Int. J. Biol. Res., 11 (1-2): 31-59, 2023.

EFFECTS OF TWO DIFFERENT MACROALGAL BIOCHARS ON PHYSIOLOGY AND PHOTOSYSTEM II FUNCTIONALITY OF RICE (*ORYZA SATIVA* L.) UNDER SALT STRESS

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ABSTRACT

Rice (*Oryza sativa* L.) var. Daimond was grown under salt stress, and the effects of macroalgal based biochars on its physiology, photochemistry and growth were examined. Red and brown marine macroalgae were collected from the coast of Karachi and dried to make algal biochar. Two concentrations i.e. 0.5% and 1% of both red and brown algae were used separately. Rice seedlings were subjected to 100 and 150mM salt stress after 21 days of sowing and salinity was maintained till 14 days. Physiological and photochemical analysis in our research has demonstrated the potential benefit of using algal biochar for alleviating salt stress in rice plants. The present study discovered that with the application of algal biochar, the stress markers like H_2O_2 and MDA have reduced under salt stress which is also evident by the decrease in heat dissipation (DIo/RC) and absorbance per reaction centers (ABS/RC) and increase in trapping (TRo/RC) and Electron transport (ETo/RC) which ultimately leads to the improvement in overall growth and physiology of rice plants under high salinity. Algal biochar maintained the relative water content (RWC) of rice plants and also enhanced the PSII functionality and chlorophyll content index (CCI) by improving qP and electron transport chain under salt stress. The OJIP transients for the current work exhibited a decrease in I and P values with the increasing salinity. Red and brown algal biochar-containing plants exhibited an upsurge in the I and P steps, suggesting that the PSII reaction centers were more active under salt stress. Overall, the study highlights the potential of macroalgal biochar as a sustainable and environment friendly approach for enhancing rice plant growth and performance under salt stress conditions.

Keywords: Algal biochar, photochemistry, salt stress, Rice (*O. sativa*), macroalgae

INTRODUCTION

Plants thrive in dynamic environments where they usually encounter a variety of challenges including abiotic stress like heat, cold, salinity, and drought (Zhu, 2016). The growth, development, and productivity of crops are reduced globally due to abiotic stresses. Salinity is an abiotic stress factor which has impacted an extensive land area of approximately 1,128 million hectares (Wicke *et al.,* 2011). Salinization is a complex mechanism that has damaging consequences on crop plant biochemical and physiological pathways (Nabati *et al.,* 2011). Excess Na^+ accumulation causes cytosolic K^+ and Ca^{2+} efflux, resulting in an imbalance in their cellular homeostasis, a lack of nutrients, oxidative stress, cell death and retarded growth (Ibrahim *et al.,* 2020). Many preceding studies have found that high levels of salinization have a negative impact on plant photosynthesis due to stomatal constraints such as stomatal closure (Ahanger *et al.,* 2017) and/or non-stomatal restrictions including chlorophyll malfunctioning (Lee *et al.,* 2020), impairment of enzymatic proteins and membranes of photosynthetic apparatus (Xu *et al.,* 2014), and chloroplast ultrastructure destruction (Kumar *et al.,* 2020).

A significant staple crop in many developing countries is rice (*Oryza sativa* L.). It provides between 50 to 80% of the daily calories consumed by more than 50% of the world's population. Under the current practices, there is simply not enough arable land to meet the demand for the increased production of rice. Furthermore, a significant portion of global food production will be subjected to abrupt environmental conditions and abiotic stresses as a consequence of climate change. Scientists have been looking for long-term ways to reduce the effects of salt on crops in order to increase yield over the past few decades. Recent studies have offered various creative and alternative methods to shield plants from the detrimental consequences of salt stress (Yu *et al*., 2019). One approach is to use the organic fertilizers and most importantly biochar in agricultural practices in order to prevent the soil deformation against salinity.

Biochar is regarded as one of the finest organic fertilizers since it has its resilience and the capacity to adhere to nutrients that prevent leaching. Biochar has gained a great deal of attention in the context of global climate change. The kinds of feedstock and manufacturing processes have an impact on the characteristics of biochar. Sewage sludge, plastics, agricultural waste, woody waste, algae, manures and other suitable feedstocks are the most frequently used feedstock for the production of biochar (Kumar *et al.,* 2015; Gunasundari and Kumar 2016, 2017). Biochar is used as a supplement in agricultural fields to promote plant growth. By raising the pH, organic carbon content, water holding capacity, and cation exchange capacity of the soil, biochar helped plants to develop and thrive (Wu *et al.,* 2019). Algal biomass, a simple aquatic microorganism, can be found abundantly in sediments, soils, shallow or deep seas, lakes, rivers, estuaries, and lagoons. It is recognized as the third generation of biofuels, offering numerous advantages over the first and second generations. These benefits include simple nutrient requirements such as sunlight and CO_2, reduced dependency on portable water sources, and the ability to thrive on non-arable land (Lee *et al.,* 2020; Xu *et al.,* 2014; Kumar *et al.,* 2020). Worldwide, there are approximately 20,000 species of algae, varying in size from 1 to 400 micrometers. They are broadly classified into two categories: macroalgae, commonly known as seaweed, and microalgae. Macroalgae include red algae (Rhodophyta), green algae (Chlorophyceae), and brown algae (Phaeophyceae). Algae generally possess a high moisture content, constituting approximately 90% of their weight (Lawton *et al.,* 2013; Nagappan *et al.,* 2019). Macroalgae are important to the environment and economy because they provide biomass for food, phycocolloids, soil additives, animal feed, neutraceuticals, and essential ecosystem services. In addition to being employed for the bioremediation of eutrophic and wastewaters (Mehta and Gaur, 2005), macroalgae also sequester additional elements including heavy metals due to their rapid growth rates (Mata *et al.,* 2010), and also have the ability to absorb nitrogen (N) and phosphorus (P) (Neori *et al.,* 2003). A value-driven strategy for carbon sequestration and recycling nutrients is to employ macroalgal biomass for the production of biochar (charcoal), which has the potential to generate energy (Bird *et al.,* 2011). Algal biomass has become increasingly recognized as an alternative for restricting environmental consequences and maximizing overall production in both agriculture (Craggs *et al.,* 1996) and aquaculture (Barrington *et al.,* 2009).

Due to their high carbohydrate content and low lipid content, macroalgae (seaweed) are typically employed to produce significant yields of biochar. In order to achieve the desired small size, the macroalgae must be ground. (Adams *et al.,* 2011; Brennan and Owende 2010). The addition of biochar to the soil significantly enhanced the average pH of the red soil and decreased the levels of NH^{+4}-N and NO^{-3}-N by 60.1% and 42.7%, respectively (Panwar *et al.,* 2019; Xia *et al.,* 2020). Research studies have shown that algae have the ability to reduce salt stress during the germination stage of bell pepper (*Capsicum annuum* L.) seeds (Hossain *et al.,* 2021). Studies have indicated that extracts derived from different algal species can enhance the salt tolerance of wheat while increasing the antioxidant capacity and protein content of the grains (Ammar *et al.,* 2022). Macroalgae and microalgae are utilized for the reclamation of calcareous and saline soils due to their ability to thrive in diverse soil conditions, including high pH and salinity. Blue-green algae have multiple impacts on plant nutrition. They produce growth-promoting compounds, mitigate the adverse effects of salt stress, enhance soil water content and biomass during decomposition, and facilitate growth promotion. Moreover, cyanobacteria contribute to soil nutrient availability by fixing nitrogen and releasing organic acids, thereby increasing soil phosphate and nitrogen content. Numerous studies have documented the beneficial effects of cyanobacterial inoculation in reducing salt stress on plants (Wei *et al.,* 2019). Different cyanobacteria present in saline soils produce various growth-regulating compounds that facilitate seed germination. For example, Abideen *et al.* (2018) demonstrated that gibberellic acid, produced by the *cyanobacterium Scytonemahofmanni*, enhanced the germination and seedling growth of rice in a saline environment. Moreover, when cyanobacterial extracts were used instead of control treatments, *Lupinus termis* seeds treated with growth regulators like indole acetic acid, gibberellic acid, and cytokinins exhibited significantly higher germination rates. Similarly, the germination and germination rate of rice seedlings improved considerably when treated with four species of the cyanobacterium and Anabaena (Aljasmi *et al.,* 2021). Studies suggested that biochar, through the adsorption of harmful sodium ions and the promotion of essential ion release such as calcium, magnesium, and potassium, enhances plant growth under abiotic stress (Neori *et al.,* 2003; Mata *et al.,* 2010). This improvement can be attributed to the organic nature of biochar and its positive impact on the physicochemical properties of saline soil. According to the literature, algal biochars' characteristics can help plants withstand salinity in the soil.

In the current study, two distinct macroalgal based biochars including red and brown macroalgae were tested for their effects on the physiology and biomass of rice plant under salinity independently. The purpose of the experiment was to monitor the fluorescence kinetics in relation to the growth parameters. The objective of this study was to assess the impact of algal biochar on the development and physiology of rice plants under salt stress.

MATERIALS AND METHODS

Seed Source and Selection

Rice (*Oryza sativa* L.) var. Diamond was obtained from the Stress Physiology Phenomic Centre, Department of Botany, University of Karachi. Prior to the experiment, One hundred and fifty rice seeds were scrupulously washed with 100 ml of autoclaved distilled water (three times) and surface sterilized for 3 minutes in a beaker with 100 ml of a 10% concentrated sodium hypochlorite solution.

Algae collection and making of biochar

Red macroalgae (*Gracilaria, Gelidium and Melanothamnus***)** and brown macroalgae (*Sargassum*) were collected from Buleji beach at Karachi coast on October 14th, 2021. All samples were washed thoroughly with clean water multiple times and completely air dried. Dried samples of both red and brown macroalgae separately crushed and grounded to powder form to make biochar.

Experimental setup:

The rice seeds were cultivated in four pots (replicates) for each treatment, with each pot containing 500g of sterile sandy loam soil. The soil composition consisted of 9% clay, 17% silt, and 74% sand. Initially, the seeds were germinated in 10kg of soil (a mixture of garden soil and organic fertilizer at a ratio of 1:10) until they reached the two-leaf stage. Following thinning, five seedlings were transplanted into individual plastic containers. The experiment took place at the Stress Physiology Phenomic Centre, Department of Botany, University of Karachi, under ambient environmental conditions. A fully randomized design was used to set up the experiments. Two different experiment sets were run, with and without application of algal biochar. Red and brown algae were investigated separately. The soil was composed of 4.10% organic carbon, 0.83% total nitrogen, 7.1% silt, 8.1% clay, and 80.5% sand particles. The electrical conductivity of the soil was 1.7dS.m^{-1}. The average day-night temperature range for rice cultivation was 33 ± 4^{o}C to 22 ± 3^{o}C. Twenty-one days old seedlings were treated to different salt concentrations using a gradual increment method until they reached 100 and 150 mM NaCl. In order to obtain the appropriate concentration, a solution of 25mM NaCl was applied to 21-day-old seedlings on alternate days while maintaining the soil's moisture levels as needed. All rice pots were given fully saturated environment. Four replicates of the treatments and controls were used in the experiment's overall setup. Following a 14-day period of saline treatment, the plants were harvested.

Harvest and Growth Parameters:

Plants were harvested two weeks after being subjected to salt stress, and biomass, shoot length, and other growth traits were measured. Plant material was dried for 24 hours at 80°C in an oven. Each plant's freshly harvested leaves were immediately frozen and kept at -20°C for additional biochemical evaluations.

Relative water content:

The relative water content (RWC) was calculated using the Barrs and Weatherley (1962) method with minor adjustments. Randomly selected leaves from both the control and treatment rice samples, with an area of 4 × 0.5 cm^2, were collected, and their fresh weight (FW) was recorded. Subsequently, the leaves were placed in 90 mm-diameter Petri plates filled with distilled water for a duration of 12 hours. After removing the leaves from the Petri plates, their turgid weight (TW) was measured. To determine the dry weight (DW), leaf samples were dried in an oven at 80°C for 48 hours. Applying the following formula, RWC was determined:

$$\boldsymbol{RWC = \frac{FW - DW}{TW - DW} \times 100}$$

Quantum Yield Photochemical Efficiency:

Prior to harvest, the chlorophyll fluorescence of twenty randomly selected young rice leaves was measured using a chlorophyll fluorescence meter (model OS-30p+, Opti-Science, United States). A leaf was first exposed to darkness for 30 minutes using leaf clips and then subjected to a modulated measurement beam of far-red light from an LED source with a peak wavelength at approximately 735 nm. The initial fluorescence yield (Fo) and maximum fluorescence yield (Fm) were determined using 1.6 pulses of saturating light (6.8 µmol m^{-2} s^{-1} PAR) and a weakly modulated red light (0.5 µmol m^{-2} s^{-1}). The variable fluorescence yield (Fv) was calculated by subtracting Fo from Fm using the equation Fm-Fo. The ratio of variable to maximum fluorescence (Fv/Fm) was determined based on the dark-adapted quantum yield of photochemical efficiency, and non-photochemical quenching were calculated following the methodology explained by Maxwell and Johnson (2000).

Chlorophyll Content Index (CCI):

The chlorophyll content index (CCI) of young leaves in both the treated and control groups were measured between 9:00 and 11:00 am using a chlorophyll content meter (Model CL-01, Hansatech Instruments, UK). The measurements were taken using intact leaves, and the average CCI values from five leaves in each replication were presented in bar graphs.

H_2O_2 Content:

The total hydrogen peroxide (H_2O_2) content was determined using a method described by Velikova *et al.*, Freshly collected leaf samples were homogenized in an ice bath with 3 mL of 0.1% (w/v) trichloroacetic acid (TCA). The resulting homogenate was then centrifuged at 12000 rpm for 15 minutes. Subsequently, 0.5 mL of the supernatant was mixed with 1 mL of 1M potassium iodide (KI) and 0.5 mL of 10 mM phosphate buffer (pH 7.0). The optical density of the supernatant was measured at 390 nm. The H_2O_2 content was determined by referring to a standard curve and expressed in µmole per gram of fresh weight (µmole g^{-1}FW).

$$H_2O_2 Content = Ve \times R \times \frac{D.F}{Vs} \times W$$

Malondialdehyde content (MDA):

The Malondialdehyde (MDA) content was determined using the method described by Carmak and Horst (1990). A sample of fresh leaves weighing 0.5g was homogenized in 10 ml of 0.1% (w/v) TCA (trichloroacetic acid) and subsequently centrifuged at 12000 rpm for 10 minutes. After centrifugation, 1 ml of the supernatant was mixed with 4.5 ml of 0.5% TBA (thiobarbituric acid). The reaction mixture was then heated in a water bath at 95 °C for 30 minutes and subsequently cooled in an ice bath to stop the reaction. The absorbance of the clear solution was measured at 532 nm and 600 nm wavelengths. An extinction coefficient of 155/mM/cm was used to determine the MDA concentration.

$$Conc.of MDA(\mu M) = \frac{(A_{532} - A_{600})}{155}$$

Statistical analysis:

Three replicates were utilized in each experiment. **Excel 2017** was used to create the graphs, and the statistical analysis of the data was done to determine the mean and standard errors. The IBM SPSS Statistics (version 20.0) software (Duncan 1955) was used to analyze all of the data gathered for this study. The results of the multiple range test were plotted on a bar graph as tiny alphabets to show the differences between mean values ($P < 0.05$).

RESULTS

Plant Growth and Development

Red algae are found to have a significant positive impact on overall shoot development when compared to other types of algae when used as a bio-fertilizer in unrestricted environments. The increase in shoot fresh weight was marginally more pronounced at 0.5% red algal biochar concentration than at 1%, where it increased by 129% as compared to 120% (Supplementary Table 1). On the other hand, brown algae that weren't exposed to salt stress had less development than the control (Fig. 1). When exposed to high salinity (150 mM), shoot fresh weight was substantially reduced by 61% when compared to the non-stressed control environment. However, under high salinity, biochar made from both red and brown algae substantially increased the shoot's fresh weight. (150mM). Shoot fresh weight increased by 135% under stress in 0.5% red algae biochar compared to the 150mM control. However, upon application of 1% red algae biochar, shoot fresh weight was increased by 75% under stress as compared to control (150mM). Shoot fresh weight increased by 66% under stress in 0.5% brown algal biochar, while 1% brown algal biochar significantly increased the shoot fresh weight under high salinity of 150mM, up to 85% compared to control (150mM without algal biochar). Root fresh weight was significantly increased by 266% and 176% in 1% red and brown algal biochar without stress respectively when compared to control (Fig. 1). However red algae showed more pronounced results across the treatments with and without stress. Red algal biochar 0.5% and 1% both increased the root fresh weight by 64% and 266%, respectively compared to control without stress. While in brown algal biochar, 0.5% led to decrease in root fresh weight by 39% compared to control, while root fresh weight was increased by 176% in 1% brown algal biochar, compared to control without stress (Supplementary Table 2). Under high salt stress of 150mM, both concentrations of red and brown algal biochar showed significant increase in root fresh weight, except for 1% brown algal biochar which reduced the root fresh

weight by 25% compared to control (150mM without biochar). While 0.5% brown algal biochar significantly increased RFW by 13% compared to control (150mM without biochar).In contrast, both 0.5% and 1% red algal biochar concentrations substantially increased RFW under high salinity by 27% and 3%, respectively, when compared to 150mM without biochar.

Shoot length was found to be increased in both concentrations of 0.5% and 1% red algae and in 0.5% brown algae compared to control without biochar application, with the exception of 1% brown algal biochar, which decreased shoot length by 12% compared to control (Fig. 2).. In comparison to the control, brown algae biochar 0.5% elongated the shoots by 16%. When compared to the control without stress, 0.5% biochar based on red algae increased shoot length by 63% and 1% increased shoot length by 47% under 150mM salt stress without biochar, shoot length was noticeably decreased by 67% compared to the control under no stress (Supplementary Table 1). While 0.5% and 1% red algal biochar additions increased the shoot length under high salinity conditions of 150 mM by 163% and 154%, respectively. On the other hand, under high salinity, brown algal biochar exhibited a more pronounced increase of 27% in 0.5% biochar as opposed to 1%, which extended the shoot by 15% (Supplementary Table 2). Compared to brown algae biochar, red algae-based biochar displayed more pronounced effects and increased growth. Root length was substantially improved under both red and brown algal biochar at both concentrations in high salinity of 150mM (Fig. 2). In comparison to the 150mM control, the root length in 0.5% red and brown algal biochar significantly elevated by 63% and 60%, respectively. In contrast to the 150mM control, the root length was significantly increased by 26% and 69%, respectively, by 1% red and brown algal biochar. 0.5% and 1% red algal biochar decreased the root length by 1% and 16%, respectively, in a non-stress control environment. Under non-stress conditions, brown algal biochar 0.5 and 1% decreased the root length by 15% and 8%, respectively, in comparison to the control. In comparison to the non-stressed control, the high salinity of 150 mM without biochar addition substantially reduced the root length by 15%.

Relative water content and Chlorophyll Content Index (CCI)

Relative water content was improved by 14% and 8% in both red and brown algae respectively in 0.5% algal biochar as compare to 1% under non-stressed control environment (Fig. 3). In 1% red algal biochar, RWC was increased by 9% compared to non-stressed control. Under high salinity of 150mM, RWC drastically reduced to 32% compared to control. The application of algal biochar significantly improved RWC under high salinity of 150mM. RWC was significantly increased in 0.5 and 1% red algal biochar by 33 and 29% respectively compared to 150mM control. In contrast, the RWC was markedly enhanced by brown algae 0.5 and 1% biochar compared to 150mM control, by 35 and 19%, respectively (Supplementary Table 1 and 2). Chlorophyll Content Index (CCI) was significantly elevated by 15% in 0.5% red algal biochar under control conditions as compare to 1% and control (Fig 3). However, both red and brown algae significantly increased CCI in 100mM salt stress but 1% algal biochar of both red and brown algae exhibited more pronounced improvement in CCI under 100mM salinity. At the salinity level of 100mM, 0.5 and 1% red algal biochar substantially enhanced CCI by 41 and 75% respectively compared to 100mM control. At the salinity level of 150mM, 0.5 and 1% red algal biochar significantly improved CCI by 434% and 361% respectively compared to 150mM control. Brown algal biochar 0.5 and 1% improved CCI by 55% and 162% under 100mM salinity respectively compared to control. While at 150mM salinity level, 0.5 and 1% brown algal biochar increased CCI by 41% and 90%, respectively compared to control. CCI drastically reduced to 72% and 86%In 100 and 150mM salt stress respectively without algal biochar (Supplementary Table 1 and 2).

Oxidative stress

The effect of two different algal biochar on Melondialdehyde (MDA) content and Hydrogen peroxide (H_2O_2) was observed under salt stress (Fig 4). MDA was significantly reduced by 54% and 39% in 0.5% red and brown algal biochar respectively compared to control and 1% algal biochar under high salinity of 150mM.In 150 mM salinity, 1% algal biochar concentration decreased the MDA content in red and brown algae by 28% and 18%, respectively, in comparison to the control. MDA content was elevated by 156% under 150mM salinity without algal biochar compared to control (Supplementary Table 1 and 2). In comparison to control, 0.5% red algal biochar application without salt stress resulted in a 74% decrease in MDA concentration.

Application of algal biochar under high salinity of 150mM resulted in the reduction of hydrogen peroxide (Fig. 4). Brown algal biochar considerably decreased the level of H_2O_2 by 43% and 38% respectively in 0.5% and 1% algal biochar compared to 150mM salt stress (241%). However, red algal biochar reduced the levels of H_2O_2 by 27% and 29% respectively in 0.5% and 1% red algal biochar compared to 150mM salt stress (241%). Hydrogen peroxide elevated by 241% under high salinity of 150mM compared to control (Supplementary Table 1 and 2). A slightly higher value of H_2O_2 was recorded in plants added with brown algal biochar under non stressed environment.

Table 1. Red algal Biochar (% Increase).

Comparisons	RL	SL	SFW	RFW	RWC	H_2O_2	MDA
Cntrl Vs 0.5%	-1%	63%	129%	64%	14%	-16%	-74%
Cntrl Vs 1%	-16%	47%	120%	266%	9%	-10%	5%
Cntrl Vs 150%	-15%	-58%	-61%	-52%	-32%	241%	156%
Cntrl Vs 0.5 + 150	38%	10%	-9%	-39%	-9%	148%	17%
Cntrl Vs 1% + 150	7%	6%	-32%	-50%	-12%	143%	84%
150 vs 0.5% + 150	63%	163%	135%	27%	33%	-27%	-54%
150 vs 1% + 150	26%	154%	75%	3%	29%	-29%	-28%

Comparisons	Fv Fm	Fv Fo	O	J	I	P	PI	CCI	qP	NPQ	ABS/RC	TRo/RC	DIo/CS	ETo/RC
Cntrl Vs 0.5%	-3%	-5%	56%	69%	68%	68%	-39%	15%	31%	-30%	-4%	6%	-10%	-1%
Cntrl Vs 1%	-2%	7%	26%	30%	41%	50%	-8%	-35%	24%	-20%	-2%	2%	-20%	2%
Cntrl Vs 100%	-8%	-19%	17%	10%	-3%	-7%	-48%	-59%	-2%	50%	25%	-24%	84%	-26%
100 Vs 0.5% + 100	10%	56%	2%	19%	48%	60%	42%	41%	25%	-53%	-20%	25%	-54%	34%
100 Vs 1% + 100	8%	58%	-1%	12%	41%	54%	50%	75%	19%	-32%	-17%	14%	-49%	31%
Cntrl Vs 150%	-21%	-45%	-19%	-25%	-25%	-20%	-87%	-85%	-14%	54%	52%	-37%	129%	-38%
Cntrl Vs 0.5% + 150	1%	27%	16%	24%	37%	44%	-38%	-21%	5%	0%	8%	-23%	3%	-13%
Cntrl Vs 1% + 150	-2%	3%	6%	5%	15%	17%	-44%	-32%	5%	20%	12%	-28%	35%	-6%
150 vs 0.5% + 150	27%	129%	42%	65%	83%	80%	373%	434%	22%	-35%	-29%	21%	-55%	41%
150 vs 1% + 150	23%	86%	30%	40%	53%	47%	327%	361%	22%	-22%	-26%	14%	-41%	52%

Table 2. Brown algal Biochar (% Increase).

Comparisons	RL	SL	SFW	RFW	RWC	H_2O_2	MDA
Cntrl Vs 0.5%	-15%	16%	-33%	-39%	8%	63%	-22%
Cntrl Vs 1%	-8%	-12%	-25%	176%	-1%	20%	-1%
Cntrl Vs 150%	-15%	-58%	-61%	-52%	-32%	241%	156%
Cntrl Vs 0.5% + 150	36%	-47%	-35%	-45%	-8%	94%	56%
Cntrl Vs 1% + 150	44%	-52%	-28%	-64%	-19%	110%	110%
150 vs. 0.5% + 150	60%	27%	66%	13%	35%	-43%	-39%
150 vs. 1% + 150	69%	15%	85%	-25%	19%	-38%	-18%

Comparisons	Fv Fm	Fv Fo	O	J	I	P	PI	CCI	qP	NPQ	ABS/RC	TRo/RC	DIo/CS	ETo/RC
Cntrl Vs 0.5%	2%	28%	12%	35%	44%	48%	-17%	-39%	25%	-40%	-14%	-4%	-17%	9%
Cntrl Vs 1%	1%	25%	-7%	-12%	18%	17%	13%	-8%	8%	-25%	-19%	0%	-12%	10%
Cntrl Vs 100%	-14%	-29%	17%	10%	-3%	-7%	-48%	-72%	-2%	50%	25%	-17%	86%	-26%
100 Vs 0.5% + 100	18%	73%	0%	4%	25%	35%	51%	55%	18%	-53%	-19%	14%	-47%	39%
100 Vs 1% + 100	20%	86%	-4%	20%	47%	52%	66%	162%	12%	-33%	-26%	22%	-58%	43%
Cntrl Vs 150%	-20%	-45%	-19%	-25%	-25%	-20%	-87%	-86%	-14%	50%	52%	-30%	130%	-38%
Cntrl Vs 0.5% + 150	-2%	10%	10%	21%	26%	26%	-34%	-80%	14%	-10%	3%	-6%	-2%	-8%
Cntrl Vs 1% + 150	1%	23%	16%	15%	34%	42%	-17%	-73%	1%	30%	1%	-26%	-24%	-1%
150 vs 0.5% + 150	23%	98%	35%	61%	68%	58%	409%	41%	32%	-40%	-32%	35%	-58%	49%
150 vs 1% + 150	27%	123%	43%	53%	78%	77%	539%	90%	17%	-13%	-34%	7%	-67%	60%

Table 3. Correlation table of effects of red algal biochar on rice growth and physiology under salt stress.

	SL 1	*SL 2*	*SL 3*	*RL 1*	*RL 2*	*RL 3*	*SFW1*	*SFW2*	*SFW3*	*MDA 1*	*MDA 2*	*MDA 3*	*RWC 1*	*RWC 2*	*RWC 3*	*CCI 1*	*CCI 2*	*CCI 3*	*H2O2 1*	*H2O2 2*	*H2O2 3*	*PI 1*	*PI 2*	*PI 3*
SL 1	1.00																							
SL 2	-0.50	1.00																						
SL 3	-0.04	0.14	1.00																					
RL 1	-0.20	-0.28	0.23	1.00																				
RL 2	-0.58	0.63	-0.34	-0.33	1.00																			
RL 3	-0.68	0.48	-0.22	-0.55	0.65	1.00																		
SFW1	-0.42	0.36	0.58	0.10	-0.17	0.29	1.00																	
SFW2	0.77	-0.67	-0.52	-0.24	-0.52	-0.34	-0.47	1.00																
SFW3	0.35	-0.13	-0.76	0.09	0.06	-0.35	-0.73	0.53	1.00															
MDA 1	0.21	-0.63	-0.26	-0.44	-0.08	0.28	-0.28	0.49	-0.18	1.00														
MDA 2	0.52	-0.38	-0.59	-0.15	0.06	-0.32	-0.99	0.58	0.76	0.29	1.00													
MDA 3	0.19	0.26	-0.19	-0.51	0.54	0.09	-0.68	-0.03	0.28	0.11	0.65	1.00												
RWC 1	-0.18	0.46	-0.25	-0.31	0.84	0.25	-0.54	-0.35	0.25	-0.08	0.44	0.87	1.00											
RWC 2	0.55	-0.13	-0.53	0.05	0.08	-0.54	-0.68	0.45	0.83	-0.21	0.67	0.36	0.36	1.00										
RWC 3	0.12	0.05	-0.28	-0.34	0.50	0.06	-0.81	0.02	0.34	0.24	0.76	0.95	0.84	0.34	1.00									
CCI 1	0.09	-0.38	-0.10	0.26	-0.47	-0.19	-0.28	0.33	0.28	0.11	0.36	-0.07	-0.32	-0.18	0.11	1.00								
CCI 2	0.65	-0.01	-0.53	-0.75	0.11	0.00	-0.54	0.66	0.51	0.29	0.61	0.56	0.31	0.58	0.43	-0.12	1.00							
CCI 3	0.51	0.20	-0.45	-0.65	0.36	-0.03	-0.65	0.37	0.53	0.08	0.67	0.80	0.64	0.67	0.67	-0.24	0.91	1.00						
H2O2 1	-0.25	0.18	0.85	-0.03	-0.29	0.21	0.78	-0.45	-0.91	0.00	-0.75	-0.34	-0.42	-0.87	-0.42	0.00	-0.50	-0.57	1.00					
H2O2 2	-0.23	0.10	-0.93	-0.22	0.61	0.40	-0.59	0.21	0.68	0.15	0.56	0.36	0.51	0.41	0.47	0.10	0.39	0.43	-0.78	1.00				
H2O2 3	0.05	0.40	-0.19	-0.48	0.68	0.17	-0.60	-0.18	0.26	0.00	0.55	0.98	0.95	0.37	0.91	-0.22	0.49	0.78	-0.35	0.40	1.00			
PI 1	0.29	0.06	-0.52	-0.82	0.07	0.43	-0.02	0.57	0.17	0.41	0.11	0.07	-0.08	0.10	-0.05	-0.16	0.76	0.50	-0.18	0.34	0.04	1.00		
PI 2	-0.61	0.48	-0.05	-0.24	0.67	0.61	-0.19	-0.53	-0.10	0.02	0.12	0.57	0.64	-0.35	0.62	0.21	-0.12	0.10	0.09	0.39	0.60	-0.17	1.00	
PI 3	-0.87	0.50	0.10	-0.11	0.71	0.76	0.19	-0.75	-0.43	0.03	-0.29	0.23	0.47	-0.59	0.28	-0.06	-0.43	-0.24	0.30	0.24	0.32	-0.23	0.87	1.00

Abbreviations: SL (shoot length), RL (root length), SFW (shoot fresh weight), MDA (Melondialdehyde), RWC (relatice water content), CCI (chlorophyll content index), H_2O_2 (hydrogen peroxide), PI (performance index). **1:** control without stress, **2:** 150mM control, **3:** 150mM with 0.5% red algal biochar.

Table 4. Correlation table of effects of brown algal biochar on rice growth and physiology under salt stress.

	SL 1	*SL 2*	*SL 3*	*RL 1*	*RL 2*	*RL 3*	*SFW1*	*SFW2*	*SFW3*	*MDA 1*	*MDA 2*	*MDA 3*	*RWC 1*	*RWC 2*	*RWC 3*	*CCI 1*	*CCI 2*	*CCI 3*	*H2O2 1*	*H2O2 2*	*H2O2 3*	*PI 1*	*PI 2*	*PI 3*
SL 1	1.00																							
SL 2	-0.50	1.00																						
SL 3	-0.04	0.14	1.00																					
RL 1	-0.20	-0.28	0.23	1.00																				
RL 2	-0.58	0.63	-0.34	-0.33	1.00																			
RL 3	-0.68	0.48	-0.22	-0.55	0.65	1.00																		
SFW1	-0.42	0.36	0.58	0.10	-0.17	0.29	1.00																	
SFW2	0.77	-0.67	-0.52	-0.24	-0.52	-0.34	-0.47	1.00																
SFW3	0.35	-0.13	-0.76	0.09	0.06	-0.35	-0.73	0.53	1.00															
MDA 1	0.21	-0.63	-0.26	-0.44	-0.08	0.28	-0.28	0.49	-0.18	1.00														
MDA 2	0.52	-0.38	-0.59	-0.15	0.06	-0.32	-0.99	0.58	0.76	0.29	1.00													
MDA 3	0.19	0.26	-0.19	-0.51	0.54	0.09	-0.68	-0.03	0.28	0.11	0.65	1.00												
RWC 1	-0.18	0.46	-0.25	-0.31	0.84	0.25	-0.54	-0.35	0.25	-0.08	0.44	0.87	1.00											
RWC 2	0.55	-0.13	-0.53	0.05	0.08	-0.54	-0.68	0.45	0.83	-0.21	0.67	0.36	0.36	1.00										
RWC 3	0.12	0.05	-0.28	-0.34	0.50	0.06	-0.81	0.02	0.34	0.24	0.76	0.95	0.84	0.34	1.00									
CCI 1	0.09	-0.38	-0.10	0.26	-0.47	-0.19	-0.28	0.33	0.28	0.11	0.36	-0.07	-0.32	-0.18	0.11	1.00								
CCI 2	0.65	-0.01	-0.53	-0.75	0.11	0.00	-0.54	0.66	0.51	0.29	0.61	0.56	0.31	0.58	0.43	-0.12	1.00							
CCI 3	0.51	0.20	-0.45	-0.65	0.36	-0.03	-0.65	0.37	0.53	0.08	0.67	0.80	0.64	0.67	0.67	-0.24	0.91	1.00						
H2O2 1	-0.25	0.18	0.85	-0.03	-0.29	0.21	0.78	-0.45	-0.91	0.00	-0.75	-0.34	-0.42	-0.87	-0.42	0.00	-0.50	-0.57	1.00					
H2O2 2	-0.23	0.10	-0.93	-0.22	0.61	0.40	-0.59	0.21	0.68	0.15	0.56	0.36	0.51	0.41	0.47	0.10	0.39	0.43	-0.78	1.00				
H2O2 3	0.05	0.40	-0.19	-0.48	0.68	0.17	-0.60	-0.18	0.26	0.00	0.55	0.98	0.95	0.37	0.91	-0.22	0.49	0.78	-0.35	0.40	1.00			
PI 1	0.29	0.06	-0.52	-0.82	0.07	0.43	-0.02	0.57	0.17	0.41	0.11	0.07	-0.08	0.10	-0.05	-0.16	0.76	0.50	-0.18	0.34	0.04	1.00		
PI 2	-0.61	0.48	-0.05	-0.24	0.67	0.61	-0.19	-0.53	-0.10	0.02	0.12	0.57	0.64	-0.35	0.62	0.21	-0.12	0.10	0.09	0.39	0.60	-0.17	1.00	
PI 3	-0.87	0.50	0.10	-0.11	0.71	0.76	0.19	-0.75	-0.43	0.03	-0.29	0.23	0.47	-0.59	0.28	-0.06	-0.43	-0.24	0.30	0.24	0.32	-0.23	0.87	1.00

Abbreviations: SL (shoot length), RL (root length), SFW (shoot fresh weight), MDA (Melondialdehyde), RWC (relative water content), CCI (chlorophyll content index), H_2O_2 (hydrogen peroxide), PI (performance index). **1:** control without stress, **2:** 150mM control, **3:** 150mM with 1% brown algal biochar.

Chlorophyll 'a 'fluorescence Parameters

Application of algal biochar improved chlorophyll a parameter such as performance index (PI) under salt stress. Under non-stressed control conditions, performance index was reduced to 40% and 8% in 0.5 and 1% red algal biochar, respectively compared to control (Fig. 5). Brown algal 0.5% biochar reduced PI by 18% while 1% biochar increased PI by 12% compared to control. However, both red and brown algal biochar tend to improve PI under 100mM salinity level compared to 100mM control. PI was significantly increased using 0.5% red and brown algal biochar by 42 and 51% respectively compared to control. Red and brown 1% algal biochar substantially improved PI by 50 and 66% compared to 100mM control (Supplementary Table 1 and 2). PI was drastically reduced to 87% under high salinity of 150mM compared to non-stressed control environment while red and brown algal biochar addition significantly improved PI under high salinity of 150mM. PI was significantly increased by 373 and 327% using 0.5 and 1% red algal biochar respectively compared to 150mM control. Brown algal 0.5 and 1% biochar significantly improved PI by 409 and 539% respectively compared to 150mM control.

Under salt stress, the dark-adapted quantum yield (*Fv/Fm*) was evaluated both with and without the addition of 0.5% and 1% of red and brown algal biochar (Fig. 6). Treatments with 0.5% and 1% red and brown algal biochar enhanced the quantum yield at both 100mM and 150mM salt stress. However, lesser decline in the quantum yield was observed in plants treated with 0.5% red algal biochar compared to 1% at both salt concentrations i.e. 100 and 150mM. While, in brown algal biochar 1% concentration showed more enhanced Dark-adapted quantum yield (*Fv/Fm*) compared to 0.5% at both salt concentrations i.e. 100 and 150mM compared to control. Lesser values of dark-adapted quantum yield (*Fv/Fm*) were recorded in salt stressed control plants. Under salt stress, the size and quantity of the active reaction centers of the photosynthetic apparatus (Fv/Fo) were assessed with and without the administration of 0.5% and 1% red and brown algal biochar (Fig. 7). Upon application of red algal biochar number of active reaction centers was significantly improved at both 0.5 and 1% concentration at 100mM salt stress, while under high salinity of 150mM, 0.5% red algal biochar expressed a significant increase in number of reaction centers compared to 1% and control. On the other hand, brown algal biochar showed quantity and enhanced size of active reaction centers of photosynthetic apparatus (*Fv/Fo*) in 1% concentration compared to 0.5% and control under both 100mM and 150mM high salinity. Salt-stressed control plants had lower measurements of the size and quantity of active reaction centers of the photosynthetic apparatus (Fv/Fo).

Photochemical quenching (qP) was gradually increased upon the addition of algal biochar under salt stress while non-photochemical quenching (NPQ) that measures the heat dissipation of chlorophyll excitation energy; ultimately decreased in plants with algal biochar addition under salinity compared to the plants with no biochar. In red algal biochar, highest qP was observed in 0.5% algal biochar which increased by 31% under non stress condition compared to control (Fig. 8). Under 100mM salinity, 0.5% algal biochar showed greater improvement in qP which is increased by 25% and in 1% algal biochar qP was increased by 19% compared to 100mM control. NPQ was found to be significantly reduced in 0.5 and 1% red algal biochar by 53 and 32% respectively compared to 100mM control. In the salinity level of 150mM, both 0.5% and 1% red Algal biochar significantly improved qP by 22% individually compared to 150mM control. NPQ was incredibly reduced under 150mM salinity by 35 and 22% using 0.5 and 1% red algal biochar respectively, compared to 150mM control (Supplementary table 1). In Brown algal biochar, photochemical quenching (qP) was increased and NPQ was gradually decreased under salinity (Fig. 9). Photochemical quenching (qP) was increased by 18 and 12% in both 0.5 and 1% algal biochar while NPQ was reduced by 53 and33% respectively in 100mM salinity. In 150mM salt stress, qP was improved by 32 and 17% compared to control while NQP was significantly reduced by 40 and 13% in 0.5 and 1% brown algal biochar compared to 150mM control. Non-photochemical quenching (NPQ) that measures the heat dissipation of chlorophyll excitation energy was highest in 100 and 150mM salinity i.e. 50 and 54% compared to non-stress control (Supplementary Table 2).

OJIP induction curve

The analysis of the OJIP induction curve revealed that the plants without biochar exhibited a decreased in fluorescence intensity (OJIP curve) as the salinity level increased from 100 to 150 mM. This indicates the negative impact of salt stress on the plants. The plants added with 0.5% and 1% red algal biochar showed the highest peaks of the induction transients under non-stressed and 100mM stressed conditions, while the plants left untreated under 150mM stress showed the lowest induction curve (Fig. 10). At the salinity level of 150mM, plants added with 0.5 and 1% red algal biochar showed significant peaks in the induction curve by rising P step by 44 and 17% respectively compared to non-stressed control and 100mM control curve. In brown algal biochar, 0.5% algal biochar

showed the highest peak under non-stressed condition. Surprisingly under high salinity, plants treated with 1% brown algal biochar, showed highest peaks of OJIP induction curve by raising the P step up to 52 and 77% under both 100 and 150mM salt stress respectively compared to 100 and 150mM control (Supplementary Table 2). Lowest peaks were observed in the un-treated control plants and 100 and 150mM salinity without biochar (Fig. 11).

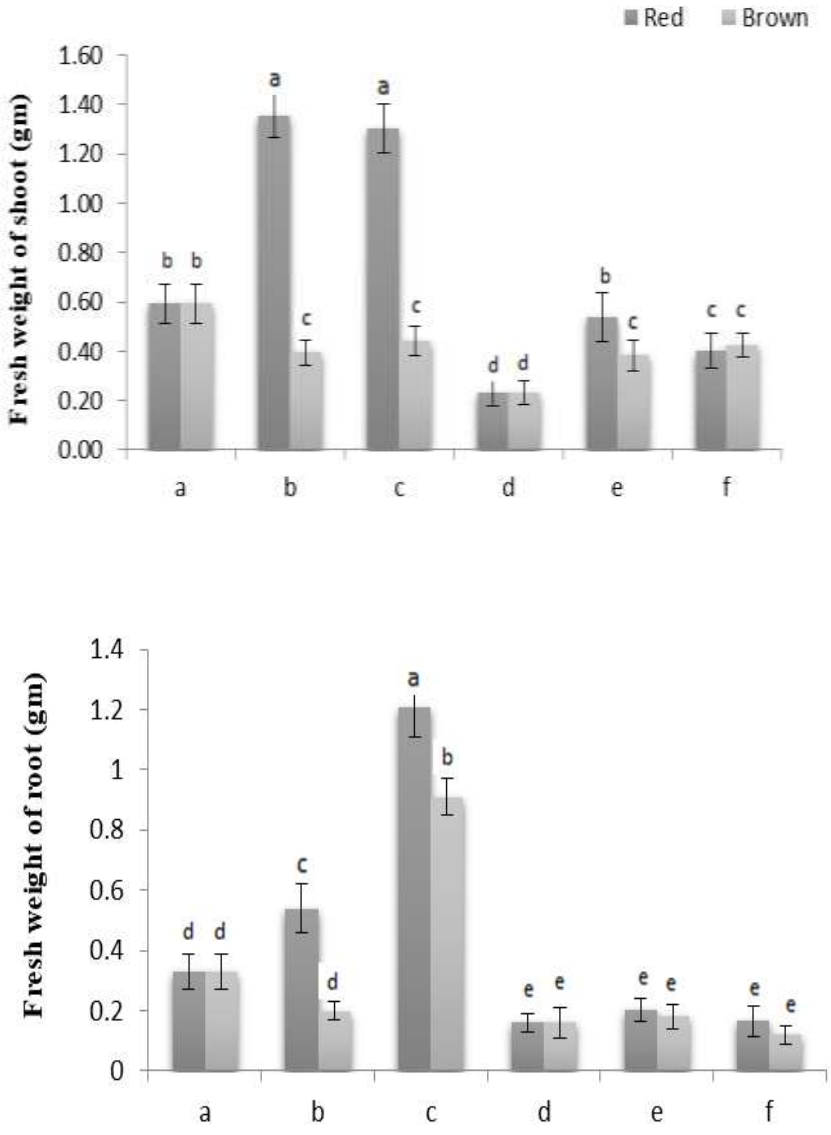

Fig. 1. Effects of algal biochar on rice plants growing in a saline environment in terms of the Fresh Weight of Shoot (SFW) and Fresh Weight of Root (RFW). On the horizontal axis, symbols represent (A) Control without algal biochar (B) 0.5% algal biochar (C) 1% algal biochar (D) 150 mM NaCl (E) 0.5% algal biochar with 150 mM NaCl (F) 1% algal biochar and 150 mM NaCl. Identical alphabets indicate non-significant differences between the means of treatments at $p<0.05$, and vertical lines on bars represent the mean standard error (S.E.).

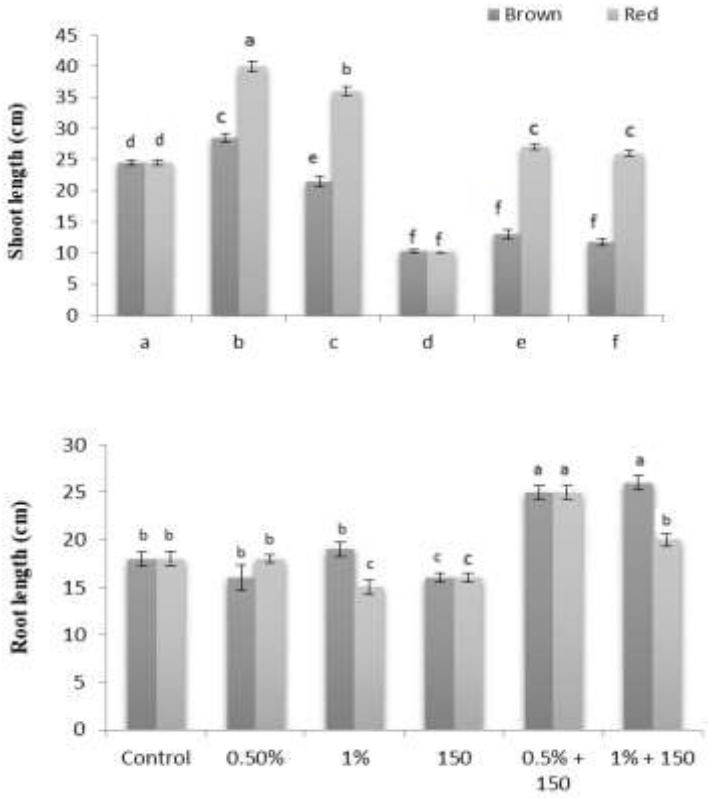

Fig. 2. Impact of algal biochar on the shoot length (SL) and root length (RL) of rice plants under salinity. The horizontal axis in the study represents: (A) Control group without algal biochar, (B) 0.5% algal biochar, (C) 1% algal biochar, (D) 150 mM NaCl, (E) 0.5% algal biochar with 150 mM NaCl, and (F) 1% algal biochar with 150 mM NaCl. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

Energy fluxes

In accordance with the leaf energy flux model, the addition of algal biochar to the plants resulted in a decrease in the absorption per reaction centre (ABS/RC) and dissipation per reaction centre (DI_O/RC) compared to the control plants without biochar (Fig. 12 and 13). The addition of 0.5% and 1% red algal biochar resulted in a decrease of 4% and 2% in ABS/RC (absorption per reaction centre) and a decrease of 10% and 20% in DI_O/RC (dissipation per reaction centre) respectively. Similarly, the inclusion of 0.5% and 1% brown algal biochar showed a significant reduction of 14% and 19% in ABs/RC and a decrease of 17% and 12% in DI_O/RC compared to the control without biochar. This indicates that algal biochar improved the trapping per reaction center (TR_O/RC) and electron transport (ET_O/RC) under natural conditions. Absorption per reaction centre (ABS/RC) and dissipation per reaction centre (DI_O/RC) were increased by 52 and 129% under 150mM control without algal biochar compared to control. While both parameters consequently reduced by 29 and 26% using 0.5 and 1% red algal biochar respectively under high salinity of 150mMwhich led to the increase in the trapping per reaction center (TR_O/RC) by 21% and 14% by using 0.5% and 1% red algal biochar respectively compared to 150mM control. While electron transport (ET_O/RC) was increased by 41 and 52% under the similar conditions. Brown algal Biochar enhanced trapping (TR_O/RC) by 35 and 7% and electron transport (ET_O/RC) by 49 and 60% in 0.5 and 1% algal biochar compared to 150mM control (Supplementary Table 1 and 2).

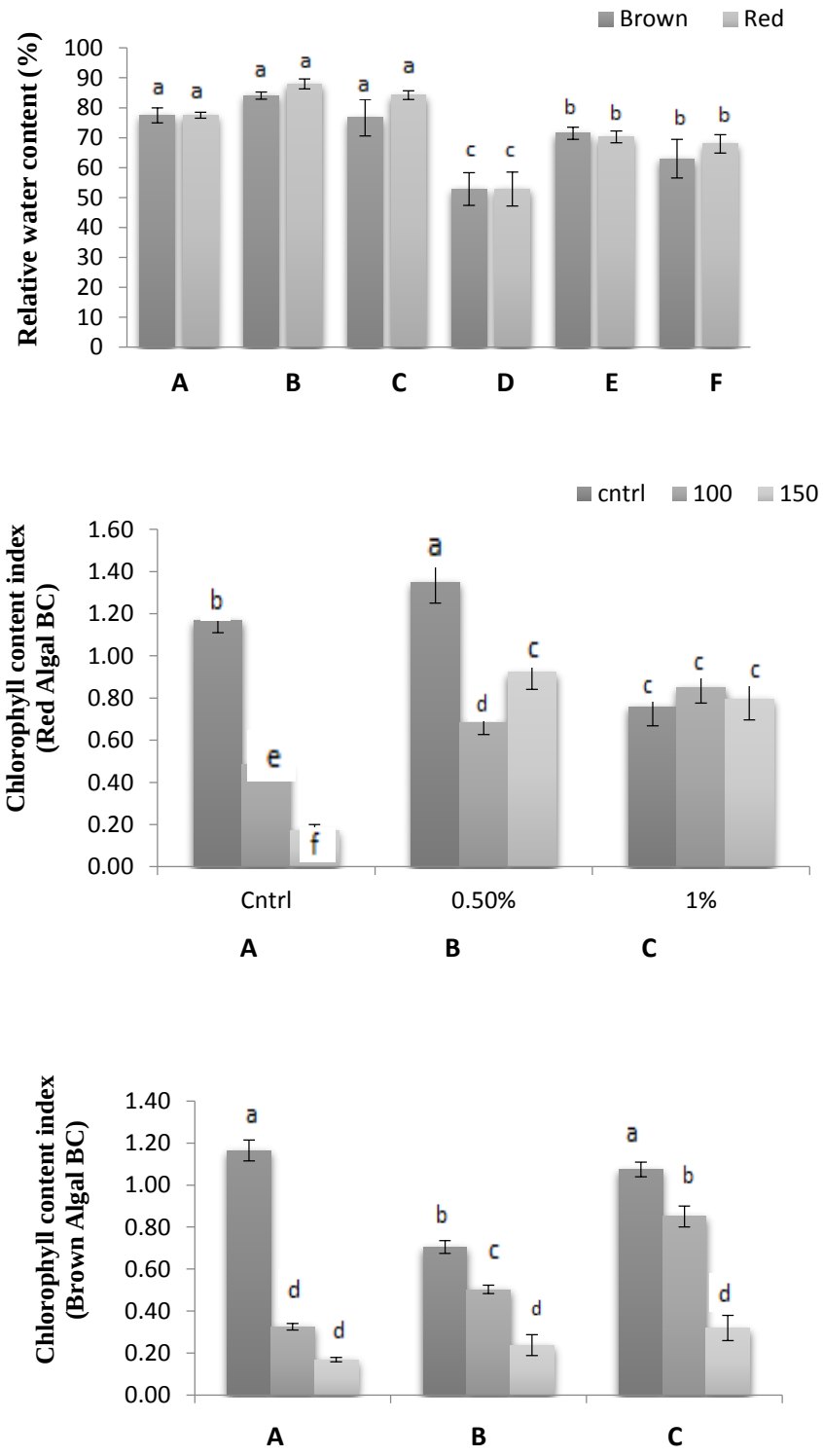

Fig. 3. Impact of algal biochar on the RWC and CCI of rice plants under salinity. The horizontal axis in the study represents: (A) Control group without algal biochar, (B) 0.5% algal biochar, (C) 1% algal biochar, (D) 150 mM NaCl, (E) 0.5% algal biochar with 150 mM NaCl, and (F) 1% algal biochar with 150 mM NaCl. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

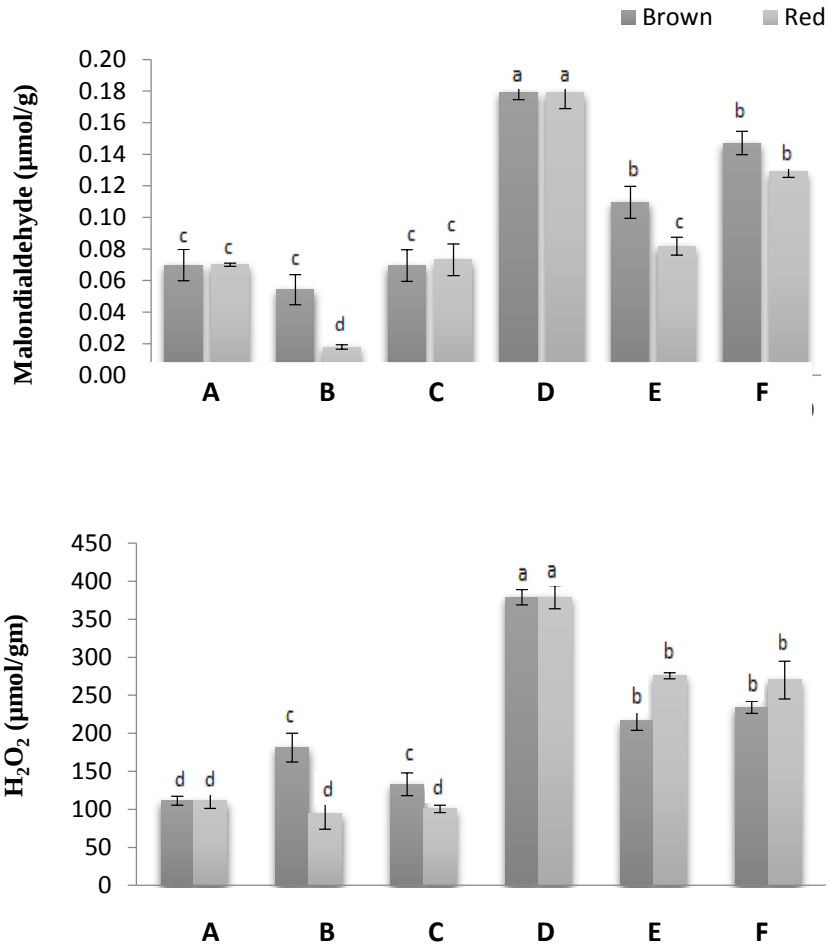

Fig. 4. Effects of red and brown algal biochar on Melondialdehyde (MDA) content and Hydrogen peroxide (H_2O_2) on rice plants grown under salinity. The horizontal axis in the study represents: (A) Control group without algal biochar, (B) 0.5% algal biochar, (C) 1% algal biochar, (D) 150 mM NaCl, (E) 0.5% algal biochar with 150 mM NaCl, and (F) 1% algal biochar with 150 mM NaCl. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

Correlation analysis

The diagonal of the table is always 1 because it represents the correlation of each variable with itself, which is always perfect. There are both positive and negative correlations between the different plant growth parameters. Shoot length (SL) in Supplementary Table 3 and 4 showed strong negative correlation between shoot length under 150mM salt stress and shoot length in control, with a correlation coefficient of -0.496. This suggests that exposure to salt stress had a detrimental effect on shoot length. However, there is a weaker but positive correlation between shoot length in 150mM salt stress with 0.5% algal biochar and SL under control, with a correlation coefficient of 0.310. This suggests that the addition of algal biochar may have had a positive effect on shoot length under salt stress.

Similarly, looking at root length (RL), there is a weak but positive correlation between root length in 150mM salt stress with 0.5% algal biochar and root length in control, with a correlation coefficient of 0.389. This suggests that the addition of algal biochar may have had a positive effect on root length under salt stress. There are both positive and negative correlations between the different conditions among shoot fresh weight (SFW) and root fresh weight (RFW). For example, there is a strong negative correlation between shoot fresh weight in control and shoot fresh weight in 150mM salt stress with 0.5% algal biochar, with a correlation coefficient of -0.521. This suggests

that the addition of algal biochar may have had a negative effect on shoot fresh weight under normal conditions. However, there is a strong positive correlation between root fresh weight in 150mM salt stress and root fresh weight in control, with a correlation coefficient of 0.691. This suggests that exposure to salt stress may have had a positive effect on root fresh weight.

Furthermore, the correlations suggest that salt stress causes damage to the cell membranes, resulting in an increase in MDA levels. However, the application of algal biochar under salt stress appears to mitigate this damage, as there is a negative correlation between MDA under salt stress with algal biochar, control and stressed plants under 150mM salinity. This indicates that the biochar application is helping to maintain membrane integrity in the presence of salt stress. Furthermore, the increase in MDA levels in control and stresses plants leads to a decrease in RWC, indicating water stress in the plants. MDA levels in plant leaves increased as a result of water stress. The relative water content of leaves decreased as a result of an increase in proline accumulation following an increase in lipid peroxidation concentration. However, the application of algal biochar under salt stress appears to alleviate this stress, as there is a negative correlation between algal biochar under salt stress, control and stressed plants under 150mM salinity. This suggests that the biochar application is helping to maintain water levels in the plants under salt stress.

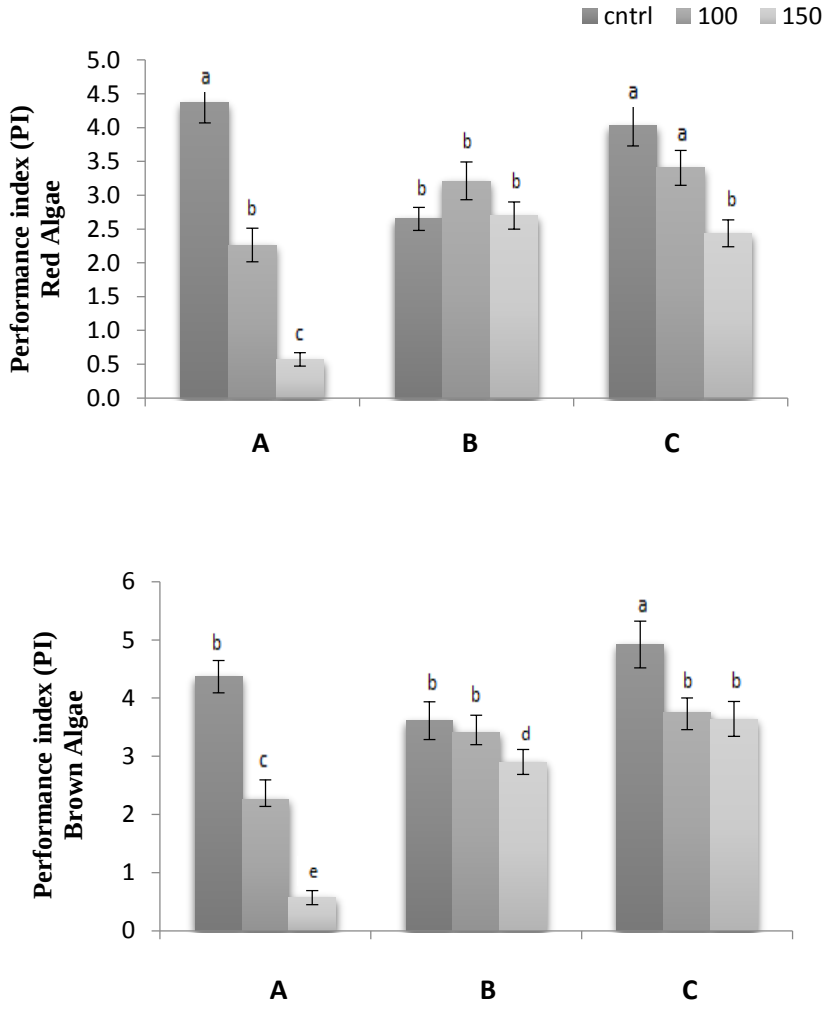

Fig. 5. Effects of red and brown algal biochar on Performance index (PI) on rice plants under salinity. The symbols on the horizontal axis: **(A)** Control without algal biochar, **(B)** 0.5% algal biochar, **(C)** 1% algal biochar. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

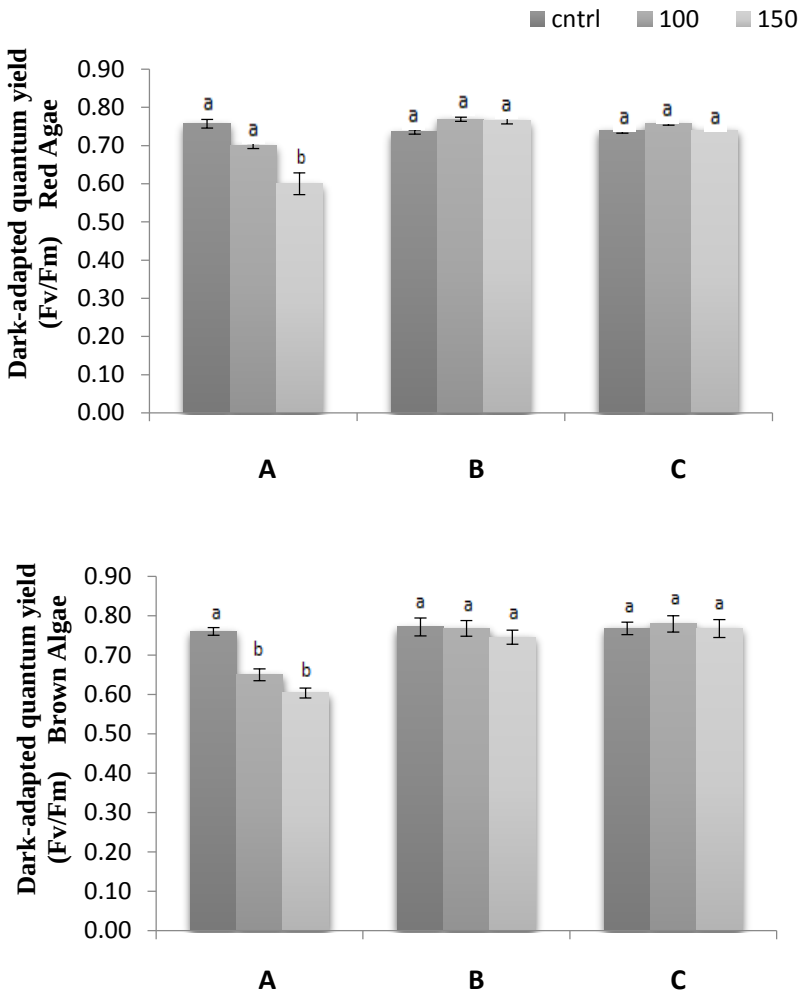

Fig. 6. Effects of red and brown algal biochar on Dark adapted quantum yield (*Fv/Fm*) on rice plants under salinity. The symbols on the horizontal axis stand for: **(A)** Control without algal biochar, **(B)** 0.5% algal biochar, **(C)** 1% algal biochar. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

There is a positive correlation between CCI and RWC under salt stress, indicating that plants with higher relative water content are able to maintain higher chlorophyll content under salt stress. The application of algal biochar under salt stress may also have a positive effect on chlorophyll content, as there is a positive correlation between CCI under stress with algal biochar and CCI under control and CCI under stress. This could be due to the biochar's ability to improve soil water retention, nutrient availability, and photosynthetic activity. Finally, the increase in hydrogen peroxide levels in control and stress may further damage the cell membranes. However, the decrease in MDA levels with increasing H_2O_2 levels in plants under stress with biochar may suggest a feedback mechanism to counteract the oxidative damage caused by hydrogen peroxide. The application of algal biochar under salt stress may be helping to activate this feedback mechanism, leading to a reduction in oxidative stress.

The correlations in the table suggest that salt stress has a negative effect on the photochemical attributes of plants, as seen by the negative correlations between PI and qP values for plants under salt stress (PI, qP under stress and PI, qP under stress with biochar). The positive correlation between PI under stress with biochar and qP under stress may suggest that the addition of algal biochar helped to mitigate some of the detrimental impacts of salinity on the photochemical attributes of plants. The negative correlation between PI under stress and qP without stress

suggests that the performance index is negatively affected by salt stress, which could be due to the disruption of the photosynthetic process in plants. The negative correlations between qP without stress and qP with stress, and between qP without stress and qP under stress with biochar, suggest that salt stress reduces the photochemical quenching of plants, which could lead to an increase in the production of reactive oxygen species (ROS) and oxidative damage to plant cells. The positive correlation between PI under stress with biochar and PI control without stress suggests that algal biochar application may have a constructive effect on the performance index of plants under salt stress, possibly by improving nutrient availability and reducing salt-induced water stress. The negative correlations between PI without stress and PI under stress with biochar, and between qP without stress and qP under stress with biochar, suggest that the addition of algal biochar may not completely mitigate the negative effects of salt stress on plant photochemistry.

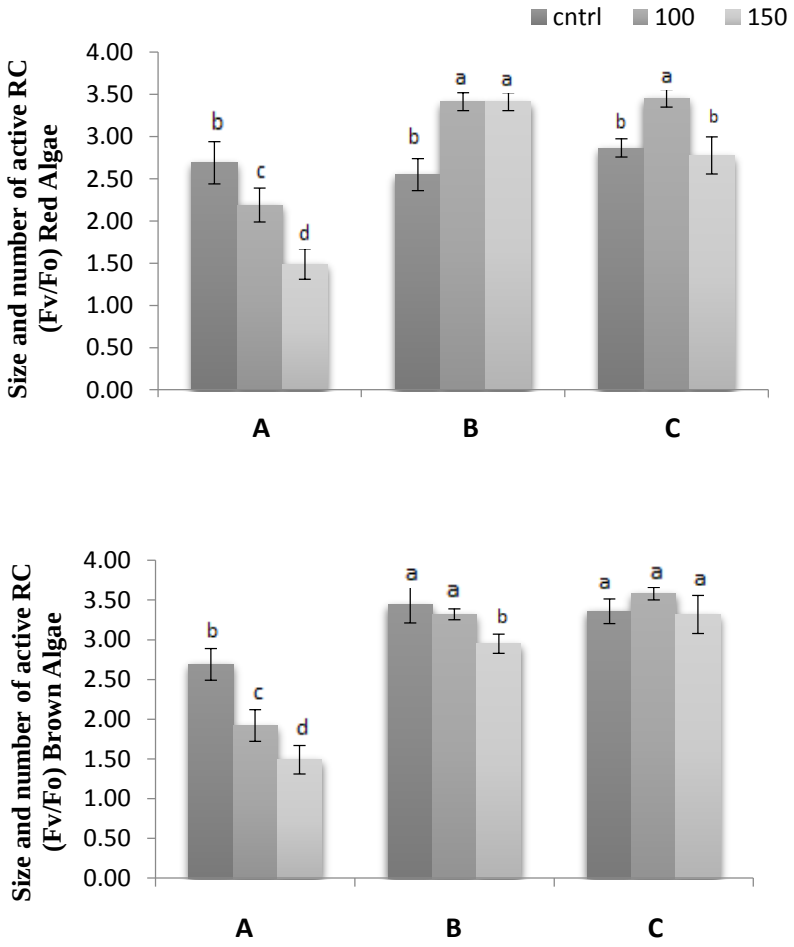

Fig. 7. Effects of red and brown algal biochar on size and number of active reaction centers (*Fv/Fo*) on rice plants under salinity. The horizontal axis represents: **(A)** Control without algal biochar, **(B)** 0.5% algal biochar, **(C)** 1% algal biochar. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

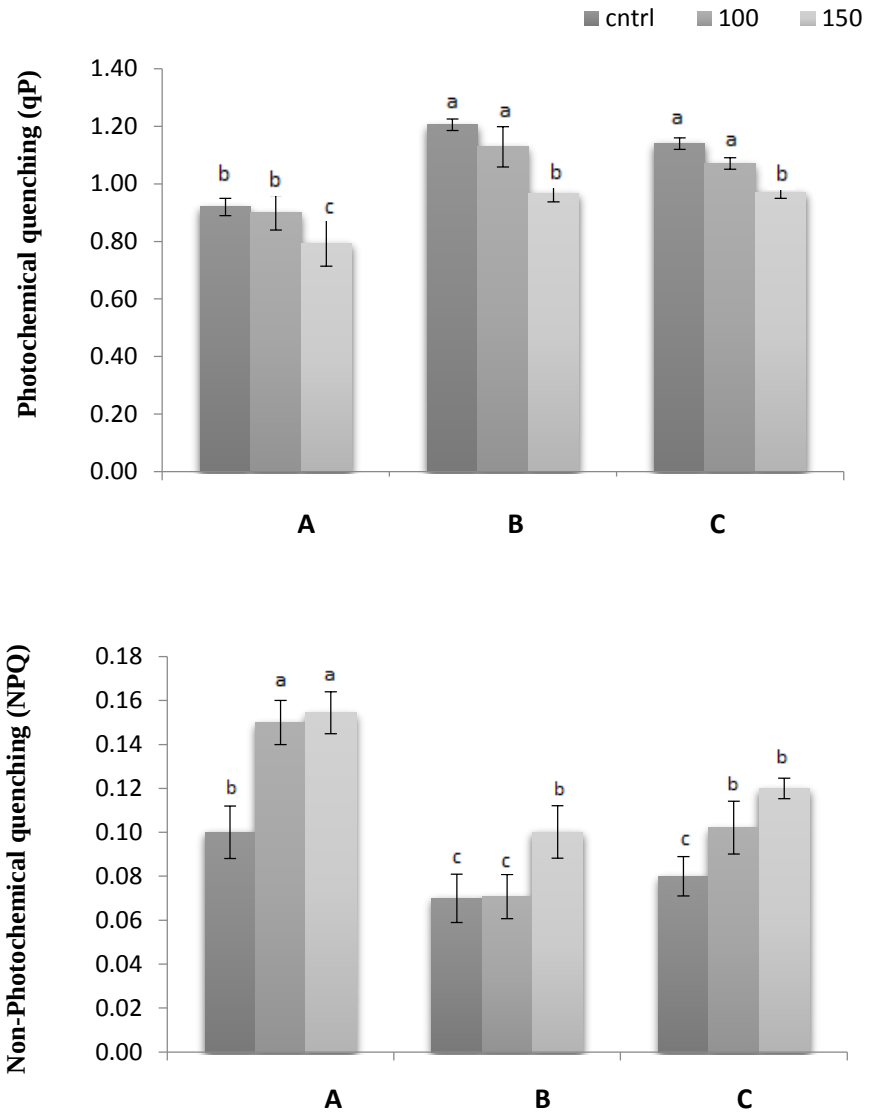

Fig. 8. Effects of red algal biochar on Photochemical quenching (qP) and Non-photochemical quenching (NPQ) on rice plants under salinity. The horizontal axis represents: **(A)** Control without algal biochar, **(B)** 0.5% algal biochar, **(C)** 1% algal biochar. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

In summary, the correlations in the table suggest that salt stress has a negative effect on plant photochemistry, but the addition of 0.5% red algal biochar may help to mitigate some of these negative effects.

Table 2 showing correlation between different photochemical and physiological aspects of rice plants under salt stress using 1% brown algal biochar. According to the table, SL (shoot length) under stress has a negative correlation with SL (shoot length) under control and RL (root length) under stress, indicating that salt stress had a detrimental effect on shoot length and root length in the rice plants. This is because salt stress can lead to ion toxicity and osmotic stress, which can disrupt plant growth and development. However, SFW under control has a positive correlation with SFW under stress with biochar, suggesting that algal biochar application may have a positive effect on shoot fresh weight in rice under salt stress. This could be due to the fact that algal biochar can improve soil fertility and nutrient availability, which can benefit plant growth and development.

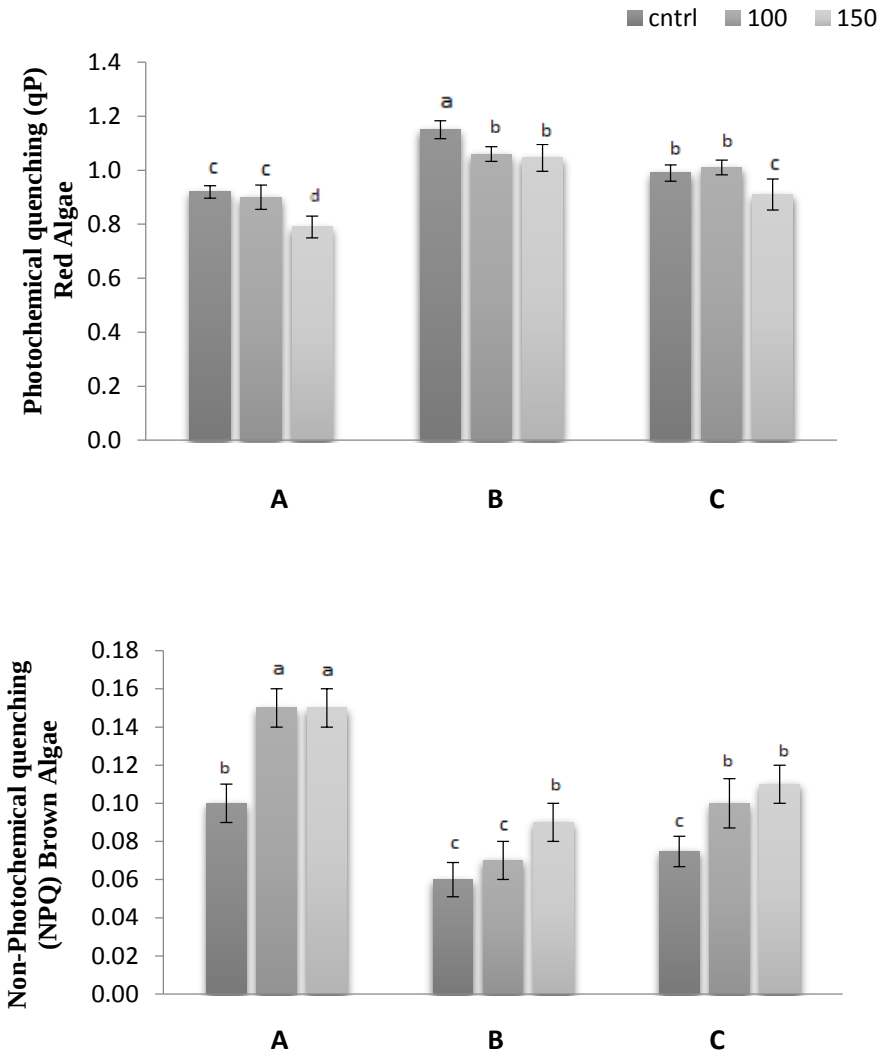

Fig. 9. Effects of brown algal biochar on Photochemical quenching (qP) and Non-photochemical quenching (NPQ) on rice plants under sal stress. The horizontal axis represents: **(A)** Control without algal biochar, **(B)** 0.5% algal biochar, **(C)** 1% algal biochar. The bars on the graph display the mean values, while the vertical lines indicate the range of ± Standard Error (S.E). Treatments with the same alphabet letter are considered statistically non-significant, with $p<0.05$.

RFW in control has a negative correlation with RFW under stress, indicating that salt stress may have a more severe impact on root fresh weight than shoot fresh weight. This could be due to the fact that roots are more directly exposed to salt stress in the soil than shoots, and therefore may be more susceptible to ion toxicity and osmotic stress. However, RFW under stress has a positive correlation with RFW under stress with biochar, suggesting that algal biochar application may have a positive effect on root fresh weight in rice under salt stress. This is in line with previous studies that have shown that algal biochar can improve soil structure, nutrient availability, and water-holding capacity, all of which can benefit root growth and development. According to the table, MDA has a positive correlation with MDA under stress and MDA under stress with biochar, which means that increasing salt stress, leads to higher levels of MDA in the rice plants. This is because salt stress causes oxidative stress in plants, which results in increased lipid peroxidation and the production of MDA as a byproduct. Similarly, H_2O_2 has a negative correlation with H_2O_2 under stress and H_2O_2 under stress with biochar, indicating that algal biochar application reduced the accumulation of hydrogen peroxide in rice under salt stress. This is because algal biochar has been shown to have antioxidant properties and can scavenge free radicals, which are produced during oxidative stress. In

terms of RWC, there is a positive correlation between RWC under control and RWC under stress with biochar, which suggests that algal biochar application can help maintain water content in the rice plants even under salt stress conditions. This could be due to the fact that algal biochar can improve soil moisture retention and water-holding capacity, which in turn can benefit plant water status. Additionally, CCI has a negative correlation with CCI under control environment without stress, indicating that algal biochar application may have a positive effect on cell membrane stability in rice under salt stress. This could be due to the fact that algal biochar can improve soil nutrient availability and reduce soil salinity, which in turn can benefit plant growth and reduce stress.

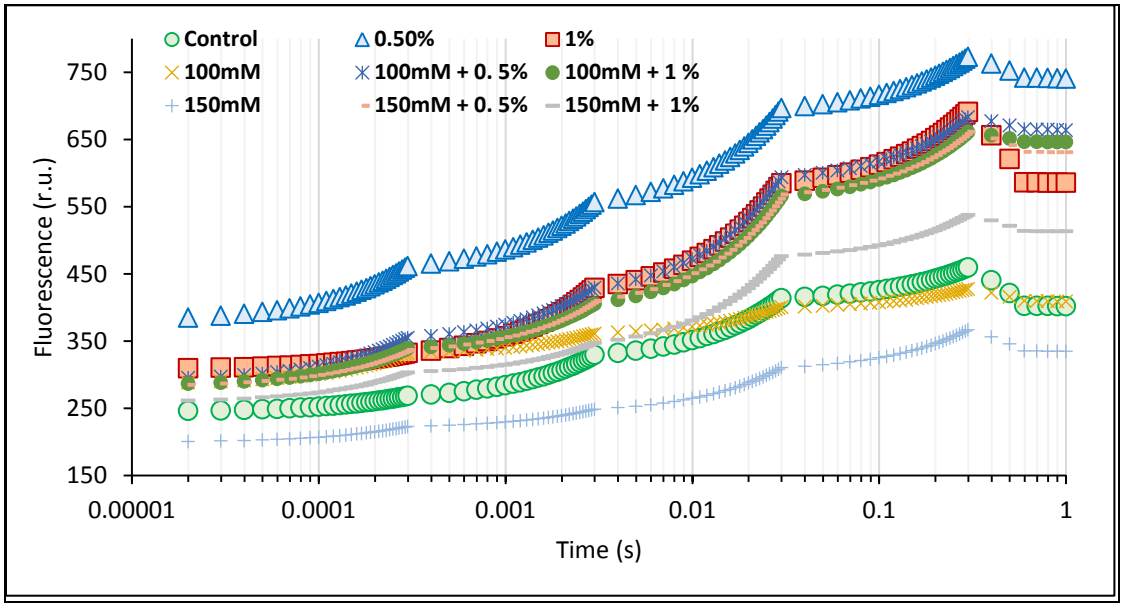

Fig. 10. Chlorophyll-a fluorescence (OJIP) of Rice (*Oryza sativa*) var. Diamond with and without the applicationof red algal biochar under salt stress.

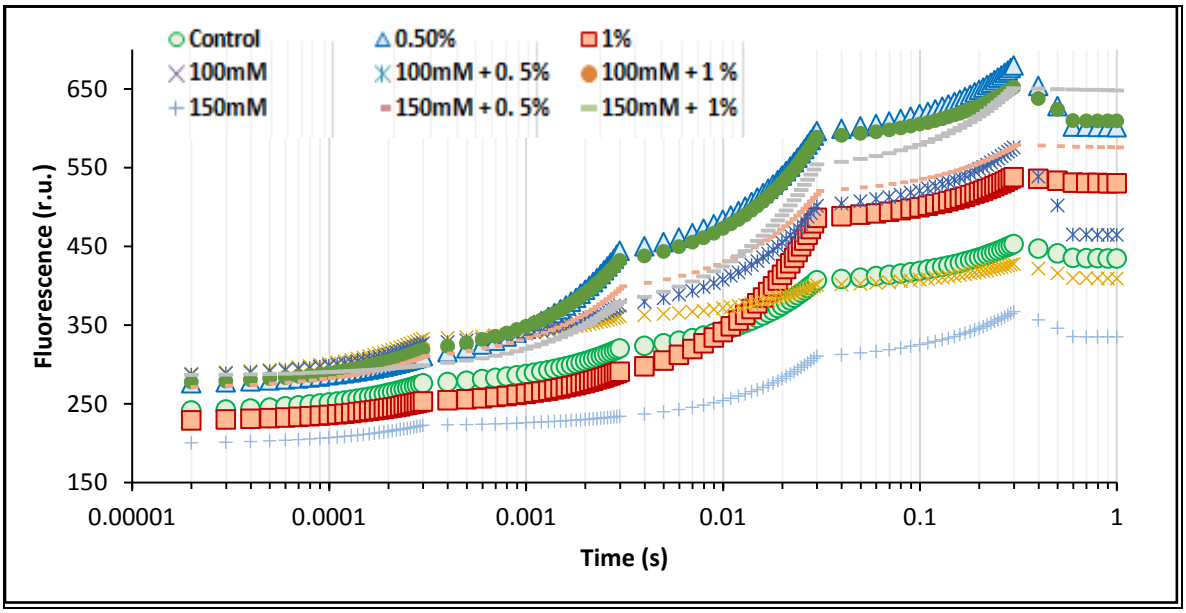

Fig. 11. Chlorophyll-a fluorescence (OJIP) of Rice (*Oryza sativa*) var. Diamond with and without the applicationof brown algal biochar under salt stress.

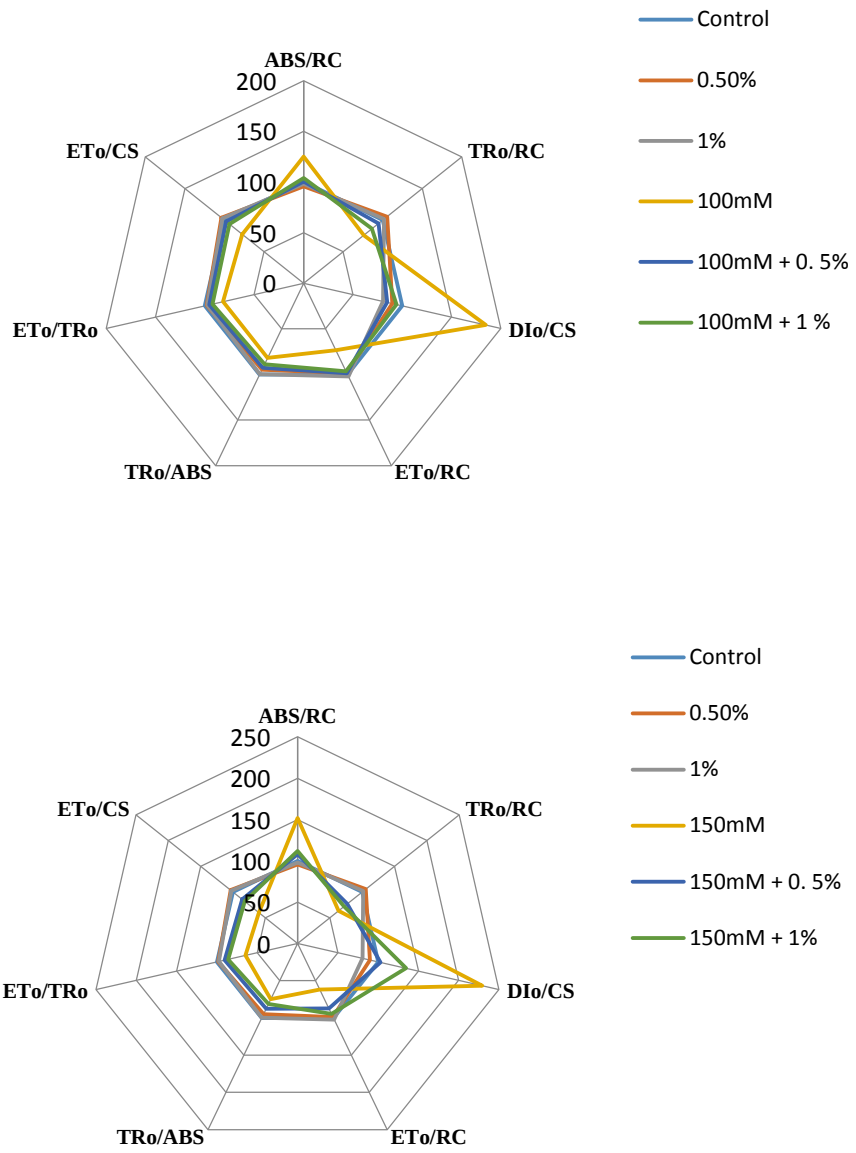

Fig. 12. Effects of red algal biochar on absorption per reaction centre (*ABS/RC*), trapping per reaction centre (TR_O/RC), electron transport per reaction centre (ET_O/RC) and dissipation per reaction centre (DI_O/RC), Trapping per absorption (*TRo/ABS*), Electron transport per trapping (*ETo/TRo*) and Electron transport per cross section (*ETo/CS*) on rice plants grown under salt stress. The parameter values are presented as a percentage change relative to the control group, which is considered as 100. The symbols on the horizontal axis correspond to different conditions: Control represents seeds without algal biochar, 0.5% denotes the presence of 0.5% algal biochar, 1% represents the presence of 1% algal biochar, and 100 & 150 mM NaCl concentration represents the inclusion of NaCl at concentrations of 100 and 150 mM.

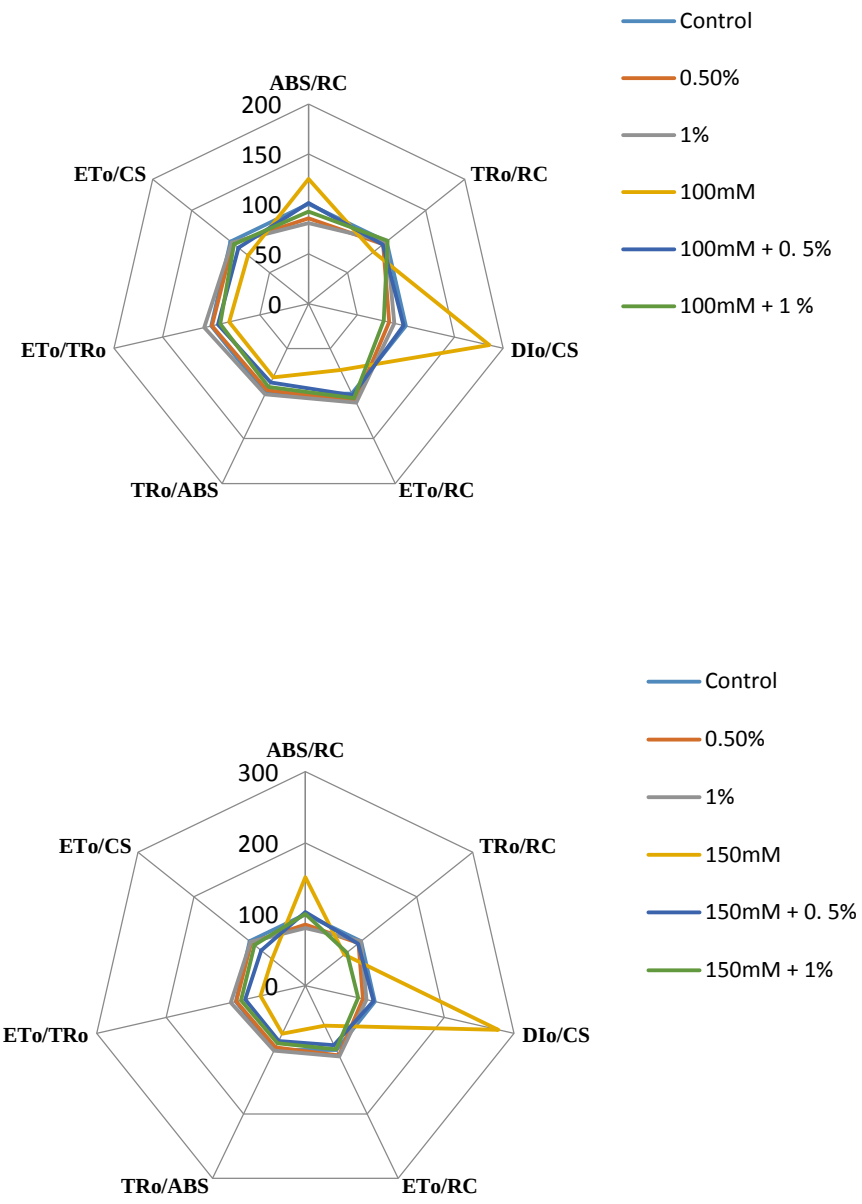

Fig. 13. Effects of brown algal biochar on absorption per reaction centre (*ABS/RC*), trapping per reaction centre (TR_O/RC), electron transport per reaction centre (ET_O/RC) and dissipation per reaction centre (DI_O/RC), Trapping per absorption (*TRo/ABS*), Electron transport per trapping (*ETo/TRo*) and Electron transport per cross section (*ETo/CS*) on rice plants grown under salt stress. The symbols on the horizontal axis correspond to different conditions: Control represents seeds without algal biochar, 0.5% denotes the presence of 0.5% algal biochar, 1% represents the presence of 1% algal biochar, and 100 & 150 mM NaCl concentration represents the inclusion of NaCl at concentrations of 100 and 150 mM.

The correlation table shows the relationship between Performance Index (PI) and photochemical quenching (qP) in control plants without algal biochar and salt, plants in 150mM salt stress, and plants in 150mM salt stress plus 0.5% algal biochar. The results indicate that PI under control and PI under stress with algal biochar have a positive correlation of 0.525, which suggests that the addition of algal biochar to salt-stressed plants improves their performance index. This finding is consistent with previous studies that have shown the beneficial effects of algal biochar in mitigating salt stress in plants by enhancing their photosynthetic performance. In contrast, PI2 has a negative correlation with both that PI under control and PI under stress with algal biochar, which suggests that salt stress has a detrimental effect on the performance index of plants. This is in agreement with previous studies that have shown that salt stress disrupts the photosynthetic apparatus of plants, leading to a decrease in their photosynthetic efficiency. The negative correlation between PI under stress and PI under stress with algal biochar (-0.282) suggests that the addition of algal biochar to salt-stressed plants partially alleviates the negative impact of salt stress on their performance index.

The results also show that qP without stress and qP with stress have a negative correlation (-0.390), which suggests that salt stress has a detrimental effect on photochemical quenching in plants. This is consistent with previous studies that have shown that salt stress inhibits the electron transport chain of photosynthesis, leading to a decrease in photochemical quenching. However, the correlation between qP with stress and qP under stress with biochar (0.310), suggests that the addition of algal biochar to salt-stressed plants partially restores photochemical quenching to its normal level. In summary, the correlation table indicates that the addition of algal biochar to salt-stressed plants improves their photosynthetic performance, as evidenced by the positive correlation between PI without stress and PI under stress with biochar. Furthermore, the negative correlation between PI with stress and PI under stress with biochar, suggests that algal biochar partially alleviates the negative impact of salt stress on the performance index of plants. The negative correlation between qP without stress and qP with stress indicates that salt stress has a detrimental effect on photochemical quenching, which is partially restored by the addition of algal biochar, as indicated by the positive correlation between qP with stress and qP under stress with biochar.

DISCUSSION

The survival of plants in arid or semi-arid places around the world is seriously hampered by exposure to severe climatic events. These significant environmental changes and population growth over the past few decades have had a severe influence on agricultural production (Pitman and Lauchli, 2002). Salinity, as highlighted by Wicke et al. (2011), is a significant abiotic factor that has adversely affected approximately 1,128 million hectares of land. The growth of crop plants is hindered by arid and saline conditions at all stages of their life, leading to reduced growth potential (Nawaz *et al.,* 2010). Among monocot crops, such as rice (*O. sativa* L.) and maize (*Zea mays* L.), they are considered moderately to highly sensitive when grown in saline soil conditions (Shafi *et al.,* 2013). Algae species are predominantly cultivated and utilized in the production of phycocolloids and food products (Neori *et al.,* 2007). Additionally, macroalgal biochar not only exhibits beneficial effects on acidic soils but also has a direct influence on soil nutrient availability and, consequently, crop productivity (Bird *et al.,* 2012).

The aim of the present study was to investigate the impact of algal biochar on the growth and physiological responses of rice plants under salt stress conditions. The literature indicates that salinity decreased plant biomass and growth in an unfavourable environment (Azooz *et al.,* 2013; Dey *et al.,* 2004). The growth and physiology of rice under salt stress were found to be improved by the addition of algal biochar. Therefore, using algal biochar to promote rice growth and physiology under salinity and halt the detrimental impacts of salt stress proved to be the most effective and convenient method. Algal biochar comprising red and brown macroalgae was added to the soil under salt stress separately. Under saline stress conditions, the growth and physiological responses of the plants were studied. Salinity had been found to drastically impede the growth and biomass of plants that lacked algal biochar.

This study focused on investigating the growth and physiological parameters, including chlorophyll content index, as well as photosynthetic traits such as maximum quantum yield of primary PSII photochemistry, performance index on absorption basis, and maximum fluorescence. The application of algal biochar demonstrated a reduction in the negative effects of salinity on plants, leading to significant improvements in growth, relative water content (RWC), and biomass production. RWC is an indicator of the plant's water status and has been associated with plant vigor in various species (Gonzalez and Gonzalez, 2001; Halder and Burrage, 2003). In the case of rice plants subjected to saline conditions, the application of algal biochar resulted in an increase in RWC by 33% (with red algal biochar) and 35% (with brown algal biochar) compared to the control.

The findings demonstrated that salt stress dramatically reduced all growth benchmarks including plant height, shoot fresh weight (FW), dry weight (DW), shoot length (SL), and root FW, DW, and RL. However, the addition of

algal biochar increased all variables when compared to the corresponding controls. The upsurge in the levels of most plant growth parameters might be attributed to the algal biochar's nutritional content. It could also be a result of the soil's increased organic matter, which has improved the soil's physicochemical and biological characteristics (Chan *et al.,* 2008; Mavi *et al.,* 2018). Previously, it was discovered that adding biochar improves the water holding capacity of the soil and, as consequently, increases nutrient availability. This enhances plant development under deficit irrigation (Jaleel *et al.,* 2009; Akhtar *et al.,* 2014; Andrenelli *et al.,* 2016). It was observed that all parameters were remarkably increased by the application of algal biochar under salt stress. In shoot and root biomass, a significant increase was found in plants under high salinity of 150mM, added with 0.5% red algal biochar (135% and 27%) respectively compared to 150mM control. Similarly, shoot length (SL) was also significantly improved under 150mM salinity with 0.5% red algal biochar addition to the soil (163%) compared to 150mM control.

The net photosynthesis of rice plants was negatively affected by salinity in our experiment under control conditions. However, the application of algal biochar helped mitigate this impact and resulted in an improvement in the net photosynthesis of rice plants. This positive effect can be attributed to the conditioning properties of algal biochar, which enhances the physiochemical and biological characteristics of the soil. This, in turn, increases the soil's water holding capacity and provides water in areas affected by drought. Previous research by Chan et al. (2008) supports the notion that algal biochar can enhance photosynthesis and improve soil conditions.

The amount of chlorophyll in plants serves as an indicator of their active involvement in the electron transport chain, high photosynthetic activity, and overall plant growth. Several factors contribute to a decrease in chlorophyll content, including the detrimental effects of salinity on chloroplasts, increased activity of chlorophyllase leading to reduced chlorophyll synthesis, and instability of the pigment protein complex. In our study, we observed a significant improvement in the chlorophyll content index (CCI) of rice plants under salt stress when 0.5% red algal biochar was applied. The CCI increased by 434% compared to the control with 150mM salt concentration. This positive effect could be attributed to the presence of bioactive growth-promoting components in seaweeds, such as fatty acids, amino acids, vitamins, minerals, and growth hormones. Previous research by Sotelo-Cuitelo *et al.* (2011) and Farooq *et al.* (2009) supports the notion that these components found in seaweeds contribute to the improvement of chlorophyll content and overall plant growth.

H_2O_2 may regulate the plant's antioxidant defense or act as a secondary messenger to assess toxicity or permanent damage to plant cells (Gechev *et al.,* 2006). In abiotic stress conditions like salinity, elevated H_2O_2 levels results in damage to the protein and lipid molecules. (Umar and Siddiqui, 2018). In our findings, salt stress results in a considerable rise in H_2O_2 levels. But application of 0.5% algal biochar both red and brown mitigated the damage to cell but 0.5% brown algal BIOCHAR significantly lowers to levels of H_2O_2 by 43% under high salinity compared to 150mM control.

This rise in MDA level is attributed to the production of reactive oxygen species (ROS) by salt stress. These reactive oxygen species cause oxidative disintegration of proteins, lipids, and nucleic acid, which disrupts metabolism. These reactive oxygen species (ROS) cause lipid peroxidation by breaking down the polyunsaturated fatty acid component of lipid membranes (Elkahoui *et al.,* 2005). Lipid peroxidation results in synthesis of MDA and is measured by MDA content of plant. Our investigation indicates that 0.5% red algal biochar significantly brings down MDA levels from 84% (under 150mM control) to 30% which shows a lower membrane damage under high salinity of 150mM. Our results were consistent with a research published by Khan *et al.,* (2012), which demonstrated that greater than 40% drop in MDA generation under salinity is indicating a less damage of cell membrane in plants.

Chlorophyll a fluorescence parameters show that salt stress has a detrimental effect on the photosynthetic system of plants. Because it gives vital information on the quantum efficiency of photochemistry and heat dissipation, chlorophyll fluorescence is often considered as a gauge of photosystem efficiency (Lichtenthaler and Burkart 1999). A photon's absorption is closely related to the movement of electrons in an electron transport chain. High light intensity or fluorescence is used to illuminate a dark-adapted leaf in order to show off the plant's photosynthetic apparatus, photosynthetic activity, maximal quantum yield of PSII, and plant oxidative damage. As salt concentration increased, Fv/Fm and PSII functionality rapidly reduced, which had a negative impact on membrane stability. This shows that under increased stress levels, the PSII reaction center degraded (Lu and Zhang 2000).Our findings indicated that 150mM salinity without algal biochar application significantly reduced Fv/Fm ratio. High ROS generation and increased damage to the photosynthetic system are indicated by a lower FV/Fm value. Increased reactive oxygen species will occur in plants under increased oxidative stress, which will ultimately lead to a drop in the FV/Fm ratio. A lower FV/Fm ratio denotes damage to the reaction centre, which leads to the formation of ROS as well as from photo-inhibition. In contrast, the application of 0.5 and 1% red and brown algal biochar increased Fv/Fm at salinity levels of 100 and 150 mM. This demonstrates the effectiveness of red and brown algal biochar in improving salt tolerance, More FV/Fmratio reflects the excellent efficacy of the photosynthetic

machinery and the decrease of primary electron acceptor i.e.QA, which is connected to enhanced PSII functionality in stressed plants.

The parameters Fv/Fm and Fv/Fo are connected. When evaluating the maximum quantum yield of PSII, the parameter Fv/Fo takes into account concurrent fluctuations in Fm and Fo. Low Fv/Fo levels, a very sensitive indicator, suggest that PSI and PSII oxidation are being suppressed. Fv/Fo ratio was also significantly improved by the application of 0.5 and 1% red and brown algal biochar under both salinity levels of 100 and 150mM compared to control. It has been found that algal biochar did not obstruct photosynthetic processes and facilitated electron flow in the electron transport chain, protecting plants from photodamage. The size of Fv/Fo and the number of active reaction centers both rise as a result of the entire procedure.

Performance index PI is highly sensitive parameter to biotic and abiotic stress and so it is drastically reduced under salinity. According to current research, the area above the fluorescence curve between maximal and minimal fluorescence decreased during drought stress. Because of the restricted electron transit of the plastoquinone pool, the area may have shrunk (Strasser *et al.,* 2000). PI was gradually increased under both salinity levels of 100 and 150mMin the plants added with 0.5 and 1% red and brown algal biochar. However, 1% algal biochar concentration showed more prominent increase in PI compared to respective controls without algal biochar indicating better resistance to salt stress. The performance index displayed all aspects of the plant's photosynthetic activity, including photon absorption, electron transfer throughout the electron transport chain, and reaction center activity. The PI is directly related to quantum yield. The high quantum yield of primary photochemistry reflects a higher conversion of light energy into chemical energy for photosynthesis.

It was found that plants treated with 0.5% red algal biochar and 1% brown algal biochar had the greatest values of parameters including PIABS, qP, and J. A significant function for algal biochar under salt stress is indicated by the magnitude of non-photochemical quenching (NPQ) and basal quantum yield of the non-photochemical process (FO/FM). Greater qP and lower NPQ readings when using algal biochar seem to indicate appropriate electron transport efficiency between PS-I and PS-II under moisture stress (Strasser *et al.,* 2010). Our data shows that upon the addition of 0.5 and 1% red algal biochar, qP gradually increased and NPQ decreased. However, 0.5% red algal biochar showed more prominent results. Brown algal biochar also showed similar results with 0.5% algal biochar. This indicates the improvement in overall photochemistry of rice plants under salt stress.

According to Baker *et al.* (2008), OJIP is a measurement tool to demonstrate oxidative stress. Putting the OJIP test on a graph on a logarithmic time scale showed the fundamental step at various fluorescence transients. In the current experiment, the OJIP curve revealed a decrease in I and P values with increasing salinity. The decrease in the I-P phase, which was brought on by a bottleneck in electron transfer at the electron acceptor side of the PSI in salt stress control plants, reveals the increase in cyclic electron flow (CEF) surrounding the PSI (Kono *et al.,* 2014; El Hamdani *et al.,* 2015). However, The inclined in I and P values in the plants supplemented with 0.5% red and 1% brown algal biochar under 100 and 150 mM salinity, demonstrated the availability of more active reaction centers (RC) in PSII under salinity compared to control plants (Kalaji *et al.,* 2011). When compared to controls, red and brown algal biochar also enhance the amount of active reaction centers as seen by the highest curve in OJIP transient. The OJIP-Test curve changes over time to indicate how electrons move through the ETC. While the PQ pool is connected to the J-I phase, the O-J phase is where the principal electron acceptor builds up. The I-P phase reveals the size of the pool of the ultimate PSI electron acceptor.

According to the leaf energy flux model, the control plants grown in high salinity without biochar displayed elevated levels of absorption per reaction centre (ABS/RC) and dissipation per reaction centre (DIo/RC). This can be attributed to a higher ratio of inactive reaction centres (RC) to active reaction centres. As a result, the control plants were able to capture more photons, but the trapped energy was not utilized to reduce the plastoquinone pool. Instead, it was dissipated as heat or lost energy. Adverse conditions caused the reaction centres in leaf tissue to close, halting the electron flow to the plastoquinone pool and limiting the chlorophyll intensity (Singh *et al.,* 2012).

However, when algal biochar was added to the plants under high salinity (150 mM), the active-to-inactive RC ratio increased in those treated with 0.5% and 1% red and brown algal biochar. This increase facilitated a faster rate of reduction of QA by the trapped exciton (TRo/RC), leading to accelerated electron transport (ETo/RC). The higher ETo/RC ratios indicate a more efficient electron transport process, preventing excessive energy absorption flux. In contrast, plants under salt stress without algal biochar treatment exhibited decreased ETo/RC values. The simultaneous enhancement of exciton trapping and transport observed in the presence of algal biochar reflects the plants' improved stress tolerance, as evidenced by increased photosynthetic production (PI) and reduced energy dissipation (DIo/RC). This suggests that algal biochar contributes to improved photosynthetic efficiency and energy utilization in plants exposed to salt stress (Grieco *et al.,* 2015).

Based on the given correlations, it appears that salt stress had a negative impact on plant growth and development which is evidenced by the decline in root and shoots length, as well as fresh weight. Additionally, salt

stress led to a decrease in relative water content (RWC) and chlorophyll content index (CCI) in plants, indicating that the plants were experiencing water stress and reduced photosynthetic activity. However, the addition of algal biochar mitigated some of the negative effects of salinity, as seen in the increased shoot and root length, as well as the increased shoot and root fresh weight in plants treated with algal biochar under salt stress. The correlations in the table suggest that salinity adversely impacts the photochemical attributes of plants which is endorsed by the decrease in performance index and qP. However, the application of algal biochar in plants under salt stress improves their photochemical attributes, as seen by the higher performance index and qP values in PI and qP under stress with biochar, respectively. The Performance Index (PI) and Photochemical Quenching (qP) values were generally higher in plants treated with algal biochar in the presence of salt stress, indicating better photosynthetic activity and higher photochemical efficiency. The negative correlation between qP values in control and salt stress conditions suggests that the plants were under oxidative stress due to the accumulation of reactive oxygen species (ROS) under salt stress. However, the positive correlation between qP values in plants treated with algal biochar and salt stress indicates that algal biochar application helped to alleviate the oxidative stress and maintain photochemical efficiency in plants under salt stress.

The negative correlation between qP and MDA in red algal biochar application suggests that oxidative damage caused by salt stress can also affect photochemical attributes. Overall, the results suggest that algal biochar application can overcome the adverse effects of salinity on the photochemical attributes of rice plants. In short, the correlation analysis showed that algal biochar application had a positive effect on the photochemical attributes of plants under salt stress. The negative correlation between salt stress and growth parameters suggests that salt stress negatively impacts plant growth, which is consistent with previous research. However, the positive correlation between growth parameters and physiological parameters indicates that the use of algal biochar can overcome the adverse effects of salinity on plants by improving their physiological performance.

Overall, the study highlights the potential of algal biochar as a sustainable and environment friendly approach for enhancing plant growth and performance under salt stress conditions. Further research is needed to explore the underlying mechanisms of algal biochar's effects on plant growth and physiology, as well as the optimal application rates and methods for different plant species and environments.

CONCLUSION

The physiological explanation of stress resistance was discovered through physiological and photochemical analysis of Rice (O. sativa) var. Diamond under salt stress using algal biochar based on red and brown macroalgae. Salt stress impaired plant growth and photosynthetic activities, interfering with PSI and PSII as well as the electron transport chain, as demonstrated by chlorophyll a fluorescence. Algal biochar, however, improved physiological aspects like photosynthetic efficiency. Plants treated with red and brown algal biochar displayed enhanced growth, chlorophyll content index and greater photosynthetic performance under salt stress. It has been proved that algal biochar induced tolerance under salt stress. Therefore, the current study came to the conclusion that employing macroalgal-based biochar for rice plant growth and development under salt stress may be a viable alternative.

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