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Effect of Seaweed Extracts on Rice Growth and Tolerance to Salinity, Drought and Blast (*Magnaporthe oryzae*)

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Abstract: Rice, a primary staple, confronts a multitude of biotic and abiotic stresses. Drought and salinity, exacerbated by climate change and blast disease caused by *Magnaporthe oryzae*, significantly contribute to yield losses. To tackle the problems, two types of seaweeds-one known as *Hypnea musciformis* and another unknown-were collected from Saint Martin, Cox's Bazar, Bangladesh and then examined for the potential of their extracts to stimulate growth and improve resistance to drought, salinity and blast disease in rice. Molecular identification based on cytochrome C Oxidase subunit I (COI) gene sequence, the unknown seaweed was identified as *Gracilaria tenuistipitata*. Seaweed extracts were prepared from seaweed at different pH (8, 9, 10) and temperature (40, 50, 60, 70, 80°C) regimes. The laboratory and pot experiments were set up using a Completely Randomized Design (CRD), with three replications for each treatment. All trials were conducted ethically and data were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) to determine the statistical significance ($p = 0.05$) of treatment means. Priming rice seeds with the extracts significantly increased germination rates and seedling vigor. Furthermore, seedlings grown in media amended with seaweed extracts exhibited enhanced growth and chlorophyll content compared to the control. Seaweed extracts also demonstrated the ability to ameliorate drought and salt stress while protecting rice plants from the blast pathogen *M. oryzae*. Among the two seaweeds tested, *G. tenuistipitata* was found to promote higher plant growth and biotic and abiotic stress tolerance in rice compared to *Hypnea musciformis*. Optimal extraction conditions, particularly low temperature (40°C) and pH 9, were identified as key factors in maximizing the bioactivity of the extracts. From these results, it can be concluded that seaweed extracts, particularly those from *H. musciformis* and *G. tenuistipitata* collected from Saint Martin, Cox's Bazar, Bangladesh, could be utilized as potential bio-stimulants and stress alleviators in rice cultivation, contributing to improved growth and resilience in the face of environmental changes.

Keywords: *Hypnea*, *Gracilaria*, Molecular Identification, Growth Promotion, Salinity, Drought

Introduction

Rice (*Oryza sativa* L.) occupies a significant portion of cultivated land dedicated to food grain production and stands as the second most widely grown cereal crop globally within the Poaceae family. Serving as the primary dietary staple for more than half of the world's population, rice holds immense importance. Asia emerges as the primary hub for rice production, with a striking statistic revealing that a substantial 60% of protein and calorie intake for most Asians is sourced from rice (Iqbal *et al*., 2003). Notably, in Bangladesh, rice assumes a pivotal role as the primary food crop, contributing to approximately 95.2% of the total food grain production, amounting to 37.96

million metric tons (FAO, 2022). Moreover, an impressive 80% of cultivated land is dedicated to rice cultivation. However, the acreage allocated for rice cultivation is gradually diminishing while the population continues to grow. Consequently, there is an urgent imperative to enhance production to meet the escalating demands of the expanding population and uphold global food security standards.

The pursuit of increased rice productivity encounters obstacles stemming from both abiotic and biotic factors, with diseases representing the most significant biotic stressors. On average, 8-20% of rice crops are lost due to diseases. Rice contends with a spectrum of over 60 different diseases, with Bangladesh alone grappling with 43 of them (Hossain *et al*., 2014). Rice blast is the most notorious among these diseases (Mahmud *et al*., 2024). Rice blast is caused by a filamentous fungal pathogen *Magnaporthe oryzae*, which maintains a global presence (Mahmud *et al*., 2024). This pathogen can infect rice plants at any developmental stage, resulting in various manifestations such as leaf, node, neck and panicle blasts (Mahmud *et al*., 2021). Inflicting substantial damage, rice blast can lead to yield reductions as high as 70-80% (Piotti *et al*., 2005).

In recent decades, drought and salinity have emerged as primary environmental stressors, resulting in significant agricultural losses. Drought can occur unpredictably in any location due to insufficient precipitation and irrigation resources, exacerbating soil salinity. Conversely, heightened salinity can induce drought-like conditions in plants by reducing cell water potential (Ghosh *et al*., 2019). The geographical extent of regions experiencing drought and salinity stress is expanding due to climate change, leading to decreased agricultural land productivity. The impact of drought on agricultural productivity is severe, with water scarcity in rice crops potentially causing yield reductions of up to 80% (Ichsan *et al*., 2021). Drought triggers various responses in plants, including growth inhibition, reduced photosynthesis and oxidative stress (Xoconostle-Cazares *et al*., 2010). Similarly, salinity imposes significant limitations on the yields of major crops (Liu *et al*., 2014; Gu *et al*., 2014). It is estimated that approximately one-fifth of cultivated lands worldwide, which receive artificial irrigation, are affected by varying levels of salinity (Mostafazadeh-Fard *et al*., 2008).

Various strategies have been devised to alleviate the adverse effects of both biotic and abiotic stressors and enhance rice productivity. Traditionally, the primary approach has been the application of synthetic fertilizers and pesticides. However, the extensive use of fertilizers has led to the rapid depletion of crucial macro and micronutrients, resulting in nutrient imbalances and soil fertility degradation. Similarly, the reliance on chemical pesticides to combat rice diseases has been associated with detrimental effects on human health, animal welfare, soil microorganisms and the broader environment. Consequently, there is a pressing need to explore viable alternatives to synthetic fertilizers and pesticides.

To address these challenges and pursue more sustainable solutions for plant growth and stress management, researchers have turned to the exploration of seaweed and seaweed-derived extracts (Shah *et al*., 2013; Layek *et al*., 2018). Marine algae, commonly referred to as seaweed, harbor a diverse array of biologically active compounds that hold promise for agricultural applications (Hossain *et al*., 2024). Over the past two decades, studies have demonstrated the beneficial effects of seaweed extracts on the growth and productivity of various crops (Shah *et al*., 2013; Demir *et al*., 2006; Rathore *et al*., 2009; Pande *et al*., 2020, Layek *et al*., 2018). Additionally, seaweed extracts have been shown to stimulate plant defenses against pests and diseases while enhancing plant tolerance to abiotic stresses (Hossain *et al*., 2024). Consequently, seaweed extracts present a promising alternative to synthetic agrochemicals in the realm of sustainable agriculture.

Beneath the vast tropical waters of the Bay of Bengal lies an abundance of marine resources. In Bangladesh, the southern region of the country is noted for its natural richness in seaweed, with saint martin island particularly renowned for its vast seaweed growth (Islam *et al*., 2020). Despite the extensive presence of seaweed floras along the Bangladesh coast, their utilization in agriculture remains relatively limited. While the exploration of seaweed resources is a recent endeavour in Bangladesh, research on the potential application of seaweed extracts to enhance plant growth and resilience against biotic and abiotic stresses is scarce. To address these gaps, this study aimed to investigate the effects of seaweed extract on boosting the growth and resistance of rice plants to drought, salinity and blast disease. Moreover, the study examined the impact of seaweed extracts prepared under various pH levels and temperatures to determine the optimal conditions for maximizing the beneficial effects of seaweed extract on rice plants.

Materials and Methods

Sample Collection and Preparation

Hypnea musciformis and one unidentified seaweed sample were collected from the saint martin coast of the Bay of Bengal, cox's bazar, Bangladesh, during the mature stage. After harvesting, the seaweed was cut off the fronds and then washed using clean seawater to remove any dirt. The seaweed was then transported in an icebox at a temperature of 4°C to the laboratory. Once it arrived, the seaweed was washed again under running water to remove any remaining seawater, sand, or other unwanted materials. The washed seaweed was then left to

dry in the sun for two days before being packaged into small plastic bags and stored in a refrigerator at a cool temperature of 4°C until it was needed. The seaweed was then ground into a fine powder using a grinding machine and 4 g of the powder was hydrated in 100 mL of deionized water and kept for 24 h at room temperature (25±1°C) followed by depigmentation using 100 mL of methanol-acetone (1:1) mixture to eliminate the organic soluble fraction. The depigmented seaweed was further treated in deionized water (~150 mL g^{-1}) and then heated in a shaking water bath. The heating process of extraction was conducted at three distinct pH levels: 8 (T8), 9 (T9) and 10 (T10) and at five different temperatures, ranging from 40-80°C. Throughout the process of extraction, the temperature and pH were carefully monitored and modified as required. After extraction, the seaweed extract was left to cool down and then sieved through thin cloth and filter paper. The pH of filtered seaweed extract was adjusted to 7 by adding sulfuric acid. The extracts were then stored in opaque plastic containers at a temperature of 4°C. To identify each extract, a code was assigned, with the first digit representing the extraction temperature and the second digit indicating the extraction pH: $T_1$8 (40°C at pH 8), $T_2$8 (50°C at pH 8), $T_3$8 (60°C at pH 8), $T_4$8 (70°C at pH 8), $T_5$8 (80°C at pH 8), $T_1$9 (40°C at pH 9), $T_2$9 (50°C at pH 9), $T_3$9 (60°C at pH 9), $T_4$9 (70°C at pH 9), $T_5$9 (80°C at pH 9), $T_1$10 (40°C at pH 10), $T_2$10 (50°C at pH 10), $T_3$10 (60°C at pH 10), $T_4$10 (70°C at pH 10), $T_5$10 (80°C at pH 10).

Plant and Pathogen

For this experiment, the rice variety BRRI dhan 28 was chosen and its seeds were obtained from the Bangladesh Rice Research Institute (BRRI). The rice blast pathogen used in the experiment, *Magnaporthe oryzae* PLPO1, was sourced from the Department of Plant Pathology at Bangabandhu Sheikh Mujibur Rahman Agricultural University in Gazipur.

Molecular Identification of Unidentified Seaweed

The unidentified seaweed sample underwent molecular identification. DNA was isolated from a tiny sample of seaweed using the CTAB (cetyltrimethylammonium bromide) method. Seaweed tissues were frozen in liquid nitrogen and then pulverized with a mortar and pestle while still frozen. A small amount of ground tissues (about 10 mg) was put into a microfuge tube containing 500 µL of CTAB extraction buffer (2% CTAB, 0.1 m Tris-HCl (pH 8.0), 1.4 m NaCl, 20 mm EDTA, 1% polyvinylpyrrolidone-PVP), adding approximately 50 µg RNAse A and 80 µg Proteinase K. The samples were then placed at 55-60°C for an hour, with intermittent mixing. The samples were then thoroughly mixed with an equal volume of chloroform: Isoamyl alcohol (24:1) before being spun for 10 min at 15,000 rpm. The top aqueous layer was moved to a new microfuge tube. An equivalent volume of 100% isopropanol was poured into the tube and incubated at room temperature for 30 min. The sample was spun for 20 min at 15,000 rpm and then decanted without disturbing the DNA pellet. After adding approximately 500 µL of 70% ethanol, the sample was spun for 5 min before being decanted. The DNA pellet was air-dried for 20 min to eliminate ethanol before adding 50 µL of sterile clean water. The quality and quantity of DNA were measured spectrophotometrically. The sample was then kept at 20°C until used.

Polymerase Chain Reaction (PCR) was used to amplify the cytochrome C Oxidase subunit I (COI) gene using COI_5P_GazFI (5'-TCAACAAATCATAAAGATATTG G-3') and COI-5P_GazR1 primers (5'-ACTTCTGGATGTC CAAAAAAYCA-3'). PCR master mix was prepared by combining 5 µL DNA with 10× Buffer, $MgCl_2$, 10 mm dNTP, 20 µm primers and rTaq polymerase. The PCR program was run with an initial step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 45 sec and elongation at 72°C for 1.5 min, followed by a final extension at 72°C for 10 min. The PCR products were analyzed on a 2% agarose gel (Hossain and Sultana, 2018) and purified by eliminating unused primers and dNTPs with PCR clean-up kits (QIAGEN sciences, MD, USA). The purified double-stranded PCR fragments were forwarded to Invent Bangladesh Ltd. (Dhaka, Bangladesh), for sequencing. Sequencing was performed using the BigDye™ terminator v3.1 cycle sequencing kit (applied biosystem, TX, USA). At least two sequences were obtained for each primer. The obtained sequences for each region were edited with chromas 2.6.6 (Technelysium Pty Ltd, Queensland, Australia). The final sequencing was deposited to the GenBank database for future reference. The resultant sequences were analyzed using the BLAST search program to determine nucleotide sequence homology. The highly homologous sequences were aligned and a neighbor-joining tree was constructed using MEGA 11. A 1000 bootstrap replication was performed to provide statistical support for the nodes in the phylogenetic tree.

In vitro Germination and Seedling Vigor Test

Rice seeds were surface sterilized and divided into four groups of 100 seeds each. The groups were subjected to different experimental treatments to test their germination. The seeds underwent treatment with a liquid seaweed extract by being placed in Petri dishes containing two layers of sterilized filter papers. To initiate the treatment, the filter papers were moistened by adding 5 mL of the liquid seaweed extract to the Petri dishes on the first day of the experiment. This ensured that the seeds were in direct contact with the liquid seaweed extract, facilitating the absorption of beneficial compounds from the extract

by the seeds. Subsequently, for every other day, 1 mL of the liquid seaweed extract was added to maintain the treatment regimen. Seeds placed in Petri dishes treated with distilled water following a similar schedule were used as controls for comparison. Germination was monitored for seven days. The germination percentage and seedling vigour index were calculated using formulas as described by Sultana and Hossain (2022).

Testing the Effect of Seaweed Extract on the Growth of Rice Seedlings

An experiment was conducted to determine the effect of seaweed extract on rice plant growth using a hydroponic medium. The rice seeds were treated with 0.1% NaOCl for 2-3 min and washed with distilled water 2-3 times. The germinated seeds were subsequently transferred to a hydroponic system contained in plastic pots filled with MGRL medium (Hossain *et al*., 2007). Metal nets were utilized on the surface of the medium to provide support for the seedlings during their growth. For the treatments involving the liquid seaweed extract, each pot received an initial dose of 2 mL of the extract on the 1st day. Starting from the third day of the experiment, 1 mL of the liquid seaweed extract was added to each pot daily. Plastic pots receiving an equivalent amount of sterilized distilled water were used as control samples for comparison. After three weeks, the number of plants, plant height, chlorophyll content, fresh biomass weight and dry biomass weight were recorded and the mean values were calculated. Chlorophyll a (Chl a) and chlorophyll b (Chl b) were quantitatively determined using the 80% acetone method by Metzner *et al*. (1965). Fresh leaves (0.2 g) from each treatment were cut and placed into test tubes containing 10 mL of 80% acetone. The samples were left for 48 h in a dark and cool place. Spectrophotometric readings were taken at 645 and 663 nm and the formulas established by Lichtenthaler and Wellburn (1983) were used to calculate Chl a and Chl b:

$$Chl\,a(\mu g/ml) = 12.21(A_{663}) - 2.81(A_{645})$$

$$Chl\,b(\mu g/ml) = 20.13(A_{645}) - 5.03(A_{663})$$

Testing of the Effect of Seaweed Extracts on Drought Tolerance of Rice Seedlings

Rice seedlings were cultivated in a hydroponic system, following the procedures outlined in the previous section. The liquid seaweed extract and control treatments were administered accordingly, adhering to the specified protocol. This involved providing rice seedlings with the designated doses of either the seaweed extract or sterilized distilled water as per the described regimen. When the seedlings reached one week of age, 30 mL of 10% PEG 6000 (polyethylene glycol) solution was added to induce drought stress. This treatment regimen was continued at 2-day intervals. After three weeks of growth, the plants were removed from the pots and the growth parameters were measured and recorded.

Testing of the Effect of Seaweed Extracts on Salinity Tolerance of Rice Seedlings

Rice seedlings were cultivated in a hydroponic system, following the procedures outlined in the previous section. The liquid seaweed extract and control treatments were administered accordingly, adhering to the specified protocol. This involved providing rice seedlings with the designated doses of either the seaweed extract or sterilized distilled water as per the described regimen. When the seedlings reached one week of age, 30 mL of 200 mm NaCl solution was added to induce salt stress. This treatment was repeated at 2-day intervals. After three weeks of growth, the plants were removed from the pots and the growth parameters were measured and recorded.

Testing of the Effect of Seaweed Extracts on Rice Blast Suppression

To assess the effect of seaweed extract on disease suppression, rice seedlings were raised in a hydroponic system, following the procedures outlined in the previous section. The liquid seaweed extract and control treatments were administered accordingly, adhering to the specified protocol. This involved providing rice seedlings with the designated doses of either the seaweed extract or sterilized distilled water as per the described regimen. When rice seedlings were three weeks old, they were inoculated with a spore suspension of *Magnaporthe oryzae.* To prepare the spore suspension, spores were harvested in sterilized distilled water from 15-day-old cultures of *Magnaporthe oryzae* cultured on an Oatmeal Agar medium. A final concentration of spore suspension containing 0.04% Tween 20 was adjusted to 10^5 spores/mL. Rice seedlings were sprayed with spore suspension with a hand automizer until runoff. The spray-inoculated seedlings were covered with polyethene bags to maintain approximately 99% Relative Humidity (RH) and placed in the dark overnight. The polyethylene bags were then removed and the plants were then taken out of a humid chamber and kept under natural light conditions at temperatures ranging from 25-30°C to allow for the development of blast symptoms. and after one week, the plants were inspected for the occurrence of disease incidence and severity. To verify the cause of the disease, the pathogen was isolated again from the affected leaves. The severity of the disease was measured as a percentage, utilizing the subsequent formula:

$$\% = \text{Disease severity index} = (A/B) \times (100/C)$$

A = Sum of disease ratings of individual leaves
B = Total number of leaves
C = Maximum rating

Design of Experiments and Analysis of Data

All studies were designed utilizing a Completely Randomized Design (CRD) approach, as all experiments were conducted either *in vitro* or pot-based settings with homogeneous experimental units. Each treatment was replicated three times to ensure the robustness and reliability of the results. Moreover, the experiment was conducted twice to validate the accuracy of the findings further. Statistical analysis was carried out using the Statistics 10 program package. The data underwent Analysis of Variance (ANOVA) to assess the variability among treatment groups. Subsequently, the means of the treatments were compared using the Least Significant Difference (LSD) test at a 5% probability level ($p \leq 0.05$) to identify any significant differences between treatments. This approach allowed for a comprehensive examination of the experimental results and determination of treatment effects with statistical confidence.

Results

Molecular Identification of Seaweed

In this experiment, two seaweed species, *Hypnea musciformis* and an unidentified species, were collected. Molecular identification was performed on the unknown species, revealing that it belongs to the genus *Gracilaria*. Phylogenetic trees constructed from cytochrome C Oxidase subunit I (COI) gene sequences indicated that the selected seaweed species exhibited 100% similarity with *Gracilaria tenuistipitata* var *liui* (Fig. 1). The sequences of the seaweed were deposited in the GenBank database under accession number JQ407638.1.

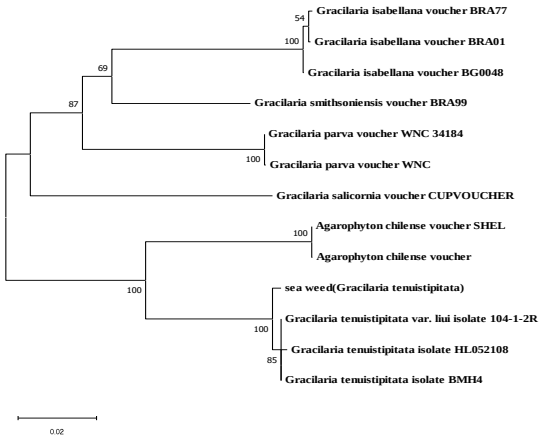

Fig. 1: The phylogenetic tree for the seaweed species *Gracilaria tenuistipitata* was constructed based on cytochrome C Oxidase subunit I (COI) gene sequences

Germination and Seedling Vigour Index of Rice

The application of seaweed liquid extract on rice seeds significantly increased the germination rate and seedling vigour in most cases. Non-treated control plants exhibited a germination percentage of 72%, while treatment with seaweed liquid extract resulted in germination percentages ranging from 68-100% for *H. musciformis* and 76-96% for *G. tenuistipitata*. In the case of *H. musciformis* extracts, treatment $T_5$8 (extracted at 80°C at pH 8) demonstrated the highest germination percentage (100%), which was statistically similar (98%) to treatments $T_1$9 (extracted at 40°C at pH 9) and $T_1$8 (extracted at 40°C at pH 8). For *G. tenuistipitata* extracts, treatment $T_1$9 (40°C at pH 9) exhibited the highest germination percentage (98%), which was statistically similar to treatment $T_2$9 (50°C at pH 9) (96%), followed by $T_1$10 (40°C at pH 10) (93%), $T_2$8 (50°C at pH 8) (92%), $T_2$10 (50°C at pH 10) (92%) and $T_3$9 (60°C at pH 9) (92%) compared to the control (72%) (Table 1).

The seedling vigour index was significantly higher in seaweed extract-treated seeds than in untreated ones. The seedling vigour index of untreated seedlings was recorded at 6408.00, whereas seeds treated with seaweed liquid extracts from *H. musciformis* ranged between 6566.40 and 10064.80. Treatment $T_1$10 (extracted at 40°C at pH 10) exhibited the highest vigour index (10064.80), which was statistically similar to $T_1$9 (9515.20), followed by $T_1$8 (40°C at pH 8) (9180.00). Conversely, the vigour index for *G. tenuistipitata* extracts spanned from 6505.60-9292.00, with $T_1$9 (40°C at pH 9) showing the highest vigour index (9292.00), which was statistically similar to $T_1$8 (40°C at pH 8) (9055.20) and $T_1$10 (40°C at pH 10) (8040.80) (Table 1).

Table 1: Effect of *H. musciformis* and *G. tenuistipitata* extracts on seedling vigor index of rice

	Germination (%)		Seedling vigour index	
Treatment	*Hypnea*	*Gracilaria*	*Hypnea*	*Gracilaria*
$T_1$8 (40°C at pH 8)	96.00^{ab}*	88.00^{c}	9180.00^{b}	9055.20^{a}
$T_2$8 (50°C at pH 8)	84.00^{b}	92.00^{b}	7140.00^{c}	8206.40^{ab}
$T_3$8 (60°C at pH 8)	88.00^{b}	84.00^{d}	6670.40^{e}	8047.20^{ab}
$T_4$8 (70°C at pH 8)	84.00^{b}	84.00^{d}	8400.00^{b}	6512.00^{c}
$T_5$8 (80°C at pH 8)	100.00^{a}	92.00^{b}	6566.40^{e}	7452.00^{b}
$T_1$9 (40°C at pH 9)	98.00^{a}	98.00^{a}	9515.20^{ab}	9292.00^{a}
$T_2$9 (50°C at pH 9)	84.00^{c}	96.00^{a}	8013.60^{c}	9216.00^{a}
$T_3$9 (60°C at pH 9)	80.00^{d}	92.00^{b}	7824.00^{d}	7856.00^{b}
$T_4$9 (70°C at pH 9)	88.00^{bc}	76.00^{c}	7761.60^{d}	6774.40^{cd}
$T_5$9 (80°C at pH 9)	84.00^{c}	80.00^{de}	7728.00^{d}	6960.00^{c}
$T_1$10 (40°C at pH 10)	92.00^{b}	93.00^{b}	10064.80^{a}	8040.80^{ab}
$T_2$10 (50°C at pH 10)	92.00^{b}	92.00^{b}	8188.00^{c}	7776.00^{b}
$T_3$10 (60°C at pH 10)	84.00^{c}	80.00^{de}	8265.60^{c}	6576.00^{c}
$T_4$10 (70°C at pH 10)	92.00^{b}	76.00^{e}	8077.60^{c}	6900.80^{c}
$T_5$10 (80°C at pH 10)	68.00^{f}	76.00^{e}	6800.00^{e}	6505.60^{cd}
Control	72.00^{e}	72.00^{f}	6408.00^{e}	6408.00^{d}

*Average values were based on three replicates. Same letter values within a column were not significantly different ($p<0.05$)

Effect of H. musciformis Extract on Growth of Rice

The seaweed extracts were evaluated for their ability to promote plant growth in rice, revealing that the application of seaweed liquid extract led to increased plant growth and biomass (Fig. 2A). Adding liquid extract of *H. musciformis* to hydroponic culture resulted in higher shoot length, with treatment $T_1$9 (40°C at pH 9) (22.50 cm) exhibiting the highest shoot length, followed by $T_1$10 (40°C at pH 10) (21.50 cm) and $T_1$8 (40°C at pH 8) (20.00 cm), compared to the control treatment (16.50 cm) (Table 2). Similarly, higher root length was observed in $T_1$9 (40°C at pH 9) (18.50 cm), followed by $T_4$10 (70°C at pH 10) (17.50 cm) and $T_1$8 (40°C at pH 8) (16.00 cm). Plant height was also significantly higher in $T_1$9 (40°C at pH 9) (41.00 cm), followed by $T_2$10 (50°C at pH 10) (37.75 cm) and $T_1$8 (40°C at pH 8) (36.00 cm), compared to the control (33.00 cm). Additionally, the total fresh biomass was observed to be higher in $T_1$9 (40°C at pH 9) (1.84 g), followed by $T_2$8 (50°C at pH 8) (1.83 g) and $T_4$10 (70°C at pH 10) (1.82 g) compared to the control (1.17 g). Similarly, the total dry biomass was higher in $T_1$9 (40°C at pH 9) (0.31 g), followed by $T_2$8 (50°C at pH 8) (0.30 g) and $T_1$10 (40°C at pH 10) (0.27 g) compared to the control (0.21 g). These results demonstrate that the extract of *H. musciformis* increased plant height by 5.65-32.25% and dry weight by 4.76-47.62% over the untreated control.

Effect of G. tenuistipitata Extract on the Growth of Rice

The application of liquid extracts of *G. tenuistipitata* resulted in higher plant growth compared to control plants (Fig. 2B). Statistically higher shoot length was observed in $T_1$9 (40°C at pH 9) (26.00 cm), followed by $T_1$10 (40°C at pH 10) (24.75 cm) and $T_1$8 (40°C at pH 8) (24.25 cm), which were all significantly greater than the control treatment (16.50 cm) (Table 3). Similarly, an equally higher root length (17.00 cm) was observed in both $T_1$8 (40°C at pH 8) and $T_1$9 (40°C at pH 9), followed by $T_1$10 (40°C at pH 10) (16.50 cm), which were significantly higher than the control treatment (11.50 cm). The highest plant height was observed in $T_1$9 (40°C at pH 9) (43.50 cm), followed by $T_1$8 (40°C at pH 8) (41.25 cm) and $T_1$10 (40°C at pH 10) (40.75 cm), compared to the control (28.00 cm). Furthermore, the fresh biomass was highest in $T_1$9 (40°C at pH 9) (2.68 g), followed by $T_1$10 (40°C at pH 10) (2.24 g), $T_2$10 (50°C at pH 10) (2.22 g) and $T_1$8 (40°C at pH 8) (2.02 g), compared to the control (1.17 g). Similarly, dry biomass was observed to be higher in $T_1$10 (40°C at pH 10) (0.33 g), followed by $T_1$9 (40°C at pH 9) (0.31 g) and $T_1$8 (40°C at pH 8) (0.30 g), compared to the control (0.21 g). Overall, applying liquid extracts of *G. tenuistipitata* resulted in a significant increase in plant height ranging from 17.86-55.36% and dry weight increased by 18.75-106.25% compared to the untreated control.

Table 2: Effect of liquid extract of *H. musciformis* on growth promotion in rice seedlings

Treatment	Shoot length (cm)	Root length (cm)	Plant length (cm)	Fresh weight (g)	Dry weight (g)
$T_1$8 (40°C at pH 8)	20.00^{a}*	16.00^{b}	36.00^{a}	1.48^{ab}	0.30^{a}
$T_2$8(50°C at pH 8)	16.75^{c}	16.00^{b}	32.75^{c}	1.83^{a}	0.30^{a}
$T_3$8 (60°C at pH 8)	18.00^{b}	15.25^{ab}	33.25^{b}	1.69^{a}	0.27^{ab}
$T_4$8 (70°C at pH 8)	19.75^{ab}	16.00^{b}	35.75^{ab}	1.73^{a}	0.29^{a}
$T_5$8 (80°C at pH 8)	21.00^{a}	15.50^{b}	35.50^{ab}	1.48^{ab}	0.24^{ab}
$T_1$9 (40°C at pH 9)	22.50^{a}	18.50^{a}	41.00^{a}	1.84^{a}	0.31^{a}
$T_2$9 (50°C at pH 9)	20.25^{a}	16.00^{b}	36.25^{a}	1.65^{a}	0.28^{a}
$T_3$9 (60°C at pH 9)	17.75^{bc}	16.00^{a}	33.75^{b}	1.66^{a}	0.27^{ab}
$T_4$9 (70°C at pH 9)	18.25^{b}	15.50^{b}	33.75^{b}	1.44^{ab}	0.24^{ab}
$T_5$9 (80°C at pH 9)	17.50^{bc}	15.00^{b}	32.50^{c}	1.62^{a}	0.27^{ab}
$T_1$10 (40°C at pH 10)	21.50^{a}	16.25^{ab}	37.75^{a}	1.64^{ab}	0.27^{ab}
$T_2$10 (50°C at pH 10)	19.50^{b}	16.00^{b}	35.50^{ab}	1.60^{ab}	0.22^{ab}
$T_3$10 (60°C at pH 10)	19.00^{ab}	16.50^{ab}	35.50^{ab}	1.74^{a}	0.27^{ab}
$T_4$10 (70°C at pH 10)	19.50^{ab}	17.50^{a}	37.00^{a}	1.82^{a}	0.25^{ab}
$T_5$10 (80°C at pH 10)	18.00^{b}	16.50^{ab}	33.50^{b}	1.79^{a}	0.24^{ab}
Control	16.50^{c}	14.50^{c}	33.00^{b}	1.17^{b}	0.21^{b}

*Average values were based on three replicates. Same letter values within a column were not significantly different ($p<0.05$)

Table 3: Effect of liquid extract of *G. tenuistipitata* on growth promotion in rice seedlings

Treatment	Shoot length (cm)	Root length (cm)	Plant length (cm)	Fresh weight (g)	Dry weight (g)
$T_1$8 (40°C at pH 8)	24.25^{ab}	17.00^{a}	41.25^{b}	2.02^{b}	0.30^{a}
$T_2$8(50°C at pH 8)	23.00^{b}	16.00^{b}	39.00^{c}	1.83^{bc}	0.29^{ab}
$T_3$8 (60°C at pH 8)	23.00^{b}	15.50^{b}	38.50^{c}	1.60^{c}	0.23^{c}
$T_4$8 (70°C at pH 8)	21.75^{cd}	14.00^{bc}	35.50^{d}	1.81^{c}	0.27^{b}
$T_5$8 (80°C at pH 8)	22.25^{c}	13.50^{bcd}	35.75^{d}	1.89^{c}	0.26^{b}
$T_1$9 (40°C at pH 9)	26.00^{a}	17.00^{a}	43.50^{a}	2.68^{a}	0.31^{a}
$T_2$9 (50°C at pH 9)	21.00^{cd}	12.00^{d}	33.00^{e}	1.50^{d}	0.19^{d}
$T_3$9 (60°C at pH 9)	20.25^{d}	13.50^{c}	33.75^{e}	1.46^{d}	0.19^{d}
$T_4$9 (70°C at pH 9)	21.50^{cd}	13.50^{c}	35.00^{d}	1.47^{d}	0.19^{d}
$T_5$9 (80°C at pH 9)	23.50^{b}	11.50^{de}	35.00^{d}	1.53^{d}	0.21^{cd}
$T_1$10 (40°C at pH 10)	24.75^{ab}	16.50^{b}	40.75^{b}	2.24^{a}	0.33^{a}
$T_2$10 (50°C at pH 10)	24.00^{ab}	13.50^{cd}	37.50^{c}	2.22^{a}	0.30^{a}
$T_3$10 (60°C at pH 10)	23.50^{b}	11.50^{de}	35.00^{d}	2.09^{b}	0.28^{b}
$T_4$10 (70°C at pH 10)	22.00^{c}	13.50^{c}	35.50^{d}	1.53^{e}	0.21^{cd}
$T_5$10 (80°C at pH 10)	22.00^{c}	12.00^{d}	34.00^{de}	1.50^{d}	0.23^{c}
Control	16.50^{e}	11.50^{de}	28.00^{f}	1.17^{f}	0.16^{e}

*Average values were based on three replicates. Same letter values within a column were not significantly different ($p<0.05$)

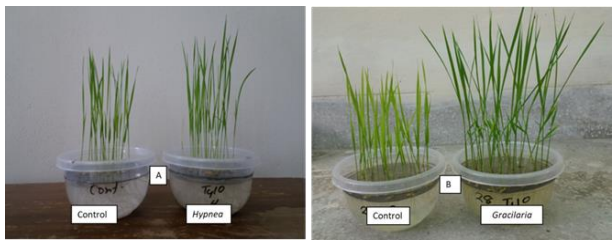

Fig. 2: Effect of *H. musciformis* and *G. tenuistipitata* extracts on rice seedlings growth

Effect of Seaweed Extracts on Chlorophyll Contents of Rice

The present experiment observed that seaweed extracts increased chlorophyll contents in the treated plants, particularly when extraction was conducted at relatively lower temperatures and pH levels. In plants treated with *H. musciformis* extract, Chl a content was highest in plants treated with $T_1$10 (40°C at pH 10) (16.88 µg mL^{-1}), followed by $T_1$8 (40°C at pH 8) (16.87 µg mL^{-1}) and $T_1$9 (40°C at pH 9) (16.57 µg mL^{-1}), compared to the control (12.52 µg mL^{-1}) (Table 4). Similarly, Chl b content was higher in $T_1$10 (7.63 µg mL-1), followed by $T_2$10 (50°C at pH 10) (6.24 µg mL^{-1}) and $T_2$8 (50°C at pH 8) treated plants (6.12 µg mL^{-1}), compared to the control (3.05 µg mL^{-1}) (Table 2). In *G. tenuistipitata* extract-treated plants, the highest Chl a content was recorded in rice plants treated with $T_1$9 (40°C at pH 9) (17.72 µg mL^{-1}), statistically similar to $T_1$8 (40°C at pH 8) (17.65 µg mL^{-1}) and $T_1$10 (40°C at pH 10) (17.60 µg mL^{-1}). Chl b content was also higher in T19 (40°C at pH 9) (7.47 µg mL^{-1}), followed by $T_3$10 (60°C at pH 10) (6.04 µg mL^{-1}), compared to the untreated control (3.05 µg mL^{-1}) (Table 4).

Effect of Seaweed Extracts on Drought Stress Tolerance in Rice

The exposure of rice plants to PEG-induced drought stress led to a general reduction in growth compared to the untreated control. However, the application of *H. musciformis* and *G. tenuistipitata* extracts minimized the impact of drought stress and prevented growth reduction (Fig. 5). For *H. musciformis* extracts, rice plants treated with $T_1$9 (40°C at pH 9) exhibited the highest shoot length (21.75 cm), root length (16.5 cm), plant length (38.25 cm), fresh weight (21.8 dg) and dry weight (4.0 dg) (Fig. 3). These measurements were statistically similar to those of plants treated with $T_1$8 (40°C at pH 8). Specifically, the application of $T_1$9 (40°C at pH 9) of *H. musciformis* extracts increased shoot length by 55.36%, root length by 43.48%, plant length by 50.00%, fresh weight by 100.00% and dry weight by 122.22% over the control.

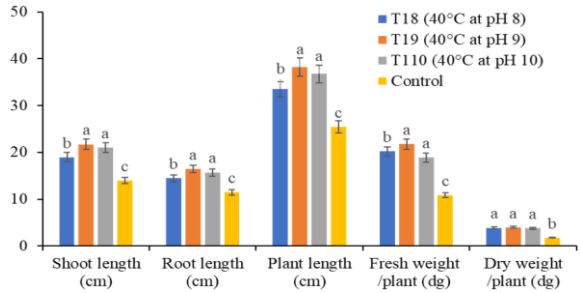

Fig. 3: Effect of liquid extract of *H. musciformis* on the growth of rice seedlings grown under drought stress. Each value represented the average of three replicates. Within the frame, bars with the same letter were not significantly different (p<0.05)

Table 4: Effect of *H. musciformis* and *G. tenuistipitata* extracts on chlorophyll contents of rice seedlings

	H. musciformis		*G. tenuistipitata*	
Treatment	Chlorophyll a (µg mL^{-1})	Chlorophyll b (µg mL^{-1})	Chlorophyll a (µg mL^{-1})	Chlorophyll b (µg mL^{-1})
$T_1$8 (40°C at pH 8)	16.87^{a}*	4.42^{b}	17.65^{a}	4.92^{bc}
$T_2$8 (50°C at pH 8)	14.18^{b}	6.12^{ab}	16.66^{ab}	4.42^{bc}
$T_3$8 (60°C at pH 8)	13.66^{c}	3.98^{c}	15.38^{b}	4.67^{bc}
$T_4$8 (70°C at pH 8)	14.24^{b}	3.29^{c}	12.17^{d}	3.22^{c}
$T_5$8 (80°C at pH 8)	11.23^{d}	2.34^{d}	11.26^{de}	2.53^{d}
$T_1$9 (40°C at pH 9)	16.57^{a}	4.09^{b}	17.72^{a}	7.47^{a}
$T_2$9 (50°C at pH 9)	14.69^{b}	3.44^{c}	17.55^{a}	4.85^{bc}
$T_3$9 (60°C at pH 9)	13.68^{b}	3.04^{c}	17.49^{a}	4.48^{bc}
$T_4$9 (70°C at pH 9)	15.83^{ab}	6.00^{ab}	13.38^{cd}	2.91^{cd}
$T_5$9 (80°C at pH 9)	11.86^{d}	2.68^{d}	9.51^{e}	2.11^{d}
$T_1$10 (40°C at pH 10)	16.88^{a}	7.63^{a}	17.60^{a}	5.28^{b}
$T_2$10 (50°C at pH 10)	15.00^{ab}	6.24^{ab}	14.95^{b}	5.58^{b}
$T_3$10 (60°C at pH 10)	9.19^{e}	2.55^{d}	15.58^{b}	6.04^{b}
$T_4$10 (70°C at pH 10)	11.71^{d}	2.85^{d}	14.80^{bc}	3.36^{c}
$T_5$10 (80°C at pH 10)	11.05^{d}	3.89^{c}	8.95^{d}	1.85^{e}
Control	12.52^{cd}	3.05^{c}	12.52^{d}	3.05^{c}

*Average values were based on three replicates. Same letter values within a column were not significantly different (p<0.05)

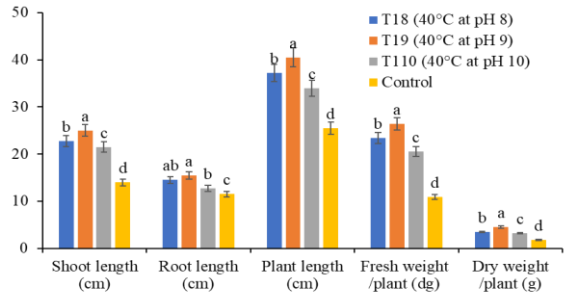

Fig. 4: Effect of liquid extract of *G. tenuistipitata* on the growth of rice seedlings under drought stress. Each value was an average of three replicates. Within the frame, bars with the same letter were not significantly different (p<0.05)

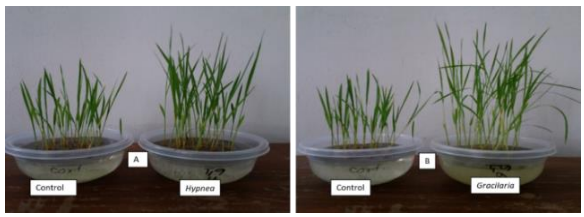

Fig 5: Effect of *H. musciformis* and *G. tenuistipitata* extracts on rice seedlings growth under drought stress

The application of liquid extracts of *G. tenuistipitata* resulted in significantly higher plant growth than untreated control under drought stress (Fig 5B). The highest shoot length (25.00 cm), root length (15.50 cm), plant length (40.50 cm), fresh weight (26.40 dg) and dry weight (4.50 dg) were found in seedlings treated with $T_1$9 (40°C at pH 9), followed by those treated with $T_1$8 (40°C at pH 8) (Fig. 4). Treatment with $T_1$9 (40°C at pH 9) increased shoot length by 78.57%, root length by 34.78%, plant length by 58.82%, fresh weight by 142.20% and dry weight by 150.00% compared to the control treatment.

Seaweed Extract for Salt Stress Tolerance in Rice Seedlings

Exposure of rice seedlings to salt stress led to a decrease in shoot height, root height, as well as fresh and dry weight for both treated and untreated plants. The reduction in seedling growth, as evidenced by decreased plant length and biomass production, was particularly pronounced in salt-stressed untreated control plants. Plants treated with seaweed extracts showed improved growth compared to the control (Fig. 6). In the case of *H. musciformis*, statistically significantly higher values were observed for shoot length (18.00 cm), root length (15.69 cm), plant length (33.69 cm), fresh weight (16.20 dg) and dry weight (3.30 dg) in seedlings treated with T_19 (40°C at pH 9) under saline conditions compared to the control (Fig. 7). Treatment with T_19 (40°C at pH 9) led to a 24.14% increase in shoot length, a 12.07% increase in root length, an 18.21% increase in plant length, a 25.58% increase in fresh weight and a 26.92% increase in dry weight over the control. No other treatment with seaweed extracts showed a significant difference in plant growth compared to the control.

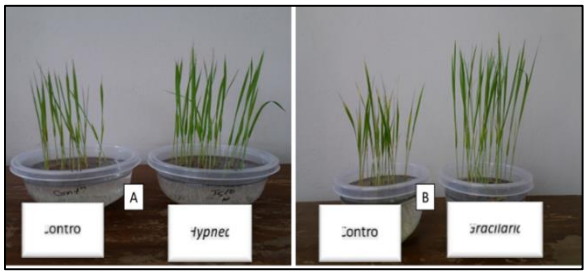

Fig. 6: Effect of *H. musciformis* and *G. tenuistipitata* extracts on rice seedlings growth under salt stress

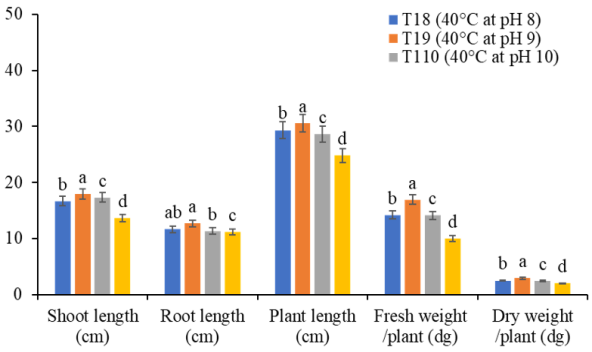

Fig. 7: Effect of liquid extract of *H. musciformis* on rice seedling growth under salinity stress. Each value is an average of three replicates. Within the frame, bars having the same letter are not significantly different ($p<0.05$)

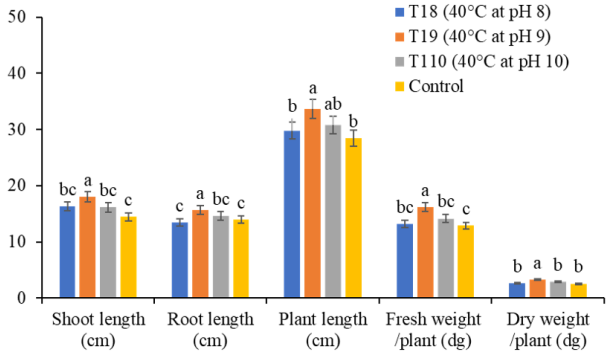

Fig. 8: Effect of liquid extract of *G. tenuistipitata* on the growth of rice seedlings under salinity stress. Each value is an average of three replicates. Within the frame, bars having the same letter are not significantly different ($p<0.05$)

Application of liquid extract of *G. tenuistipitata* under salt stress resulted in higher shoot length compared to the control treatment (Fig. 6B). Among the treatments, T_19 (40°C at pH 9) resulted in the higher shoot length (17.93 cm), root length (12.67 cm), plant length (30.60 cm), fresh weight (16.90 dg) and dry weight (2.90 dg). This was followed by T_110 (40°C at pH 10) and T_18 (40°C at pH 8) (Fig. 8). Treatment with T_19 (40°C at pH 9) led to a 31.16% increase in shoot length, a 13.43% increase in root length, an 23.24% increase in plant length, a 69.00% increase in fresh weight and a 45.00% increase in dry weight over the control.

Effect of Seaweed Extracts on Suppression of Rice Blast

After one week of inoculation, the rice seedlings were examined for blast disease. For *H. musciformis*, the untreated control had the highest disease severity index (37.22%), while the lowest occurred in T_19 (40°C at pH 9) (8.08%) (Fig. 9). Significant reduction in blast severity was also observed in treatment with T_18 (40°C at pH 8) (15.13%) and T_110 (40°C at pH 10) (9.84%), but it was lower than the reduction observed with T_19 (40°C at pH 9). The blast reduction achieved by extracts of *H. musciformis* under treatments T_18 (40°C at pH 8), T_19 (40°C at pH 9) and T_110 (40°C at pH 10) over the control were calculated as 59.35, 78.29 and 73.56%, respectively. Regarding *G. tenuistipitata*, similar trends were also observed, with the untreated control showing the highest disease severity index. At the same time, the lowest was recorded in T_19 (40°C at pH 9) at 7.22%, followed by T_110 (40°C at pH 10) at 8.78% and T_18 (40°C at pH 8) at 10.97% (Fig. 9). The reductions in blast severity achieved by extracts of *G. tenuistipitata* under treatments T_18 (40°C at pH 8), T_19 (40°C at pH 9) and T_110 (40°C at pH 10) over the control were estimated at 70.53, 80.60 and 76.41%, respectively.

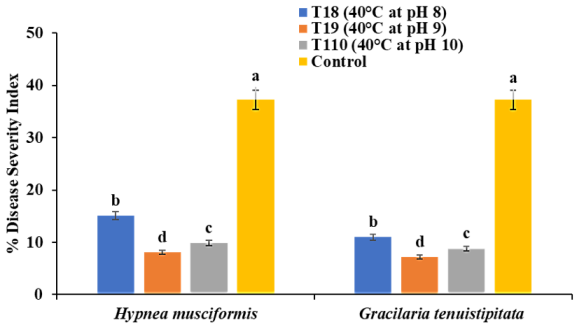

Fig. 9: Effect of seaweed liquid extract of *H. musciformis* and *G. tenuistipitata* on suppression of disease severity of rice blast caused by *Magnaporthe oryzae*. Each value is an average of three replicates. Within the frame, bars having the same letter are not significantly different ($p<0.05$)

Discussion

Seaweeds serve as biofertilizers and biopesticides in sustainable agriculture, addressing this concern and enhancing agricultural productivity. Hence, the current study gathered *H. musciformis* and an unidentified seaweed, examining the effects of their aqueous extracts prepared under varying temperatures and pH levels on rice growth and stress tolerance. The unidentified seaweed was molecularly identified as *G. tenuistipitata* var. *liui*. The Cox's Bazar coast in Bangladesh is renowned for being a rich habitat of both *Hypnea* and *Gracilaria* (Islam *et al*., 2017; 2020). These marine algae represent a promising reservoir of bioactive compounds with potential diverse applications in agriculture (Hossain *et al*., 2024).

Rice is the most important crop in Bangladesh, facing major challenges from diseases, drought and salinity stresses. These problems have become increasingly prevalent in recent years due to the changing climate. Therefore, research into novel agronomic approaches for stress mitigation is critical to ensuring rice productivity. One such strategy is the application of biostimulant sprays to help plants overcome biotic and abiotic challenges by boosting plant growth. Previous research has demonstrated that using seaweed extracts as bio-stimulants improves plant growth and development while also increasing stress tolerance (Nichol *et al*., 2023). However, this method has yet to be tested on rice in Bangladesh. In the present study, rice seeds treated with extracts of *H. musciformis* and *G. tenuistipitata* improved seed germination and seedling vigor of rice. Furthermore, seedlings cultivated in media enriched with seaweed extracts demonstrated enhanced growth and chlorophyll content compared to the control. These findings are consistent with those of prior investigations. A study indicated that liquid seaweed extracts made from *G. textorii* and *H. musciformis* improved seed germination and the quantity of photosynthetic apparatus such as leaf number in brinjal, tomato and chilli plants (Rao and Chatterjee, 2014). Liquid extracts of *G. tenuistipitata* var *liui* spray increased soybean output in the field (Mannan *et al*., 2023). Treatment with seaweed extract in *Vigna radiata* improved vegetative growth, including plant height, leaf count, leaf area and biomass (Punitha *et al*., 2024; Karthik and Jayasri, 2023). Liquid extracts of *Kappaphycus alvarezii, G. edulis, Caulerpa racemosa* and *Sargassum crassifolium* have also been shown to stimulate rice seedling development, germination, productivity and quality (Dumale *et al*., 2016; Layek *et al*., 2015; Sunarpi *et al*., 2019). In soil media containing several doses of inorganic fertilizers and brown algae liquid extracts of *Sargassum crassifolium, Sargassum cristaefolium, Sargassum aquifolium and Turbinaria murayana*, there was an increase in chlorophyll content in leaf, N, P and K content in tissue, growth and yield of rice plants. Furthermore, spraying rice plants with brown algae liquid extracts reduced the need for inorganic fertilizers by 50% (Sunarpi *et al*., 2020). The findings of this study suggested that seaweed extracts from *H. musciformis* and *G. tenuistipitata* could be used to improve rice seed germination and growth.

The liquid extracts of G. *tenuistipitata* and *H. musciformis* were observed to mitigate drought and salt stress while enhancing rice growth. In a previous study, extracts of *G. tenuistipitata* var *liui* improved the growth and yield of soybeans under drought conditions (40% of FC) (Mannan *et al*., 2023). According to Zhang and Ervin (2004), treating creeping bent grass with seaweed extract can improve drought resistance. Rasul *et al*. (2021), in another work on the protective impact of seaweed extracts against drought stress in Arabidopsis, demonstrated how seaweed extract counteracted long-term plant dehydration. Under salt stress circumstances, tomato plants treated with three seaweed extracts showed decreased leaf water potential, higher water use efficiency and increased yield, showing that seaweed extracts had osmo-priming actions (Di Stasio *et al*., 2020). The use of *Ascophyllum nodosum*-based extracts reduced salinity stress in tomato plants (Dell'Aversana *et al*., 2021). According to Du Jardin *et al*. (2020), the application of plant biostimulants such as seaweed extract enhances nutrient use efficiency, stress tolerance and crop quality. Overall, these findings suggest that seaweed extract application can be beneficial in safeguarding plants from osmotic stress induced by seasonal salinization and drought, common occurrences in arid and semi-arid environments.

Rice blast disease represents a significant threat to rice production. In the present study, both seaweed extracts were found to confer protection against the blast pathogen *M. oryzae* in rice plants. In previous studies, the red seaweed *Kappaphycus* sp. and *Eucheuma* sp. derived extracts were found to protect rice against fungal blast disease (Sahana *et al*., 2022). Crown gall disease caused

by the bacterial pathogen *Agrobacterium tumefaciens* was reduced considerably in tomato seedlings after spraying with seaweed extracts (Esserti *et al*., 2017). These results show that seaweed extract may contribute multiple benefits to plants in terms of growth, stress mitigation and productivity.

Seaweed extracts are widely recognized as one of the fastest-growing bio stimulant products currently on the market. Although there has been a recent increase in research studies focused on plant bio stimulants generated from seaweed, the specific molecular mechanism responsible for their effects is still poorly understood (Sujeeth *et al*., 2022). Seaweed extracts contain various bioactive compounds that trigger a range of reactions in plants, such as stress reduction, increased growth of shoots and roots, improved production of chlorophyll, higher yield, delayed ageing and enhanced quality of produce (Calvo *et al*., 2014). Seaweed extracts are recognized for their presence of amino acids, minerals, vitamins and phytohormones, which are believed to play an active role in bio stimulant activity (Castellanos-Barriga *et al*., 2017; Bharath *et al*., 2018; Punitha *et al*., 2024; Karthik and Jayasri, 2023; Mannan *et al*., 2023). Prior studies have demonstrated that when an alkaline extract of *A. nodosum* is applied to Arabidopsis, it can enhance the activation of the cytokinin-responsive promoter of Arabidopsis Response Regulator 5 (ARR5). This finding supports the idea that seaweed extracts may contain substances that can induce hormone-like and hormone-inducing effects (Khan *et al*., 2011). A study conducted by Wally *et al*. (2013) discovered that an extract obtained from *A. nodosum* has the capacity to control the production, amount and proportions of naturally produced cytokinin, auxin and abscisic acid metabolites. Moreover, the study suggested that these alterations could potentially influence the physical characteristics and development of plants. Multiple genetic studies have discovered alterations in the expression of transcripts in plants when treated with seaweed extract (De Saeger *et al*., 2020). The genes that showed differential expression in response to the seaweed extract treatment in this investigation were involved in the regulation of endogenous hormones, photosynthesis and the intake and transport of nutrients (Jannin *et al*., 2013; De Saeger *et al*., 2020). Nevertheless, a study conducted by Rayorath *et al*. (2008) demonstrated that the reported stimulatory effects on barley seed germination and growth are attributed to substances other than the phytohormone Gibberellic Acid (GA3) found in the seaweed extract. Recent research indicates that seaweed extracts contain abundant polysaccharides that can function as metabolic stimulants, hence, affecting plant development (Rasul *et al*., 2021; Pacheco *et al*., 2021). Seaweed-derived carbohydrates, such as laminarin, fucoidan, alginate, carrageenan, ulvan and other non-carbohydrate compounds, have the potential to operate as molecular priming agents or important bioactive substances in plant extracts. These substances can trigger specific responses in plants that have been treated with them (Kerchev *et al*., 2020). Research has shown that plant priming with seaweed extract can modify how plants process nitrogen in their leaves when exposed to high salt levels or osmotic stress. This modification helps plants absorb nutrients more efficiently and reduces the harm caused by the accumulation of Reactive Oxygen Species (ROS). Additionally, it increases the production of antioxidants in plants (Dell'Aversana *et al*., 2021; Di Stasio *et al*., 2020). Seaweed extracts are believed to alleviate osmotic stress by enhancing root morphology, increasing the accumulation of non-structural carbohydrates for greater energy storage, speeding up metabolism and facilitating water adaption (Dalal *et al*., 2019). Plants treated with seaweed extract showed increased stomatal conductance, relative water content and antioxidant activity when subjected to drought stress (Mannan *et al*., 2023). Shukla *et al*. (2018) found that treating soybean plants with seaweed extract reduced the negative effects of drought stress. This was achieved by activating certain genes that respond to stress. Omidbakhshfard *et al*. (2020) conducted a thorough investigation where they analyzed transcriptome, metabolomic and lipidomic data to understand the specific molecular mechanisms behind the development of oxidative stress tolerance in Arabidopsis, which was caused by the seaweed bio stimulant. The oxidative stress alone activated genes associated with autophagy and ROS-induced programmed cell death, but these genes were not active in plants that had been pretreated with seaweed extract and then exposed to oxidative stress (Omidbakhshfard *et al*., 2020). In addition, different types of carrageenans and other sulfated polysaccharides extracted from various marine algae have been found to possess antimicrobial properties and can activate various defense mechanisms against different types of pathogens. This ultimately results in improved suppression of diseases (Sarkar *et al*., 2018; El-Sheekh *et al*., 2021; Hossain *et al*., 2024). These findings suggest that the presence of nutritional components, phytohormones and elicitors in seaweed extracts can help promote growth, reduce stress and suppress diseases in rice plants. Further in-depth research is necessary to determine the specific and combined effects of seaweed-derived polysaccharides and other components on enhancing cell division and plant growth.

The quantity and availability of bioactive in seaweed extracts vary depending on the extraction procedure used, resulting in different effects on plant development and stress tolerance (Guinan *et al*., 2013; Dell'Aversana *et al*., 2021).

Therefore, it is crucial to ascertain the ideal extraction conditions to maximize their impact on plants. The current investigation revealed that the most favourable extraction conditions for seaweeds included boiling at a low temperature of 40°C, with a pH of 9 producing improved outcomes compared to pH 8 and pH 10 in several studies. In their study, Godlewska *et al*. (2016) investigated two techniques for producing aqueous extracts from Baltic seaweeds: Boiling and soaking. The algal extract obtained by immersing biomass did not exhibit any inhibitory activity against Escherichia coli and Staphylococcus aureus. The only extract that exhibited an inhibitory effect against *E. coli* was the one that had been cooked. Prior studies have shown that boiling seaweed extracts at low temperatures effectively preserves heat-sensitive bioactive components (Zheng *et al*., 2019; Moreira *et al*., 2021). The boiling extraction process used for seaweed fertilizer manufacture at higher temperatures might cause damage to heat-sensitive components, such as vitamins and phytohormones (Zheng *et al*., 2019; Moreira *et al*., 2021). In addition, the extraction efficiency of seaweed can be enhanced by performing alkaline hydrolysis at higher pH values (Echave *et al*., 2021). Thus, it is reasonable to suggest that seaweed extracts produced under low-temperature conditions and at a pH of 9 possess the highest concentration of bioactive chemicals. Consequently, these extracts have the potential to stimulate plant growth and improve the ability of rice plants to withstand both biotic and abiotic stresses. However, further research is essential to elucidate the optimal conditions for seaweed extraction to maximize bioactivity.

Conclusion

The present study underscores the potential of seaweed extracts derived from *H. musciformis* and *G. tenuistipitata* var. *liui* as biostimulants, bioameliorators and biopesticides in sustainable agriculture, particularly in addressing the challenges faced by rice cultivation in Bangladesh. Our findings highlight the efficacy of these extracts in enhancing rice seed germination, seedling vigour and growth under drought, salinity and rice blast disease caused by *Magnaporthe oryzae*. While the specific molecular pathways involved in seaweed extract-mediated stress mitigation remain to be fully elucidated, existing research suggests the involvement of various bioactive compounds, including phytohormones, nutrient elements and polysaccharide elicitors. Our results also indicate that aqueous extracts prepared under a low temperature of 40°C and at a pH of 9 exhibit superior efficacy in promoting plant growth and stress resilience, implying the importance of refining extraction protocols to preserve heat-sensitive bioactive components and enhance extraction efficiency. These findings provide valuable insights into the positive impact of seaweed extracts on plant growth and both abiotic and biotic stress tolerance in rice as well as their potential for sustainable agriculture by reducing the reliance on chemical inputs in rice cultivation. Further research is warranted to unravel the intricate mechanisms underlying the bioactivity of seaweed extracts and to optimize their application in the field for improved crop resilience and yield stability. However, overcoming the challenges of insufficient supply of desired seaweeds at the right price, along with regulatory support for their agricultural use, is crucial for fostering widespread utilization and research on seaweeds in Bangladesh.

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Author's Contributions

Mir Sayeada Alam: Drafted, research implementation and data collection.

Jannatun Nayeema: Data analysis, drafted and revision.

S. M. Rafiquzzaman: Data interpretation and revision.

Md. Motaher Hossain: Conception, research designed and revision.

Ethics

This article presents original content that has not been published elsewhere. The corresponding author ensures that all co-authors have reviewed and approved the manuscript, with no ethical concerns raised. The corresponding author relates that no ethical concerns exist on the publication of this study.

Conflict of Interest

The authors have no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author. Reasonable request.

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