Algal amendment improved yield and grain quality of rice with alleviation of the impacts of salt stress and water stress

Taha Mohamed El-Katony*, Fatma Mohamed Ward, Mohamed Ali Deyab, Magda Faiz El-Adl

Department of Botany and Microbiology, Faculty of Science, Damietta University, New Damietta City, Egypt

HIGHLIGHTS

• Benefit of rice yield from seaweed extract exceeded that from seaweed powder.
• The impact of PEG-6000 water stress on rice yield surpassed that of NaCl salinity.
• Beneficially, abiotic stress, particularly water stress, reduced grain As content.
• High rice yield was paralleled with high protein and mineral nutrient contents.
• Although rice grain shape is inherited it can be modified environmentally.

ARTICLE INFO

Keywords: Rice yield Salt stress Water stress Seaweeds Dictyota dichotoma

ABSTRACT

The hazardous effect of abiotic stress and the beneficial effect of organic amendments on rice have been extensively studied during the vegetative stage, but little information is available regarding rice yield. Therefore, the response of rice yield to abiotic stress × organic amendment interaction needs thorough investigation. The differential potency of aqueous extract and biomass of the seaweed Dictyota dichotoma in alleviation of NaCl salinity and PEG-6000 water stress, at \( \Psi_w \) of -0.492 MPa in medium-textured soil, on yield of cv. Sakha 101 of Oryza sativa was investigated. Grain yield, number of spikes/plant, number of grains/spike, and seed index were lowered by 59%, 47%, 40%, and 35%, respectively under salt-stress with relatively severe reductions of 63%, 50%, 50%, and 40%, respectively under water stress. Also, the improvement in grain yield, number of spikes/plant, number of grains/spike and seed index by algal amendment was greater with algal extract (106%, 72%, 79%, and 81%, respectively) than algal powder (71%, 52%, 46%, and 65%, respectively). The improved grain yield of algal-amended plants was paralleled with the production of wider, heavier and drier grains. Both salinity stress and water stress significantly reduced grain protein but increased soluble sugars and starch contents. The grain content of protein, \( \text{K}^+ \), \( \text{Ca}^{2+} \), \( \text{P} \) and \( \text{N} \) was improved while that of \( \text{Na}^+ \) was reduced in response to algal amendment with marginal effects on soluble sugars and starch. Rice grain vigor was positively correlated to protein and mineral nutrient contents versus negative correlation with soluble sugar and starch contents. Both algal amendment and abiotic stress agreed in reducing grain As content. The benefit afforded by Dictyota dichotoma to rice yield justifies manipulation of the algal extract for alleviation of abiotic stress on rice yield and improvement of grain quality.

1. Introduction

Abiotic stress arising from soil salinity and aridity is a major challenge to crop productivity in the arid and semi-arid regions of the globe. The two stresses are mutually associated because of the fact that high soil salinity arises either primarily from water scarcity which hinders leaching of soluble salts out of the rhizosphere or secondarily from usage of brackish water in irrigation. In turn, the early stress event perceived by the plant under the impact of salinity is the osmotic shock. It has been claimed that the salt-affected lands approached one third of the irrigated lands globally (Zhao et al., 2020). The soil becomes saline with saturation extract of >40 mM NaCl (\( \Psi_w \) of < -0.2 MPa). Most crop plants are glycophytes, with variable degree of salt sensitivity according to the species, cultivar, nutritional status and developmental stage (Acosta-Motos et al.,
2017). Salinity impacts plant performance via several threats including induction of an osmotic stress, a specific ion effect or interference with plant metabolism and generation of oxidative stress. The osmotic shock represents the early threat of salinity stress, with retardation of root and shoot extension (due to loss of cellular turgor) in addition to inducing stomatal closure which inhibits photosynthesis. The biochemical processes of photosynthesis are also seriously impeded by ion imbalance arising from the salinity-induced specific ion effect. Although the physiological similarity between Na⁺ and K⁺ allows Na⁺ to compete strongly with K⁺ during uptake; yet, the fine physiological differences between the two ions render Na⁺ (with smaller ionic radius and larger hydration shells) inhibitory to enzyme activity. Similarly, the Cl⁻/NO₃⁻ and Cl⁻/SO₄²⁻ competition can seriously impact plant functioning, probably via inducing N and S deficiency. Oxidative stress arises as a consequence of inhibited photosynthesis; when the flux of absorbed light exceeds the demand for photosynthesis, with initiation of a burst of reactive oxygen species in leaf tissues (Zhao et al., 2020).

The attempts to improve yield of edible agricultural and horticultural crops have to be done with paying due concern to the quality and safety criteria of the final produce. For example, a possible drawback of altered protein and starch contents of rice grain is the loss of cooking quality as a result of high grain chalkiness (Suriyasak et al., 2017). Generally, cereals are characterized with a low protein content (10–15% DW) relative to legumes (40–50%); but fortunately, the biological value and net protein utilization of cereals are better compared with legumes (Wiesler, 2012). Among cereals, rice and maize exhibit low protein content (Hager et al., 2012), which justifies the efforts for nodulation induction in rice in a way to increase grain protein content.

Because polishing of brown rice causes loss of proteins, vitamins, and minerals, white rice needs supplementation with some bioactive constituents. Biofortification of rice grains with nutrients can be approached also through either plant breeding programs or agronomic practices. Several trials have been attempted to improve yield and quality of rice grains; for example, the induction of nodulation to increase protein content (Cooper and Scherer, 2012) and implementation of soybean-ferritin genes (Vasconcelos et al., 2003) to increase the concentrations of Fe and Zn in the grain. Fortunately, the task of improving yield and quality of rice grains; for example, the induction of nodulation to increase protein content (Cooper and Scherer, 2012) and implementation of soybean-ferritin genes (Vasconcelos et al., 2003) to increase the concentrations of Fe and Zn in the grain.

Rice is the stable food for about one-half of the world population, and it is particularly essential to persons suffering from gluten intolerance (Rossi et al., 2020). Rice (Oryza sativa L.) is a salt-sensitive grass with marked natrophobic behavior (George et al., 2012). The salt sensitivity along with the huge water requirements render rice amenable to salinity and drought stresses, respectively. In Egypt, groundwater of the Nile Delta aquifer, which is a principal water resource, suffers from dramatic abstraction and invasion from the Mediterranean Sea during the last 30 years (Armanious and Negen, 2019). Therefore, research should be directed to improve rice yield via safe and economic practices that avoid the drawbacks of the sophisticated genetic engineering approaches and the environmental consequences of the extensive use of chemical fertilizers. Extracts and powder of the seaweed Dictyota dichotoma have been manipulated to mitigate the impact of water stress and salt stress on rice during germination (El-Katony et al., 2020) and the vegetative stage (El-Katony et al., 2020). The present work investigates the relative severity of salinity stress and water deficit on yield characteristics of a profitable Egyptian cultivar of rice (Sakha 101) as well as the relative potency of the aqueous extract and powder of D. dichotoma in ameliorating the impact of abiotic stress on the yield of rice. Dictyota dichotoma is a valuable seaweed of marked nutritive and therapeutic potentialities. The alga can be encountered across the Red Sea coast of Egypt all the year and flourishes abundantly during autumn.

2. Materials and methods

2.1. Plant material

Seeds of rice (Oryza sativa L. cv. Sakha 101) were supplied by the Experimental Station of Agricultural Research at Giza, Egypt. Fronds of the brown alga Dictyota dichotoma (Hudson) J.V. Lamouroux were harvested from semi-exposed areas at Hurghada shore, Red Sea coast of Egypt (27° 13’ N, 33° 45’ E), during October 2014. The harvested fronds were instantaneously washed with distilled water and transported, in an ice box, to the laboratory for shade-drying on blotting paper at 27 °C for one week. The dried biomass was milled into a fine powder and stored in air-tight glass containers at room temperature until extracted.

2.2. Preparation of treatment solutions

A stock aqueous algal extract (AE) was prepared by boiling 100 g of the powdered algal fronds in 1 L distilled water for 6 h. A balanced nutrient solution was prepared containing the macronutrients (mM): N 16 (12 mM NH₄Cl, 4 mM NO₃), K 6, P 1, Ca 2, Mg 1 and S 9.5; and the micronutrients (μM): Fe (as FeEDTA) 100, Mn 10, Zn 1, Cu 1, B (as boric acid) 50 and Mo 0.5. Salt stress and water stress were imposed by applying isosmotic solutions of NaCl and PEG 6000, respectively at \( \Psi_w \) of -0.492 MPa. Details of preparation of algal extract, nutrient solution and stress solutions were outlined in El-Katony et al. (2020). Biomass of the experimental alga contained the macronutrients (% DW): N 3.1, P 0.17, K 3.8, Ca 2.5 and Mg 0.06; the micronutrients (μg g⁻¹ DW): Fe 23, Mn 85, Zn 6, Cu 22, Ni 24 and Co 7; the growth regulators (μg g⁻¹ DW): cytokinins 260, auxins 45, gibberellins 12 and abscisic acid 24; and bioactive compounds (mg/g DW): phenolics 1.5, flavonoids 1.4, terpenes 23.7, sterols 40.3, vitamin E 0.49, vitamin C 0.63 and fucoidan 57 (Ward et al., 2017).

2.3. Growth conditions of rice

Twenty-seven sealed plastic pots of 20 cm diameter and 25 cm height, full of a silty clay soil, were first divided into two groups. The first group (nine pots) was allocated to the algal powder treatment (AP) by thoroughly mixing algal powder, at a rate of 20 g/pot, with the soil before planting. The remaining 18 pots were left native and further subdivided into two groups, each of nine pots; one group received no amendment (NA) while the other group received the aqueous algal extract equivalent to 20 g/pot at split doses superimposed on the treatment solutions (AE).

Uniform rice seeds were sown in the water-saturated pots for seven d. Seedlings then received the nutrient solution so as to form one cm-layer above the soil surface and thinned to one seedling per pot within five d. Each of the three amendment regimes (NA, AE and AP) was then subdivided into three equal subgroups, each of three pots, depending on the stress regime. The stress treatments included the control (only the nutrient solution), salt stress (120 mM NaCl) and water stress (PEG 6000). Both NaCl and PEG 6000 were superimposed on the nutrient solution at \( \Psi_w \) of -0.492 MPa. For the AE group, the extract was added to the treatment solution and supplied to plants as 10 equal sequential doses so as to provide the equivalent of the 20 g AP throughout the growth period. Treatment solutions were applied in such a way to maintain one cm-layer above the soil surface. Aliquots of the standing solutions above the soil surface were periodically assayed for Na⁺, where salinity and water potential were maintained at the planned levels by the addition of either water or treatment solutions.

Plants were grown in a greenhouse at the Faculty of Science, Damietta University. The environmental conditions were: irradiance of about 2000 μmol m⁻² s⁻¹ from natural sunlight in a 14/10 h light/dark period, with day/night temperature of 35/25 °C and average relative humidity of about 80%.
2.4. Harvest and measurements

Plants were harvested at the stage of grain maturity (105 d from imposing of abiotic stress). The above-ground part was extracted and left to air-dry until constant weight for estimation of the different yield attributes. The grains were extracted from husk and ground into a fine powder prior to assay of carbohydrates, proteins and minerals. Harvest index was calculated as the proportion of grain yield in the total above-ground yield. Seed index was calculated as the weight (g) of 1000 grains. Grain density was calculated from grain weight and grain volume. Grain volume (cm$^3$) was estimated by water displacement.

2.4.1. Estimation of the grain carbohydrate fractions

Total soluble sugars (TSS) and starch were assayed according to Brinylkova et al. (2011). The powdered grains were boiled twice in 5 mL of 70% ethanol for 30 min and the combined extracts were used for TSS determination. For assay of starch, the debris left after extraction of soluble sugars was incubated in 1.6 M perchloric acid at 70 °C for 2 h and the released sugars were determined as glucose equivalents. Total soluble sugars and starch hydrolysate were assayed using the anthrone procedure adopted by Maness (2010) with reference to a calibration curve of sugars and starch hydrolysate were assayed using the anthrone procedure adopted by Allen et al. (1986). Potassium, sodium and albumin in 0.15 M NaCl in the range of 0–100 μg mL$^{-1}$ was estimated in an aliquot of the clear extract according to Bradford (1976), with reference to a standard curve of bovine serum albumin in 0.15 M NaCl in the range of 0–100 μg mL$^{-1}$.

2.4.2. Estimation of grain protein content

The powdered grains were extracted in 4 mL of 1N NaOH and the slurry was vortexed for 5 min and incubated at -4 °C for 48 h. Protein content was determined in an aliquot of the clear extract according to Bradford (1976), with reference to a standard curve of bovine serum albumin in 0.15 M NaCl in the range of 0–100 μg mL$^{-1}$.

2.4.3. Determination of grain mineral content

The powdered grains were digested in the sulfuric acid/hydrogen peroxide mixture adopted by Allen et al. (1986). Potassium, sodium and calcium were assayed in the clear extract by using a Jenway PFP7 Flame Photometer. Arsenic was assayed by using a Pye-Unicam SP 90 Atomic Absorption Spectrophotometer. Total phosphorus was determined according to the phosphomolybdate blue method adopted by Allen et al. (1986). Nitrogen was assayed as NH$_3$ after distillation of the digest as outlined by Allen et al. (1986).

2.5. Experimental design and statistical analysis

The experiment was factorial with two factors and three replications, in a completely randomized design. The main factors were 1) amendment regime with three levels: NA, AE and AP; 2) abiotic stress with three levels: control, SS and WS. Statistical analysis was performed using SPSS version 22. Two-way ANOVA—to evaluate the effect of the main factors and their interaction on rice yield—was followed by mean separation according to the Duncan’s multiple range test at P < 0.05. Principal component analysis (PCA) was performed to gather the relationships among the different yield and biochemical parameters of rice under the different factor combinations.

3. Results

Most of the yield attributes of rice were significantly (P < 0.05) to highly significantly (P < 0.001) affected by algal amendment and abiotic stress, with just a significant or non-significant interaction (Table 1). The beneficial effect of algal amendment on rice yield was more evident for algal extract (AE) than algal powder (AP), under stress conditions than for non-stressed plants and under salt stress (SS) than water stress (WS). The increase in grain yield attributable to AE and AP amounted to 44% and 32%, respectively in control plants, 150% and 110%, respectively under SS and 70% and 50%, respectively under WS. In turn, the adverse effect of abiotic stress was more evident in the NA plants (67% average reduction) than in AE- and AP-amended plants (average reduction of 54%). The beneficial effect of algal amendment on straw yield followed the pattern exhibited by grain yield, but the adverse effect of abiotic stress was limited and versatile (Figure 1A, B). As a consequence, harvest index was improved due to algal amendment, particularly in the stressed

| Variable and source of variation | df | F   | P     | Variable and source of variation | df | F   | P     |
|---------------------------------|----|-----|-------|---------------------------------|----|-----|-------|
| Grain yield                     |    |     |       | Grain width                      |    |     |       |
| Amendment                       | 2  | 84.73 | 0.000 | Amendment                       | 2  | 69.40 | 0.000 |
| Stress                          | 2  | 383.8 | 0.000 | Stress                          | 2  | 147.7 | 0.000 |
| Amend. × Stress                 | 4  | 2.161 | 0.115 | Amend. × Stress                 | 4  | 1.855 | 0.162 |
| Straw yield                     |    |     |       | Grain width/length ratio        |    |     |       |
| Amendment                       | 2  | 146.8 | 0.000 | Amendment                       | 2  | 14.03 | 0.000 |
| Stress                          | 2  | 4.808 | 0.021 | Stress                          | 2  | 21.77 | 0.000 |
| Amend. × Stress                 | 4  | 24.65 | 0.000 | Amend. × Stress                 | 4  | 3.668 | 0.024 |
| Harvest index                   |    |     |       | Seed index                      |    |     |       |
| Amendment                       | 2  | 17.66 | 0.000 | Amendment                       | 2  | 255.7 | 0.000 |
| Stress                          | 2  | 132.2 | 0.000 | Stress                          | 2  | 233.1 | 0.000 |
| Amend. × Stress                 | 4  | 0.465 | 0.761 | Amend. × Stress                 | 4  | 23.98 | 0.000 |
| Number of spikes/plant          |    |     |       | Grain density                   |    |     |       |
| Amendment                       | 2  | 72.95 | 0.000 | Amendment                       | 2  | 256.2 | 0.000 |
| Stress                          | 2  | 172.5 | 0.000 | Stress                          | 2  | 356.7 | 0.000 |
| Amend. × Stress                 | 4  | 1.342 | 0.293 | Amend. × Stress                 | 4  | 6.311 | 0.002 |
| Number of grains/spike          |    |     |       | Grain water content             |    |     |       |
| Amendment                       | 2  | 121.8 | 0.000 | Amendment                       | 2  | 31.67 | 0.000 |
| Stress                          | 2  | 188.2 | 0.000 | Stress                          | 2  | 1.929 | 0.174 |
| Amend. × Stress                 | 4  | 3.864 | 0.019 | Amend. × Stress                 | 4  | 3.166 | 0.039 |
| Grain length                    |    |     |       | Straw water content             |    |     |       |
| Amendment                       | 2  | 40.64 | 0.000 | Amendment                       | 2  | 10.05 | 0.001 |
| Stress                          | 2  | 114.5 | 0.000 | Stress                          | 2  | 6.998 | 0.006 |
| Amend. × Stress                 | 4  | 1.189 | 0.349 | Amend. × Stress                 | 4  | 3.455 | 0.029 |
plants but was reduced equally under the impact of SS and WS (Figure 1C).

Application of AE and AP increased number of spikes per plant by 44% and 31%, respectively in control plants, by 140% and 108%, respectively under SS and 75% and 52%, respectively under WS. The reduction in number of spikes per plant due to SS and WS averaged around 59% in NA plants and 43% in both AE- and AP-amended plants (Figure 1D). The increase in number of grains per spike due to AE and AP averaged around 25% in control plants but amounted to 130% and 82%, respectively under SS and 160% and 78%, respectively under WS. The reduction in number of grains per spike due to the impact of SS and WS was most severe in NA plants (57% and 66%, respectively), moderate in AP-amended plants (35% and 49%, respectively) and least (25% and 32%, respectively) in AE-amended plants (Figure 1E). Both AE and AP increased seed index by averages of 16% in control plants and 155% under SS and WS. The reduction in seed index was comparable under the impact of SS and WS, and amounted to 65% in NA plants and 23% in both AE- and AP-amended plants (Figure 1F).

The effect of treatments on grain size was relatively mild. Algal amendment, particularly AE, increased grain dimensions to a greater extent in stressed than control plants and under WS than SS. Application of AE and AP increased grain width by 17% and 7%, respectively in control plants, by 29% and 7%, respectively under SS and by 41% and 23%, respectively under WS (Figure 2A). The stress-induced reduction in grain width was more evident in NA plants (24% and 36% due to WS and SS, respectively) than in amended plants (23% as an average for SS and WS, irrespective of type of amendment). Grain length was increased by an average of 12% for AE and 8% for AP, irrespective of the stress regime but was reduced by an average of 15% below the control under the impact of SS and WS, irrespective of the amendment regime (Figure 2B). The width/length ratio of rice grain was increased in response to algal amendment, particularly AE, with greater effect under stress conditions.

Figure 1. Yield attributes: grain yield (A), straw yield (B), harvest index (C), number of spikes per plant (D), number of grains per spike (E) and seed index (F) of O. sativa L. cv. Sakha 101 grown on a silty clay soil amended with aqueous extract and powder of D. dichotoma under the impact of salt stress and water stress at \( \Psi_w \) of -0.492 MPa for 105 d. Each value is the mean of three replicates \( \pm SE \). Columns with common letters are non-significantly different at \( P \leq 0.05 \).
and under WS than SS. By contrast, abiotic stress reduced the grain width/length ratio, with stronger effect of WS than SS and in NA plants compared to AE-amended plants (Figure 2C).

Grain density exhibited the same pattern of seed index in response to treatments, with marked beneficial effect of algal amendment, particularly AE, and adverse effect of abiotic stress, particularly WS. Therefore, the beneficial effect was most pronounced for AE under SS, whereas the adverse effect of abiotic stress was most evident in NA plants with comparable effect of SS and WS (Figure 2D). Grain water content was reduced in response to algal amendment, particularly the AE in water stressed-plants but with marginal effect of abiotic stress, except for the increase due to WS in NA plants. Straw water content was less responsive to treatments compared with grain water content, with a mild decrease due to abiotic stress only in NA plants (Figure 2E-F).

The effect of the main factors on the biochemical composition of rice grain varied from significant to very highly significant but with stronger effect of abiotic stress than algal amendment and a less evident interaction (Table 2). The grain TSS and starch contents were marginally affected by algal amendment but appreciably increased under the impact of stress, particularly SS with an average increase in the two components of 24% above the control for all amendment regimes. By contrast, the grain protein content was significantly increased by algal amendment, particularly AE but reduced under stress, particularly WS. Consequently, the grain protein/starch ratio was increased due to algal amendment, particularly AE but was comparably reduced under the impact of SS and WS (Table 3).

The grain contents of $K^{+}$, $Ca^{2+}$, N and P were increased in response to algal amendment but reduced under the impact of abiotic stress, but Na$^{+}$ exhibited the opposite pattern. The amendment-induced increase in grain $K^{+}$ content was limited in control plants but was highest in water-stressed plants, with comparable effect of AE and AP. Only in salt-stressed plants, the increase due to AE exceeded that of AP. The reduction in grain

Figure 2. Grain attributes: grain width (A), grain length (B), grain width/length ratio (C), grain density (D), grain water content (E) and straw water content (F) of *O. sativa* L. cv. Sakha 101 grown on a silty clay soil amended with aqueous extract and powder of *D. dichotoma* under the impact of salt stress and water stress at $\Psi_w$ of -0.492 MPa for 105 d. Each value is the mean of three replicates ±SE. Columns with common letters are non-significantly different at $P \leq 0.05$. 

...
Starch K/Na ratio

Protein Nitrogen (N) stress conditions. The stress-induced increase in grain Na was marginal in control plants but averaged around 17% under the two treatments on K plants versus 18% in amended plants. As a consequence of the differential effect of treatments on K and Na contents of the grain, the grain K/ Na ratio was profoundly affected in a pattern similar to that of grain K in water-stressed plants with comparable effect of AE and AP in both cases; but, a differential effect emerged under the impact of SS in favor of AP. The effect of SS was marginal on grain As content of both NA- and AE-amended plants. The reduction in the As/P ratio was comparable due to AE and AP amendments. The reduction in the As/P molar ratio was appreciably affected by treatments. The reduction in the As/P ratio was comparable due to AE and AP amendments.

Table 2. Two-way ANOVA showing the effect of the main factors (algal amendment and abiotic stress) and their interaction on the chemical composition of grains of cv. Sakha 101 of O. sativa.

| Variable and source of variation | df | F  | P   | Variable and source of variation | df | F  | P   |
|----------------------------------|----|----|-----|----------------------------------|----|----|-----|
| Total soluble sugars (TSS)       | Amendment  | 2  | 3.062 | 0.072 | Calcium (Ca^{2+}) | Amendment  | 2  | 90.09 | 0.000 |
|                                  | Stress    | 2  | 105.2 | 0.000 | Stress             | 2  | 438.1 | 0.000 |
|                                  | Amend. × Stress | 4  | 0.199 | 0.936 | Amend. × Stress | 4  | 7.741 | 0.001 |
| Starch K/Na ratio                | Amendment  | 2  | 33.62 | 0.000 | Amendment          | 2  | 14.97 | 0.000 |
|                                  | Stress    | 2  | 113.5 | 0.000 | Stress             | 2  | 107.8 | 0.000 |
|                                  | Amend. × Stress | 4  | 2.210 | 0.109 | Amend. × Stress | 4  | 2.513 | 0.078 |
| Protein Nitrogen (N)            | Amendment  | 2  | 63.86 | 0.000 | Amendment          | 2  | 52.17 | 0.000 |
|                                  | Stress    | 2  | 101.8 | 0.000 | Stress             | 2  | 105.1 | 0.000 |
|                                  | Amend. × Stress | 4  | 0.384 | 0.818 | Amend. × Stress | 4  | 1.230 | 0.333 |
| Starch/protein ratio            | Amendment  | 2  | 80.34 | 0.000 | Amendment          | 2  | 74.78 | 0.000 |
|                                  | Stress    | 2  | 369.9 | 0.000 | Stress             | 2  | 98.82 | 0.000 |
|                                  | Amend. × Stress | 4  | 1.731 | 0.187 | Amend. × Stress | 4  | 0.580 | 0.681 |
| Potassium (K^{+})               | Amendment  | 2  | 162.8 | 0.000 | Amendment          | 2  | 300.3 | 0.000 |
|                                  | Stress    | 2  | 1342  | 0.000 | Stress             | 2  | 356.0 | 0.000 |
|                                  | Amend. × Stress | 4  | 25.47 | 0.000 | Amend. × Stress | 4  | 140.0 | 0.000 |
| Sodium (Na^{+})                 | Amendment  | 2  | 6.381 | 0.008 | Amendment          | 2  | 350.7 | 0.000 |
|                                  | Stress    | 2  | 15.61 | 0.000 | Stress             | 2  | 0.000 | 1.000 |
|                                  | Amend. × Stress | 4  | 1.477 | 0.251 | Amend. × Stress | 4  | 44.56 | 0.000 |

K^{+} content was more severe under SS than WS and in NA plants than amended plants. The amendment-induced reduction in grain Na content was marginal in control plants but averaged around 17% under the two stress conditions. The stress-induced increase in grain Na content was comparable under the impact of SS and WS and amounted to 41% in NA plants versus 18% in amended plants. As a consequence of the differential effect of treatments on K and Na contents of the grain, the grain K/Na ratio was profoundly affected in a pattern similar to that of grain K (Table 4). The increase in grain Ca^{2+} content due to AE and AP was comparable in control and salt-stressed plants with averages of 15% and 90%, respectively; but in water-stressed plants the increase due to AE (31%) exceeded that of AP (8%). The stress-induced reduction in Ca^{2+} content was more severe under SS than WS, particularly in NA plants (Table 4).

The amendment-induced increases in grain contents of N and P were comparable for the two algal amendments under the two stress regimes, with averages of 14% for N and 27% for P. The stress-induced reductions in grain N and P contents were more severe under WS (23%) than SS (14%), as averages for all amendment regimes (Table 5). Both algal amendment and abiotic stress decreased grain As content. The effect of algal amendment was mild in control plants and appreciable in water-stressed plants with comparable effect of AE and AP in both cases; but, a differential effect emerged under the impact of SS in favor of AP. The effect of SS was marginal on grain As content of both NA- and AE-amended plants versus 54% reduction in AP-amended plants; but the reduction due to WS was stronger in amended than NA plants. Consequently, the grain As/P molar ratio was appreciably affected by treatments. The reduction in the As/P ratio was comparable due to AE and AP amendments.

Table 3. Concentrations of total soluble sugars, starch, and protein and the protein/starch ratio in the grains of cv. Sakha 101 of O. sativa grown on a silty clay soil amended with aqueous extract and powder of D. dichotoma under the impact of salt stress and water stress at Ψ_w of -0.492 MPa for 105 d.

| Algal amendment and abiotic stress | Soluble sugars (mg g^{-1} DW) | Starch (mg g^{-1} DW) | Protein (mg g^{-1} DW) | Protein/starch ratio |
|------------------------------------|-------------------------------|----------------------|------------------------|----------------------|
| No amendment                       |                               |                      |                        |                      |
| Control                            | 142.6 ± 0.96^a                | 591.8 ± 5.27^a       | 57.5 ± 0.91^ad         | 0.097 ± 0.001^d     |
| NaCl                               | 175.4 ± 0.98^c                | 727.9 ± 6.49^h       | 50.6 ± 0.81^ah         | 0.070 ± 0.001^a     |
| PEG                                | 159.7 ± 2.17^b                | 662.8 ± 5.90^f       | 47.7 ± 0.78^f          | 0.072 ± 0.001^a     |
| Aqueous algal extract              |                               |                      |                        |                      |
| Control                            | 139.6 ± 0.41^a                | 579.3 ± 1.87^b       | 67.7 ± 1.43^f          | 0.171 ± 0.003^f     |
| NaCl                               | 173.1 ± 1.51^c                | 718.4 ± 2.33^b       | 59.5 ± 1.27^f          | 0.083 ± 0.002^b     |
| PEG                                | 153.4 ± 1.86^b                | 636.7 ± 1.81^d       | 55.5 ± 1.20^d          | 0.087 ± 0.002^d     |
| Algal powder                       |                               |                      |                        |                      |
| Control                            | 135.9 ± 0.76^a                | 563.8 ± 0.50^d       | 63.3 ± 0.77^d          | 0.112 ± 0.001^d     |
| NaCl                               | 171.2 ± 2.67^c                | 710.4 ± 0.63^b       | 55.7 ± 0.67^b          | 0.078 ± 0.001^b     |
| PEG                                | 153.5 ± 1.25^b                | 637.1 ± 0.55^c       | 51.9 ± 0.60^d          | 0.081 ± 0.001^b     |

Each value is the mean of three replicates ±SE. Means with common letters are non-significantly different at P ≤ 0.05.
Table 4. Concentrations of K⁺, Na⁺ and Ca²⁺ and the K/Na ratio in the grains of cv. Sakha 101 of O. sativa grown on a silty clay soil amended with aqueous extract and powder of D. dichotoma under the impact of salt stress and water stress at \( \Psi_w \) of -0.492 MPa for 105 d.

| Algal amendment and abiotic stress | K⁺ (mmol g⁻¹ DW) | Na⁺ (mmol g⁻¹ DW) | Ca²⁺ (mmol g⁻¹ DW) | K/Na ratio |
|-----------------------------------|-----------------|-----------------|-----------------|-------------|
| No amendment                      |                 |                 |                 |             |
| Control                           | 0.545 ± 0.004²  | 0.495 ± 0.042²  | 0.436 ± 0.009⁴  | 1.116 ± 0.084⁴ |
| NaCl                              | 0.319 ± 0.011³  | 0.722 ± 0.013⁴  | 0.150 ± 0.008⁴  | 0.441 ± 0.008⁴  |
| PEG                               | 0.396 ± 0.005²  | 0.676 ± 0.023⁴  | 0.308 ± 0.008⁴  | 0.586 ± 0.013²  |
| Aqueous algal extract             |                 |                 |                 |             |
| Control                           | 0.584 ± 0.001³  | 0.497 ± 0.016⁴  | 0.516 ± 0.009⁴  | 1.180 ± 0.036⁴  |
| NaCl                              | 0.382 ± 0.005³  | 0.583 ± 0.052⁴  | 0.299 ± 0.008⁴  | 0.666 ± 0.061⁴  |
| PEG                               | 0.520 ± 0.003³  | 0.569 ± 0.050⁴  | 0.402 ± 0.017³  | 0.929 ± 0.082²  |
| Algal powder                      |                 |                 |                 |             |
| Control                           | 0.583 ± 0.004³  | 0.481 ± 0.009³  | 0.489 ± 0.009⁴  | 1.213 ± 0.008⁴  |
| NaCl                              | 0.341 ± 0.005³  | 0.600 ± 0.014³  | 0.274 ± 0.008³  | 0.568 ± 0.020³  |
| PEG                               | 0.502 ± 0.003³  | 0.556 ± 0.039³  | 0.333 ± 0.008³  | 0.913 ± 0.064³  |

Each value is the mean of three replicates ±SE. Means with common letters are non-significantly different at P ≤ 0.05.

and averaged around 26% in control plants and 51% in water-stressed plants; but in salt-stressed plants, AE led to 22.5% reduction versus 64% reduction due to AP. The effect of abiotic stress on the grain As/P ratio was less evident than that of algal amendment and was versatile depending on type of stress and amendment regime (Table 5).

4. Discussion

The impact of abiotic stress on performance of rice has been intensively studied in the vegetative stage, but little information is available concerning yield attributes and the physiological changes in rice grain. Elucidation of the stress-induced changes in rice grain composition would help to assess the grain nutritional quality in a changing environment. The effect of treatments (algal amendment and abiotic stress) on rice yield resembled their effect on vegetative growth reported by El-Katony et al. (2020) for the same rice cultivar. Whereas grain yield of rice was adversely affected by abiotic stress, particularly in NA plants, it was markedly benefited from algal amendment, particularly under stress conditions. The beneficial effect of algal amendment was more evident for AE than AP and under the impact of SS than WS. The lesser improvement in straw yield relative to grain yield due to algal amendment led to marked improvement of the harvest index.

Several safe and efficient organic amendments, such as plant growth promoters (Quiroga et al., 2020), organic manures (Srivastava et al., 2019) and seaweed extracts (El-Katony et al., 2020) have been proven to enhance plant performance and to mitigate the severity of abiotic stress. Furthermore, organic amendments have the advantage of improving soil structure and capacity to hold water and nutrients beside enhancement of the beneficial soil microflora and fauna. In addition to affecting the magnitude of rice yield, the hazardous effect of abiotic stress as well as the beneficial effect of algal amendment included also grain characteristics. The enhanced yield afforded by algal amendment was accompanied with the production of broader, drier and heavier grains; meanwhile the stress-induced hindered productivity was associated with the production of narrower, lighter and less dry grains. Production of drier grains can be appreciated as a valuable economic and technical advantage for it allows good storage conditions and avoidance of invasion from pests and pathogens. Spoilage of cereal grains with high moisture content at harvest can result from invasion from insects and fungi with the production of mycotoxins (Terzi et al., 2014).

The differential effect of treatments on dimensions and weight of rice grains resulted in altered grain density. The length and width of rice grain are important attributes that are relevant to the class, chemical composition and cooking properties of the grain. Although the experimental rice cultivar (Sakha 101) is a medium grain one, with a length/width ratio of 0.573, the present work claims that this trait can be appreciably modified environmentally, with the production of broader grains in response to algal amendment versus narrower grains under the impact of abiotic stress. Usually, an intimate correlation exists between grain size of cereals and quality criteria such as flour yield of baking wheat or malting suitability of brewing barley (Wiesler, 2012). The

Table 5. Concentrations of N, P and As and the As/P ratio in the grains of cv. Sakha 101 of O. sativa grown on a silty clay soil amended with aqueous extract and powder of D. dichotoma under the impact of salt stress and water stress at \( \Psi_w \) of -0.492 MPa for 105 d.

| Algal amendment and abiotic stress | N (mmol g⁻¹ DW) | P (mmol g⁻¹ DW) | As (mmol g⁻¹ DW) | As/P ratio × 10⁻² |
|-----------------------------------|----------------|----------------|-----------------|-----------------|
| No amendment                      |                 |                 |                 |                 |
| Control                           | 0.647 ± 0.009³  | 3.47 ± 0.473³  | 1.019 ± 0.009³  | 2.9 ± 0.07³     |
| NaCl                              | 0.569 ± 0.008³  | 2.91 ± 0.399³  | 0.992 ± 0.029³  | 3.4 ± 0.15³     |
| PEG                               | 0.527 ± 0.004³  | 2.57 ± 0.350³  | 0.872 ± 0.016³  | 3.4 ± 0.07³     |
| Aqueous algal extract             |                 |                 |                 |                 |
| Control                           | 0.766 ± 0.016³  | 4.37 ± 0.777³  | 0.930 ± 0.012³  | 2.1 ± 0.07³     |
| NaCl                              | 0.673 ± 0.014³  | 3.67 ± 0.650³  | 0.975 ± 0.013³  | 2.6 ± 0.03³     |
| PEG                               | 0.598 ± 0.021³  | 3.23 ± 0.573³  | 0.548 ± 0.013³  | 1.7 ± 0.06³     |
| Algal powder                      |                 |                 |                 |                 |
| Control                           | 0.715 ± 0.009³  | 4.45 ± 1.637³  | 0.988 ± 0.013³  | 2.2 ± 0.06³     |
| NaCl                              | 0.629 ± 0.008³  | 3.74 ± 1.373³  | 0.458 ± 0.009³  | 1.2 ± 0.03³     |
| PEG                               | 0.585 ± 0.007³  | 3.29 ± 1.210³  | 0.538 ± 0.009³  | 1.6 ± 0.06³     |

Each value is the mean of three replicates ±SE. Means with common letters are non-significantly different at P ≤ 0.05.
improvement of grain vigor with the aid of algal amendment, paralleled with the increase in protein content along with the marginal lowering in TSS and starch contents, resulted in increased protein/starch ratio of the grain. A positive correlation of vigor of rice grain with protein and mineral nutrient content versus a negative correlation with soluble sugars and starch contents is evident from Figure 3. The broader grains of algal amend-plants are expected to simulate the short grain rice with low content of amylose and sticky texture upon cooking. However, such prediction needs experimental verification.

The response of mineral content of rice grain to treatments simulated those of the vegetative stage reported by El-Katony et al. (2020) but to a limited extent. The beneficial effect of algal amendment included increased grain contents of K\(^+\), Ca\(^{2+}\), N and P versus reduced content of Na\(^+\), with the reverse being assigned to abiotic stress. The beneficial role of algal amendment in increasing grain Ca\(^{2+}\) content, even to a greater extent than that of K\(^+\), can be appreciated in light of the fact that the overall Ca\(^{2+}\) content of cereals is low and is subjected to further lowering under the impact of salinity (Luo et al., 2014; Razzaq et al., 2020). The limited changes in grain mineral composition compared with those in plant foliage can be resolved in light of the fact that fruits and grains, in contrast to plant foliage, receive their organic and mineral supplies mainly via the phloem. This allows major modification of the ascending sap which aids in protection of the reproductive structures from the adverse consequences of abiotic stress on their mineral composition.

Although algal amendment and abiotic stress exerted contrasting effects on yield and grain composition of rice, the two regimes coincided in reducing As content and the As/P ratio of the grain, which represents a beneficial aspect of abiotic stress regarding safety measures of the grain. However, while the effect of algal amendment in reducing As/P ratio was consistent at all stress regimes the effect of abiotic stress was versatile, being evident only in amended plants. The As-P competition during uptake renders the As/P ratio of the grain an important safety criterion. Arsenic is efficiently accumulated in cereals which renders cereal staples, particularly rice, the primary source of As Ingestion (Deng et al., 2019). Pollution with As can arise from irrigation of paddies with high As waters and the use of arsenical pesticides (Kumarabhilaka et al., 2018). Fortunately, the As levels in the experimental rice samples (0.061 mg/kg in the average) are by far below those reported by Huang et al. (2013) and the maximum allowable limit of 0.70 mg/kg. These overall low As levels might be a consequence of the hygienic experimental conditions employed in the present work with lack of the regular agricultural regimes of rice production including application of As-containing pesticides and fertilizers.

The beneficial effect of algal amendment—also their stress-relieving effect—on rice yield can be attributed to the high content of the alga (D. dichotoma) of macro- and micro-nutrients, growth stimulators (cytokinins, auxins, and gibberellins), bioactive ingredients (flavonoids and phenolics) and the unique algal product fucoidan (Ward et al., 2017). The improving role of macro- and micro-nutrients can be ruled out since all rice plants received an adequate supply of nutrients during the time course of the experiment, leaving an appreciable role for the unique algal components. But, the limited beneficial effect of AP compared with AE can be probably attributed to fermentation of the organic fraction of algal biomass such as sugars, alginates, proteins and lipids in the anaerobic environment of paddy rice with the release of toxic intermediates such as volatile fatty acids and phenolics (Pang et al., 2007). These toxic intermediates, rather than O\(_2\) deficiency, might be the growth limiting factor for cotton amended with wheat straw under anaerobic microhabitats (Narwal, 2012). Furthermore, for seaweeds in particular, the production of sulfides as a consequence of anaerobic decomposition of the sulfated sugar ‘alginate’ must be taken into account (Nabti et al., 2017).

The relatively severe impact of WS compared with SS on rice yield, demonstrated in the present work, as well as on the vegetative growth of rice reported by El-Katony et al. (2020) can be explained in light of the fact that salt ions might serve as cheap osmotic to countervail the osmotic factor of SS. This hypothesis is, however, not reasonable because of the significant negative correlation between yield attributes of rice and grain Na\(^+\) content (Figure 3). Alternatively, it seems that inducing WS via the artifact "PEG 6000" might return a different response than that expected from physical drought induced by water shortage. Although the high molecular weight PEG 6000 is proposed not to cross cellular membranes, yet the more aggressive impact of PEG-induced WS compared with that of NaCl-induced SS on rice yield suggests a specific toxic effect of PEG. There exists the possibility of membrane permeation of the PEG polymer or some of its contaminants such as monomer molecules and the chemicals used in manufacture of the polymer. In addition, the low oxygen availability and the restricted water uptake from the viscous PEG solutions cannot be ruled out (Slama et al., 2007). However, the hazard of oxygen shortage is not likely since paddy rice is well-adapted to anaerobic conditions.

5. Conclusions

The lower beneficial effect of D. dichotoma powder relative to algal extract on rice yield can be attributed to fermentation of the algal organic material in the paddy rice habitat with the release of toxic intermediates. Likewise, the stronger impact of WS (PEG 6000) compared with NaCl salinity can be due to either the specific effects of toxic chemicals in PEG preparations or the restricted water absorption from the viscous PEG solutions. A beneficial aspect of abiotic stress, particularly WS, is the reduced grain As content. The improved yield of algal amended-rice was accompanied with the production of broader, drier and heavier grains as well as high protein and mineral content versus low contents of Na\(^+\), As, soluble sugars and starch. Production of drier grains is a technical advantage for it points to early maturation of grains which allows good storage conditions and avoidance of invasion from pests and pathogens. Although rice grain vigor is an inherited trait it can be appreciably modified environmentally. The limited alteration in rice grain ionic relative to that of the foliage agrees with the fact that grains receive their mineral supply primarily via the phloem.

Declarations

Author contribution statement

Taha Mohamed El-Katony: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Fatma Mohamed Ward: Performed the experiments.
Mohamed Ali Deyab: Contributed reagents, materials, analysis tools or data.
Magda Faiz El-Adl: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement
Data will be made available on request.

Declaration of interests statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

Acknowledgements
The authors thank the Experimental Station of Agricultural Research at Giza, Egypt for providing rice grains.

References
Acosta-Motos, J.R., Ortuño, M.F., Bernal-Vicente, A., Díaz-Vivancos, P., Sanchez-Blanco, M.J., Hernandez, J.A., 2017. Plant responses to salt stress: adaptive mechanisms. Agron 7, 18.
Allen, S.E., Grimshaw, H.M., Rowland, A.P., 1986. Chemical analysis. In: second ed. Methods in Plant Ecology. Blackwell Scientific Publications, Oxford, pp. 285–344.
Armanuos, A.M., Negm, A., 2019. Integrated groundwater modeling for simulation of saltwater intrusion in the Nile Delta aquifer, Egypt. In: Negm, A. (Ed.), Groundwater in the Nile Delta. Springer, Cham, pp. 489–544.
Bassouny, N.N., Zembeli, J., 2019. Effect of planting methods on the quality of three Egyptian rice varieties. Agriculture 65, 119–127.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Brányiová, I., Mariášková, B., Doucha, J., Brányik, T., Bišová, K., Zachleder, V., Vítová, M., 2011. Micr algal novel biologically efficient starch producers. Biotechnol. Bioeng. 108, 766–776.
Cooper, J.E., Scherr, H.W., 2012. Nitrogen fixation. In: Marschner, P. (Ed.), Marschner’s Mineral Nutrition of Higher Plants. Academic Press, pp. 389–408.
Deng, F., Yu, M., Martinoia, E., Song, W.Y., 2019. Ideal cereals with lower arsenic and cadmium by accurately enhancing vascular sequestration capacity. Front. Genet. 10, 322.
El-Katony, T.M., Deyab, M.A., El-Adl, M.F., Ward, F.M., 2020. The aqueous extract and powder of the brown alga Dictyota dichotoma (Hudson) J.V. Lamouroux alleviate salt stress in rice (Oryza sativa L.) during germination. J. Plant Growth Regul. 40 (3), 986–999.
George, E., Horst, W.J., Neumann, E., 2012. Adaptation of plants to adverse chemical soil conditions. In: Marschner, P. (Ed.), Marschner’s Mineral Nutrition of Higher Plants. Academic Press, pp. 409–472.
Hager, A.S., Wolter, A., Jacob, F., Zannini, E., Arendt, E.K., 2012. Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. J. Cereal. Sci. 56, 239–247.
Huang, Z., Pan, X.D., Wu, P.G., Han, J.L., Chen, Q., 2013. Health risk assessment of heavy metals in rice to the population in Zhejiang, China. PloS One 8, e75007.
Kumarathilaka, P., Seneeweera, S., Meharg, A., Bundschuh, J., 2018. Arsenic speciation dynamics in paddy rice soil-water environment: sources, physico-chemical, and biological factors. A review. Water Res. 140, 403–414.
Luo, Y.W., Xie, W.H., Jin, X.X., Wang, Q., He, Y.J., 2014. Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes. CyTA - J. Food 12 (1), 22–26.
Maness, N., 2010. Extraction and analysis of soluble carbohydrates. In: Plant Stress Tolerance. Humana Press, pp. 341–370.
Nabbi, E., Jha, B., Hartmann, A., 2017. Impact of seaweeds on agricultural crop production as biofertilizer. Int. J. Environ. Sci. Technol. 14, 1119–1134.
Narwal, S.S., 2012. Allelopathy in Crop Production. Scientific Publishers, India.
Pang, J., Cui, T., Shabala, L., Zhou, M., Mendham, N., Shabala, S., 2007. Effect of secondary metabolites associated with anaerobic soil conditions on ion fluxes and electrophysiology in barley roots. Plant Physiol. 145, 266–276.
Quiroga, G., Erice, G., Aroca, R., Zamarro, Á.M., García-Mina, J.M., Ruiz-Lozano, J.M., 2020. Radial water transport in arbuscular mycorrhizal maize plants under drought stress conditions is affected by indole-3-acetic acid (IAA) application. J. Plant Physiol. 146, 153115.
Razzaq, A., Ali, A., Safdar, L.B., Zafar, M.M., Rui, Y., Shaheen, A., Ashraf, M., Gong, W., Yuan, Y., 2020. Salt stress induces physiochemical alterations in rice grain composition and quality. J. Food Sci. 85, 14–20.
Rossi, S., Capobianco, F., Sabatino, G., Mauroma, F., Luongo, D., Rossi, M., 2020. Pilot scale production of a non-immunogenic soluble gluten by wheat flour transamination with applications in food processing for celiac-susceptible people. J. Cereal. Sci. 96, Article 103117.
Slaama, I., Ghazy, T., Hessini, K., Messedi, D., Savoure, A., Abdebly, C., 2007. Comparative study of the effects of manniol and PEG osmotic stress on growth and solute accumulation in Sesuqium portulacastrum. Environ. Exp. Bot. 61, 10–17.
Srivastava, P., Wu, Q.S., Giri, B., 2019. Salinity: an overview. In: Microorganisms in Saline Environments: Strategies and Functions. Springer, Cham, pp. 3–18.
Suriyakul, C., Harano, K., Tanamachi, K., Matsuo, K., Iwaya-Inoue, M., Ishibashi, Y., 2017. Reactive oxygen species induced by heat stress during grain filling of rice (Oryza sativa L.) are involved in occurrence of grainchalkiness. J. Plant Physiol. 216, 52–57.
Terzi, V., Tumino, G., Stacca, A.M., Morcia, C., 2014. Reducing the incidence of cereal head infection and mycotoxins in small grain cereal species. J. Cereal. Sci. 59 (3), 284–293.
Varga, G., Svecziak, Z., 2006. The effect of late-season urea spraying on grain yield and quality of winter wheat cultivars under low and high basal nitrogen fertilization. Field Crop. Res. 96, 125–132.
Vasconcelos, M., Datta, K., Oliva, N., Khalekuzzaman, M., Torizzo, L., Krishnan, S., Oliveira, M., Goto, F., Datta, S.K., 2003. Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci. 164, 371–378.
Ward, F.M., Deyab, M.A., El-Katony, T.M., 2017. Biochemical Composition and Bioactivity of Dictyota from Egypt. Lambert Academic Publishing, Germany.
Wieder, E., 2012. Nutrition and quality. In: Marschner, P. (Ed.), Marschner’s Mineral Nutrition of Higher Plants. Academic Press, pp. 271–282.
Zhao, C., Zhang, H., Song, C., Zhu, J.K., Shabala, S., 2020. Mechanisms of plant responses and adaptation to soil salinity. Innovation 1 (1), 100017, http://creativecommons.org/licenses/by-nc-d/4.0/.

T.M. El-Katony et al. Heliyon 7 (2021) e07911