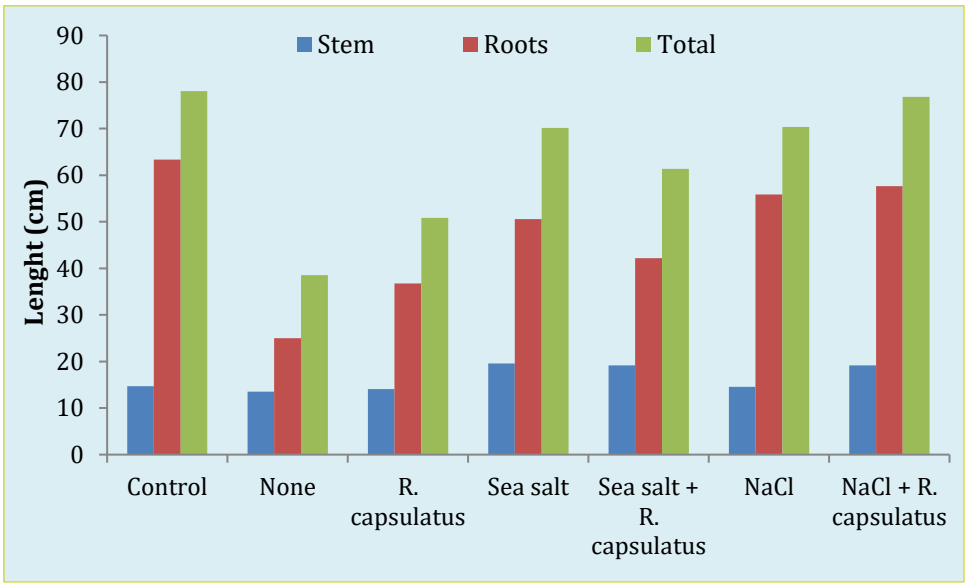

Figure 9.1. Schemes follow the same formatting. Cabbage growth with the control group and *F. graminearum*-treated group. Stem, root, and total lengths of cabbages were measured and recorded for each group.

Cabbages treated with sea salt, sea salt combined with *R. capsulatus*, NaCl, and NaCl combined with *R. capsulatus* showed considerably greater growth compared to those treated with only *F. graminearum*. This suggests that salt solutions, whether used alone or combined with *R. capsulatus,* are effective in inhibiting fungal growth and enhancing plant development.

In previous experiments, cabbages treated with *R. capsulatus* and *F. graminearum* showed a smaller growth increase (50.8 cm) compared with those treated with only *F. graminearum* (38.5 cm). This suggests that *R. capsulatus* alone does not considerably enhance plant growth without salt stress, but it does improve growth under salt stress by mitigating its effects.

Both sea salt and NaCl proved highly effective in inhibiting fungal growth and enhancing plant development. Plants treated with sea salt and NaCl showed total lengths of 70.1 cm and 70.4 cm, respectively, considerably exceeding those treated solely with *F. graminearum* and approaching the control group's length of 78.1 cm. Notably, the addition of *R. capsulatus* to sea salt resulted in shorter growth (61.3 cm), whereas the addition of *R. capsulatus* to NaCl promoted further growth (76.8 cm), significantly exceeding that of the group treated with only *F. graminearum* and approaching the control group's growth (Figure 9.1).

Qualitatively, cabbages treated with NaCl and sea salt showed denser root hairs and stronger roots. Those treated with NaCl, NaCl combined with *R. capsulatus*, sea salt, and sea salt combined with *R. capsulatus* showed noticeably longer roots and stems, indicating enhanced growth and development with the salt solution and *R. capsulatus* treatment (Figure 9.2).

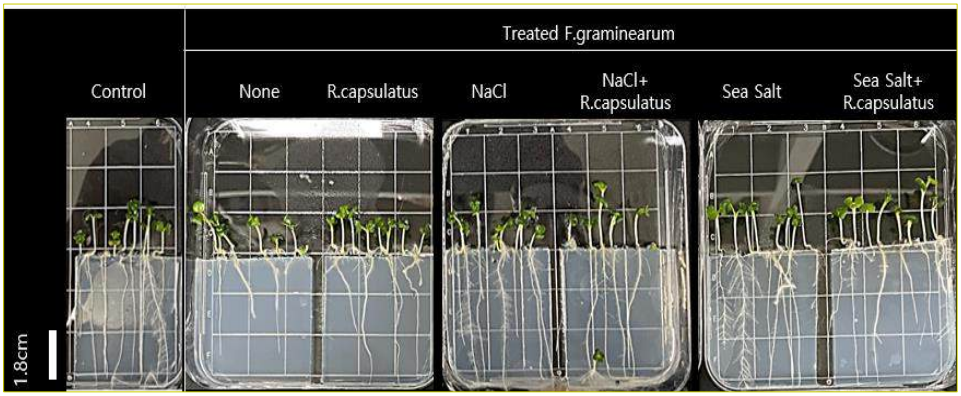

Figure 9.2. Cabbage growth with the control group and *F. graminearum*-treated group. *F. graminearum*-treated group includes no treatment of other solutions-*R. capsulatus*, NaCl, NaCl and *R. capsulatus*, sea salt, and, sea salt and *R. capsulatus*.

3.5. Impact of IAA Production by *R. capsulatus* on Salt Stress-related Gene Expression

The BrZHD10 gene in cabbage is known for conferring high salt tolerance when expressed in *Arabidopsis*, making it a potential marker of salt resistance [41]. In this study, while BrZHD10 expression was not observed under 2% w/v NaCl treatment, it was observed under 2% w/v sea salt and when NaCl or sea salt was combined with *R. capsulatus* (Figure 10.1). Among those groups, BrZHD10 expression was the lowest in the group treated with 2% w/v NaCl and *R. capsulatus*, with a relative intensity of 17.95%. In contrast, the sea salt group exhibited higher growth than did the NaCl group, and the addition of *R. capsulatus* further enhanced BrZHD10 expression, with a relative intensity of 54.2%, compared to 27.9% in the sea salt group (Figure 10.2).

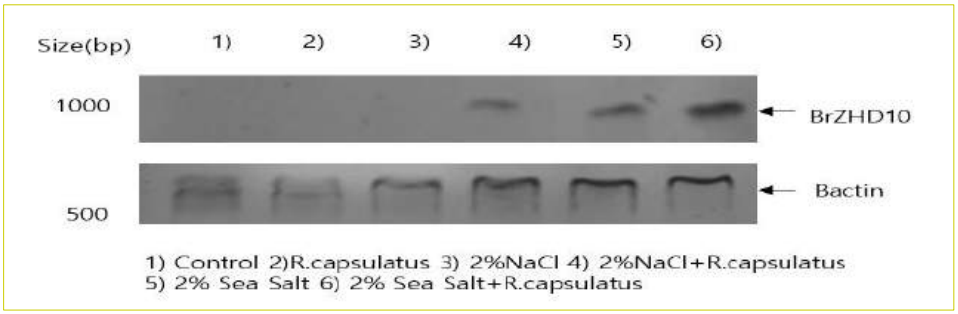

Figure 10.1. Impact of IAA production by *R. capsulatus* on salt stress-related gene expression, each row representing BrZHD10 and Bactin, with each expression labeled below.

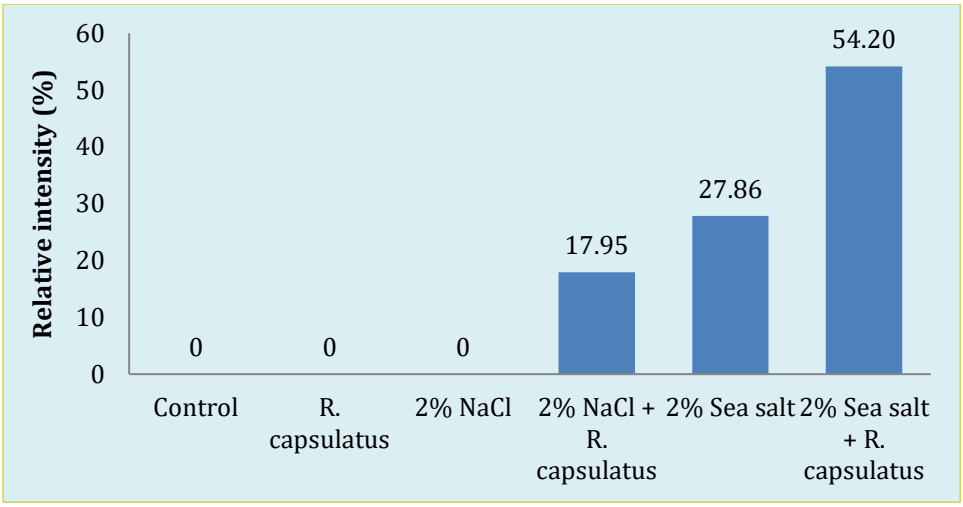

Figure 10.2. Impact of IAA production by *R. capsulatus* on salt stress-related gene expression, shown through relative intensity (%) of each gene expression.

4. Discussion

Our findings confirmed that *R. capsulatus* can tolerate saline conditions up to 1% w/v, indicating its feasibility for seawater farming. The evaluation of IAA production in the presence and absence of salt, using Salkowski reagent and tryptophan, demonstrated that *R. capsulatus* can produce IAA under both conditions, indicating its potential to enhance plant growth and mitigate salt stress. However, minimal IAA production in NaCl tryptophan broth pointed to possible experimental variability. Section 3.3 explored the antifungal properties of NaCl and sea salt. The reduced growth of *F. graminearum* on PDA plates treated with these salts confirmed their effectiveness in inhibiting fungal growth, highlighting seawater farming's potential to reduce pesticide use.

Cabbage growth under 1% w/v NaCl and sea salt showed minimal inhibition, with sea salt slightly enhancing growth. Chlorophyll analysis revealed a higher chlorophyll a to b ratio in salt-treated plants, suggesting improved photosynthetic efficiency. Owing to seawater's typical 3.5% salinity, 1% w/v dilution was suitable for agriculture. Combining this with photosynthetic bacteria could prevent fungal growth and enhance crop yields through auxin production and mineral benefits. While 1% w/v salt water did not substantially affect growth in the short term, long-term exposure could lead to cumulative salt stress, impacting plant health. *R. capsulatus* might mitigate this stress by producing IAA.

Cabbage seeds planted on agar and treated with various *R. capsulatus* dilutions and IAA solutions showed optimal growth with *R. capsulatus* dilutions of 5X, 2X, and 1X, whereas higher dilutions reduced the effectiveness. *R. capsulatus* generally improved growth under saline conditions, with optimal concentrations of 20 µL for NaCl and 30 µL for sea salt. In a follow-up experiment with NaCl, sea salt, *R. capsulatus*, and *F. graminearum*, both salts enhanced growth by inhibiting fungal growth. Sea salt, when combined with *R. capsulatus*, slightly reduced growth, indicating that while *R. capsulatus* mitigates salt stress and fungal growth, root length measurements may have inaccuracies due to limitations in ImageJ.

Gene expression analysis showed that *R. capsulatus*'s IAA production enhances stress tolerance, particularly with sea salt, which was more effective in NaCl in reducing salt stress. The combined use of *R. capsulatus* and sea salt improved cabbage growth and stress tolerance.

Future research should explore these solutions in soil to assess their effectiveness in real agricultural settings and further investigate genes related to high salt stress in soil-grown plants.

5. Conclusions

R. capsulatus can be effectively utilized in seawater farming at 1% w/v NaCl and 1% w/v sea salt, as it survives and produces IAA in these salinities. Our results suggest that photosynthetic bacteria can tolerate and function under seawater farming conditions. Both NaCl and sea salt inhibited fungal growth, suggesting that combining them with *R. capsulatus* could mitigate salt stress and enhance plant growth. Notably, combining sea salt with *R. capsulatus* resulted in exceptional growth, likely due to the beneficial minerals in sea salt, indicating a synergistic effect. Optimal combinations were found to be 20 µL of *R. capsulatus* with NaCl and 30 µL of *R. capsulatus* with sea salt.

Spraying and using extracts of these solutions appear most practical for seawater farming applications. The mixture of 1% w/v sea salt with 50 µL of 2.0 AU *R. capsulatus* was the most effective in a 62.5 × 62.5 mm square plate and can be scaled up for field applications. Salt-stress-related gene expression further supports these findings, indicating increased gene activity in plants treated with sea salt and *R. capsulatus.*

This research highlights the potential of using *R. capsulatus* in combination with salt solutions to reduce water pollution, promote environmental sustainability, and address global food demands. Future research could explore the effects of these solutions on plants grown in soil to assess their practical application in agriculture.

Declarations

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Author Contribution: The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.
Conflict of Interest: The author declares no conflict of interest.
Consent to Publish: The author agrees to publish the paper in the International Journal of Recent Innovations in Academic Research.
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