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Extracts of the Brown Alga *Dictyota dichotoma* (Hudson) J.V. Lamouroux Alleviate Salt Stress in Rice (*Oryza sativa* L.) During Germination

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Abstract

Algal extracts provide a safe regime for enhancing crop productivity under stressful conditions. The present study evaluated the efficiency of aqueous and ethanolic extracts of the brown alga *Dictyota dichotoma* in alleviation of salt stress on germination of rice seeds. Firstly, seeds were germinated using the aqueous extract of *D. dichotoma* at concentrations of 0, 5, 10, 20, and 50 g L^{-1}, prepared either at room temperature (RTE) or by boiling (BLE). The % germination of rice increased from 84% in non-treated seeds to 100% when treated with 20 g L^{-1} BLE, although this treatment caused reduced uniformity of germination. Embryo growth was maximum at 20 g L^{-1} of both extracts with superiority of BLE over RTE. In the second experiment, the effect of 20 g L^{-1} aqueous and ethanolic extracts relative to a balanced nutrient supply on germination of seeds treated with 0, 40, 90, and 170 mM NaCl was investigated. Salinity reduced % germination with delayed onset but high uniformity of germination, whereas algal amendments counterbalanced the effect of salinity to a greater extent relative to the nutrient supply. Upon withdrawal of salt stress, seeds promptly recovered, with more efficient recovery of seeds exposed to 170 mM than from 90 mM NaCl. The lower recovery of salt-treated seeds compared with the control seed germination suggests that rice suffered from the toxic ion effect of salinity on embryo rather than from the osmotic effect. Extracts of *D. dichotoma* can enhance and also alleviate salinity stress on rice seed germination.

Keywords Algal extract · Embryo growth · Germination speed · Germination uniformity · Salinity

Introduction

Secondary salinization arises from improper irrigation practices such as flood irrigation and the use of low-quality water such as well water and brackish surface waters. Under warm climates, soil flooding results in deposition of soluble salts at the soil surface as a consequence of enhanced evaporation of water (Minhas et al. 2015). These factors pose a serious constraint to the productivity of land available for cultivation, particularly in developing countries. Toxicity caused by salinity adversely impacts plant performance, which can be attributed to the osmotic effect, specific ion effect, induction of nutritional imbalance and oxidative damage to biomolecules and membranes. The specific ion effects of salinity, which arise at relatively low salt concentrations, might be attributed either to Na^+, e.g., in rice (Lin and Kao 2001), or Cl^-, e.g., in *Citrus* (Moya et al. 2003) and *Vitis* (Fort et al. 2013), or to both Na^+ and Cl^-, e.g., in *Medicago* (Aydi et al. 2008).

Salt sensitivity of plant species varies according to their developmental stage. For example, rice is more salt-tolerant at germination than at maturity (Khatun and Flowers 1995; Walia et al. 2005). In contrast, sugar beet is highly salt-tolerant during most of its life cycle but sensitive during germination (George et al. 2012). Seed germination is a critical stage, determining the establishment of plants under saline conditions. Salinity can reduce the rate and percentage of germination which in turn can lead to the development

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of patchy crop stands and reduction in yield (Foolad et al. 1999). Although the extent of salinity damage in some plant species might vary at germination and during the vegetative stages, the plant response to salinity at germination can be manipulated as a brief and reliable indicator of plant establishment in salt-affected habitats.

A wide variety of amendments have been applied to enhance plant performance and to confer protection from the harmful effects of biotic and abiotic stresses. These materials include growth-stimulating hormones, polyamines, and antioxidants, which can be applied either to the germinating seeds or during vegetative growth (George et al. 2012; Fu et al. 2019; Parađiković et al. 2019). In this context, algal extracts provide promising amendments to alleviate the impact of abiotic stress on performance of higher plants. Algae are well-known valuable sources of plant macronutrients and micronutrients as well as a variety of important bioactive compounds (Hamid et al. 2015; Kocira et al. 2019). For example, *Cladophora pellucida*, *Padina tetrastromatica* and *Laurencia papillosa* can be exploited as sources of the unique algal polysaccharides cladophoran, fucoidan and agar, respectively, in addition to a high content of bioactive compounds and antioxidant activities. The polysaccharide cladophoran had been identified by Ismail et al (2016) and Robin et al. (2017) in *Cladophora pellucida*. These include DPPH radical scavenging activity, β carotene, phenolics, flavonoids, ascorbic acid as well as omega 3 and omega 6 fatty acids (Ismail et al. 2016). Extracts of different algal species can enhance plant growth and protect plants against biotic and abiotic stresses through activation of the antioxidative systems, such as catalase, peroxidase and ascorbate, as well as through providing nutrients, hormones and other essential compounds needed for plant growth (Hashem et al. 2019). In addition, the beneficial effect of algal extracts has been attributed to modulating the expression of a plethora of stress-responsive genes (Jithesh et al. 2019). Priming of rice seeds with the alga *Turbinaria ornata*-based biogenic ZnO nanoparticles enhanced seed germination and seedling growth. Also, foliar application of the biogenic ZnO nanoparticles improved rice grain weight and quality (Itroutwar et al. 2019).

The brown alga *Dictyota dichotoma* (Hudson) J.V. Lamouroux grows along the Red Sea coast of Egypt throughout the year, and flourishes especially during the colder seasons, and can serve as a suitable amendment to improve plant productivity, particularly under stressful conditions. Extracts of *D. dichotoma* improved the growth and yield of okra (*Abelmoschus esculentus*) (Sasikumar et al. 2011). Rice is one of the most important cereal crops globally, with numerous cultivars with divergent yield potential and attributes. Out of several cultivars investigated, the rice cultivar Sakha 101 is very productive (Fazaa et al. 2016). Rice production consumes huge amounts of water, which represents a serious demand on water supplies available for irrigation, particularly in arid regions. Furthermore, it is expected that the water resources of Egypt will be subjected to a sharp decline within the coming few years (Omar and Moussa 2016) as a consequence of construction of “Grand Ethiopian Renaissance Dam” at the headwaters of the river Nile. Egypt is one of the most arid countries all over the world and relies primarily on the river Nile for water resources. Therefore, it is necessary to use the limited potable fresh water supplies for human consumption and to utilize the available poor quality water for irrigation. This necessitates an evaluation of the salt tolerance of the economic crops to select the most tolerant cultivars as well as to search for and adopt safe and economic practices to enhance crop yield under salt stress conditions.

The adverse effect of salinity stress, as well as the stress-relieving effect of algal extracts on rice germination, needs thorough investigation, not only with respect to the final germination percentage but also regarding the time-dependent indices of germination, such as speed, uniformity, synchrony and lag of germination. The present work evaluated the effect of different extracts of the brown alga *D. dichotoma* in alleviation of the salinity impact on germination of rice.

Materials and Methods

Plant Material

Seeds of rice (*O. sativa* L.) cv. Sakha 101 were obtained from the Experimental Station of Agricultural Research at Giza, Egypt. Uniform seeds were used to minimize experimental error resulting from heterogeneity of the starting material. Fronds of the brown alga *D. dichotoma* were collected from semi-exposed shores of Hurghada, Red Sea coast of Egypt (27°13′ N, 33°45′ E), during October 2014. At this period, the alga forms lush vegetation and fronds were harvested from the intertidal zone, washed with distilled water to remove extraneous foreign particles and salts from the surface and stored in an ice box during transportation to the laboratory. In the laboratory, the fronds were shade-dried on blotting paper at 27 °C for 1 week to achieve a constant weight, and then ground into a fine powder using a coffee grinder. The powder was stored in air-tight glass containers at room temperature until used for extraction.

Preparation of Algal Extracts and Nutrient Solution

Stock aqueous algal extracts were prepared by mixing 100 g powdered algal fronds in 1 L distilled water either at room temperature (30 °C) (RTE) or in boiling water (BLE) for six hours. The slurry was first filtered through nylon cloth, followed by Whatman No. 1 filter paper and the volume of supernatant was made up to 1 L with distilled water. An ethanolic algal extract was prepared in the same way as the boiled aqueous extract but with the use of 80% ethanol instead of water, followed by evaporation of ethanol at 70 °C; the residue was re-dissolved in 1 L water. In addition to using algal extracts as amendments, seeds were treated with a balanced nutrient solution containing the macronutrients (mM): N 6, P 0.5, K 4.5, Ca 1, Mg 0.5 and S 3.5 and the micronutrients (µM): Fe 25, Mn 5, Cu 0.5, Zn 0.5, B 25, Na 50, Cl 50, Mo 0.25 and Co 0.1.

Experiment 1. Effect of Extraction Temperature on Rice Germination

Seeds of rice were germinated in a range of concentrations (0, 5, 10, 20, and 50 g L^{-1}) of the aqueous algal extract, either the RTE or the BLE, in the dark at a temperature of 30/20 °C day/night ± 2 °C in the laboratory. Fifty seeds were germinated in 12 cm plastic Petri dishes lined with filter papers saturated with the test solutions. To saturate the filter papers, 5 ml of the test solutions were applied at 12 h intervals. Seeds were considered germinated with the emergence of the radicle of up to 2 mm. The number of germinated seeds was monitored daily for 7 days. The lengths of radicles and plumules were measured at the end of the germination period.

The experiment was factorial with two factors and four replications in a completely randomized design. The main factors were (1) temperature of extraction with two levels: room temperature (30 °C) and boiling water (100 °C); and (2) concentration of extract with five levels: 0, 5, 10, 20, and 50 g L^{-1}.

Experiment 2. Amendment × Salinity Interaction on Rice Germination

The above extraction temperature experiment revealed that BLE of *D. dichotoma* at 20 g L^{-1} resulted in maximum germination of the rice seeds. Therefore, in this experiment, the effectiveness of the aqueous and ethanolic algal extracts (AE and EE, respectively) at 20 g L^{-1}, in relieving the impact of salt stress on rice germination, was compared with the effect of amendment with a balanced nutrient solution (NS). The nutritional factor of the algal extract can be resolved from other probable effects by comparing the algal extract with a balanced nutrient solution. It is possible that treatment of rice seeds with nutrients, derived from algal extracts, might relieve the adverse effect of salinity on seed germination by counteracting the specific ion effect and the salinity-induced nutritional imbalance. Otherwise, superior effectiveness of algal extracts over the nutrient supply, if any, might point to more than a nutritional component of the algal material.

Seeds were germinated in 0, 40, 90, and 170 mM NaCl in combination with either 20 g L^{-1} aqueous or ethanolic algal extract or with the balanced nutrient solution along with a non-amended control (NA). Germination conditions were the same as in the previous experiment. Filter papers were kept moist by addition of 5 mL of the test solutions twice a day. To prevent the buildup of salt, the seeds were daily transferred to new Petri dishes lined with filter papers saturated with the test solutions. The number of seeds germinated was monitored daily for 7 days. On day 7, non-germinated seeds were transferred from saline solutions to new Petri dishes lined with filter papers that were kept moist by addition of 5 mL distilled water every 12 h, and number of newly germinated seeds was monitored daily for 7 days.

This experiment was also factorial with two factors and four replications in a completely randomized design. The main factors were (1) type of amendment with four levels: no amendment, aqueous algal extract, ethanolic algal extract, and a complete nutrient solution; and (2) salinity stress with four levels: 0, 40, 90, and 170 mM NaCl.

Definitions, Calculations and Statistical Analysis

The germination parameters measured were % germination as well as the rate or speed, lag time, uniformity and synchrony of germination (Ranal and Santana 2006). These parameters were calculated as follows:

I. The final germination percentage (FGP) was calculated as the final number of seeds germinated as a percentage of the total number of seeds.
II. Rate or speed of germination was estimated as Timson index of germination velocity (TI):

$$\text{Timson index} = \frac{\sum G_i}{T} = \frac{G_1 + G_2 + G_3 + \ldots + G_n}{T} \quad (\%\ d^{-1})$$

where G_1, G_2, G_3, G_i and G_n are the cumulative number of seeds germinated at the first, second, third, i^{th} and final time, respectively; and T is the total germination period (days).

III. Germination lag estimated as T_{10} or time to 10% germination which is a measure of the lag period between imbibition and onset of germination.
IV. Uniformity of germination

The coefficient of variation of the germination time (CV_t) evaluates the germination uniformity or variability of germination time. CV_t is an inverse measure of germination uniformity, where high values mean less uniform germination. CV_t was calculated as follows:

$$CV_t = \frac{S_t \times 100}{\bar{t}} \ (\%)$$

where S_t is the standard deviation of germination time and $\bar{t}$ the mean germination time (MGT).

Mean germination time was calculated according to the following equation:

$$\mathrm{MGT} = \frac{\sum g_i \times t_i}{\sum g_i} = \frac{\sum g_i \times t_i}{N} \text{ (d)}$$

where g_i = the number of seeds newly germinated at time t_i (days) from sowing, not the cumulative germination count, and N = the total number of seeds germinated.

The variance of germination time (S_t^2) was calculated according to the following formula:

$$S_t^2 = \frac{\sum (t_i - \bar{t})^2}{\sum g_i - 1} \text{ (d}^2\text{)}$$

V. Synchrony of germination was estimated using the synchronization index ($\overline{E}$) as follows:

$$\overline{E} = \sum f_i \times \log_2 f_i \quad \text{bit} \quad \text{and} \quad f_i = \frac{g_i}{\sum g_i}$$

where f_i is the relative frequency of germination and g_i the number of seeds germinated on day i. Synchronization index evaluates the degree of overlapping of seed germination. Low values of $\overline{E}$ indicate more synchronized germination.

Any germination recovery following the removal of salinity stress was calculated as the number of seeds newly germinated after transfer to water as a percentage of the number of seeds transferred (those non-germinated in the saline solution).

Final germination percentage and final percentage recovery of germination from salinity stress were arcsine-transformed before performing statistical analyses to ensure homogeneity of variance. Data were analyzed using SPSS version 22 and the effects of the main factors and their interaction were assessed using two-way ANOVA. Mean separation was performed using Duncan's multiple range test at $P < 0.05$.

Results

Effect of Temperature of Algal Extraction on Rice Germination

The time course of germination for the rice seeds tested followed a typical sigmoid pattern, with increasing magnitude but shorter lag period with increasing concentration of algal extract (Fig. 1). The effect of treatments varied from highly significant ($P < 0.01$) for the main factors (i.e., temperature of extraction and concentration of extract) and their interaction for speed of germination and embryo growth but with non-significant interactions for germination and synchronization index or a significant effect only of extract concentration for uniformity of germination (Table 1).

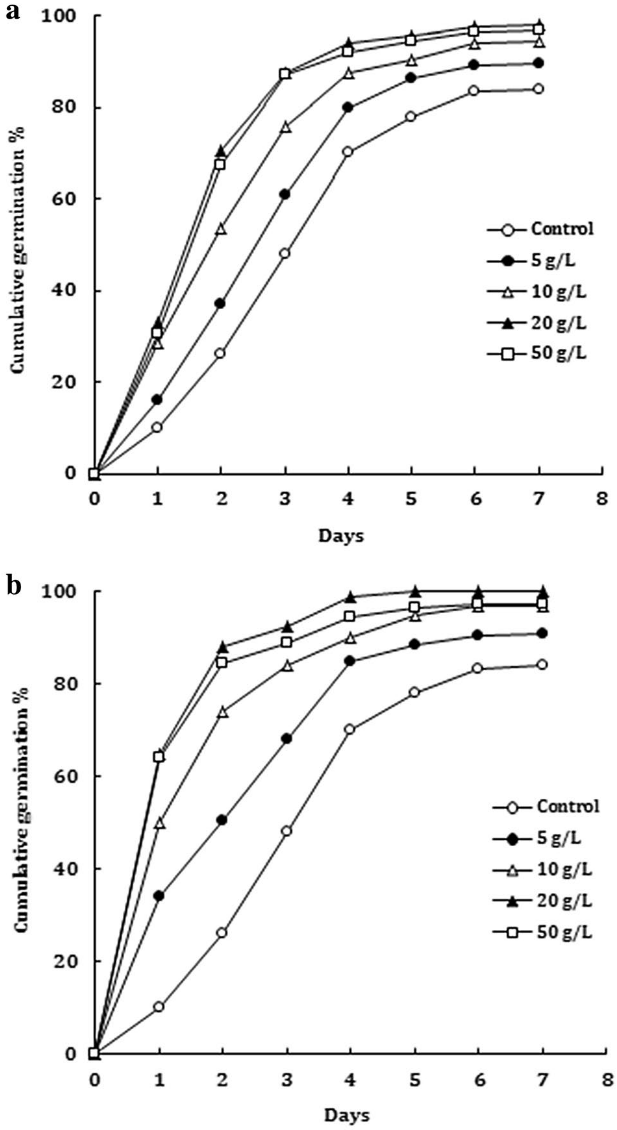

Fig. 1 Time course of germination of *O. sativa* L. seeds treated with extracts of the brown alga *D. dichotoma*. The extracts were prepared either at room temperature (RTE, **a**) or by boiling (BLE, **b**). Each value is the mean of four replicates

The final germination percentage and speed of germination were significantly improved following use of algal amendment, with a marked advantage of the BLE over the RTE and a progressive increase with extract concentration up to 20 g L^{-1}, beyond which the final germination percentage and speed of germination approached steady values (Fig. 2a). Germination of control rice seeds was only 84%, whereas at the optimal extract concentration of 20 g L^{-1},

Table 1 Two-way ANOVA showing the effect of extraction temperature and concentration of *D. dichotoma* Hudson extract and their interaction on germination of *O. sativa* L. seeds

Source of variation	df	*F*	*P*	*F*	*P*
		Germinability (deg.)		Synchronization index ($\overline{E}$)	
Temperature	1	8.509	0.007	8.678	0.006
Conc. of extract	4	44.82	0.000	6.710	0.001
Temp. × conc	4	1.369	0.268	0.921	0.464
		Timson index		Radicle length	
Temperature	1	169.1	0.000	143.2	0.000
Conc. of extract	4	451.5	0.000	72.75	0.000
Temp. × conc	4	11.35	0.000	9.226	0.000
		Coefficient of variation in germination time (CV_t)		Plumule length	
Temperature	1	0.065	0.801	297.5	0.000
Conc. of extract	4	5.820	0.001	131.5	0.000
Temp. × conc	4	0.576	0.682	20.58	0.000

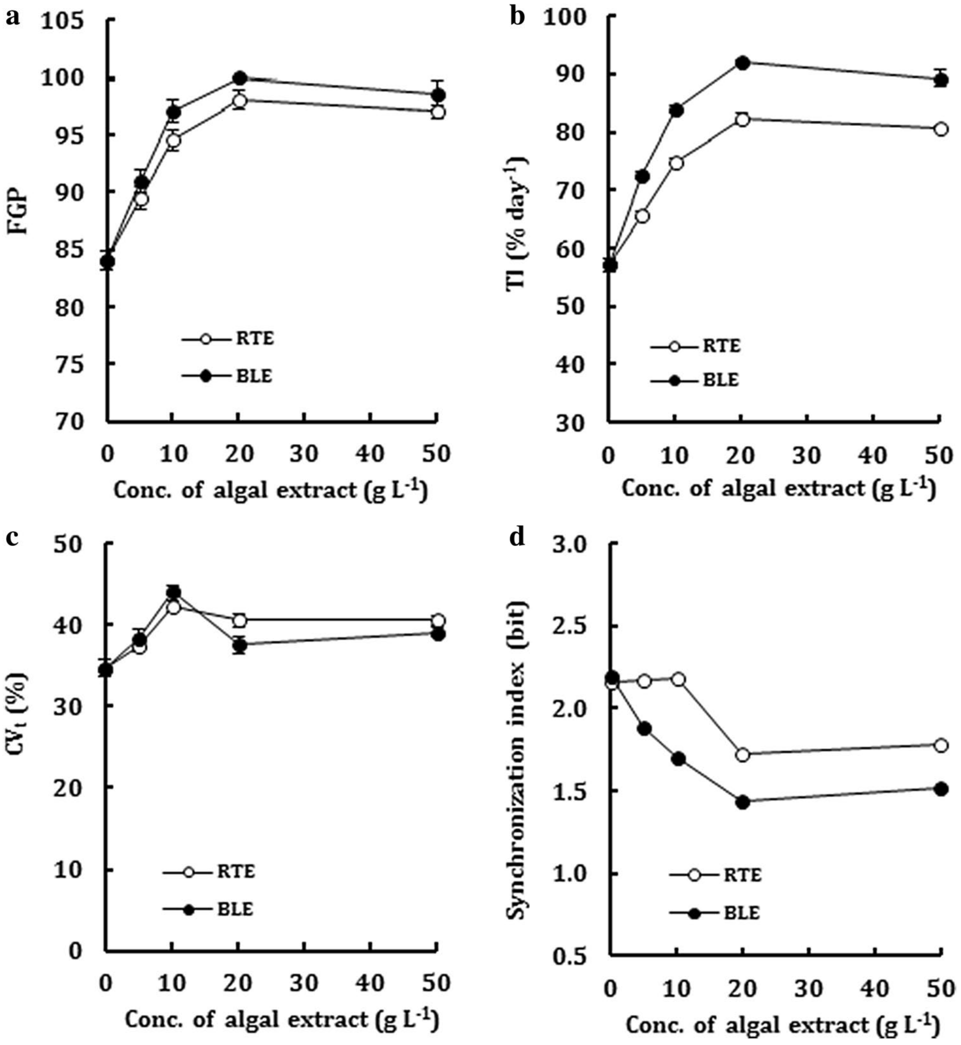

Fig. 2 Final germination percentage (**a**), Timson index (**b**), coefficient of variation in germination time (CV_t, **c**) and synchronization index (E, **d**) of *O. sativa* L. seeds treated with extracts of the brown alga *D. dichotoma* prepared either at room temperature (RTE, a) or by boiling (BLE, **b**). Each value is the mean of four replicates ± SE

the magnitude of germination approached 98% in the RTE and up to 100% in the BLE. A more profound effect of the algal amendment was found for germination speed, where an increasing concentration of the RTE and BLE from 0 to 20 g L^{-1} increased Timson index by 44% and 60%, respectively (Fig. 2b).

Uniformity of germination was not significantly affected by the temperature of extraction ($P = 0.801$, Table 1) but was markedly reduced (CV_t increased) with the increase in extract concentration, reaching a minimum at 10 g L^{-1}, with a relatively small increase at higher concentrations (Fig. 2c). Germination synchrony was significantly higher (lower $\overline{E}$) in BLE than in the RTE-treated seeds. In both extract treatments, germination synchrony increased with an increase in extract concentration, with maximum synchronization achieved at 20 g L^{-1} and remained steady at higher concentrations. However, the decrease in $\overline{E}$ was progressive as extract concentration approached 20 g L^{-1} in the BLE, it was evident only after 10 g L^{-1} in the RTE (Fig. 2d). The germination lag exhibited sharp reductions of 70% and 85% in the RTE and BLE, respectively with the increase in extract concentration from 0 to 20 g L^{-1}, post which the lag period approached steady low values (Fig. 3a). The beneficial effect of *D. dichotoma* extract on rice germination extended further to embryo growth, where it was more evident on radicle growth than on plumule growth. Increasing extract concentration from 0 to 20 g L^{-1} increased the lengths of radicles and plumules by 44% and 38%, respectively, in the RTE and by 81% and 66%, respectively, in the BLE (Fig. 3b, c).

Algal Extracts Alleviate Salt Stress on Rice During Germinating

The time course of germination in the different salt stress treatments exhibited the typical sigmoid curve, with low germination and a long germination lag under salt stress, compared with the control. There was significantly higher germination and a shorter lag in response to an algal amendment, particularly with the aqueous algal extract (Fig. 4). The effects of amendment regime and salinity and their interaction on germination parameters of rice were generally highly significant ($P < 0.01$), with the exception of CV_t which exhibited highly significant effects of the main factors but with non-significant interaction (Table 2).

The beneficial effect of amendments on germination was highest for the aqueous algal extract, followed by the ethanolic extract and least for the nutrient solution. The average improvement in germination attributable to the aqueous algal extract, ethanolic extract, and nutrient solution amounted to 55%, 45% and 24%, respectively, relative to non-amended seeds (Fig. 5a). Likewise, the increase in Timson index attributable to the aqueous algal extract, ethanolic extract, and nutrient solution approached 89%,

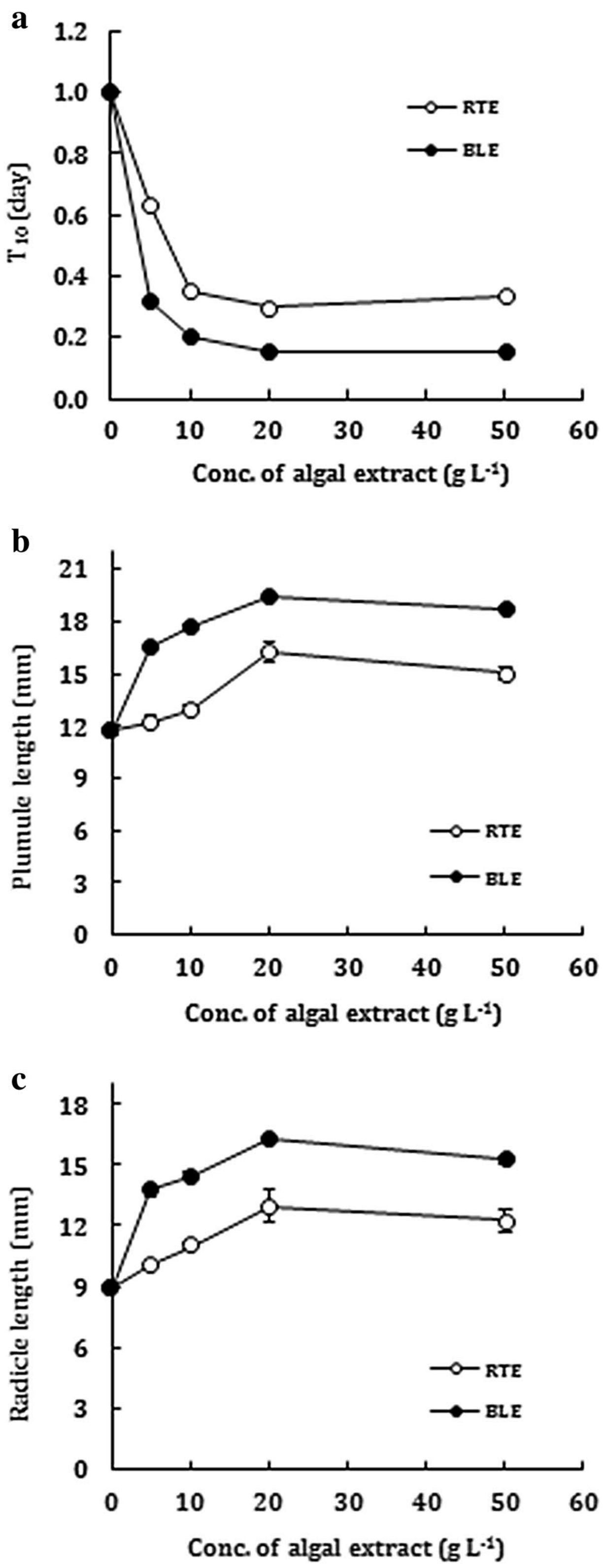

Fig. 3 Time to 10% germination (T_{10}, **a**), lengths of plumule (**b**) and radicle (**c**) of *O. sativa* L. seeds treated with extracts of the brown alga *D. dichotoma* prepared either at room temperature (RTE, **a**) or by boiling (BLE, **b**). Embryo lengths are means of four replicates ± SE

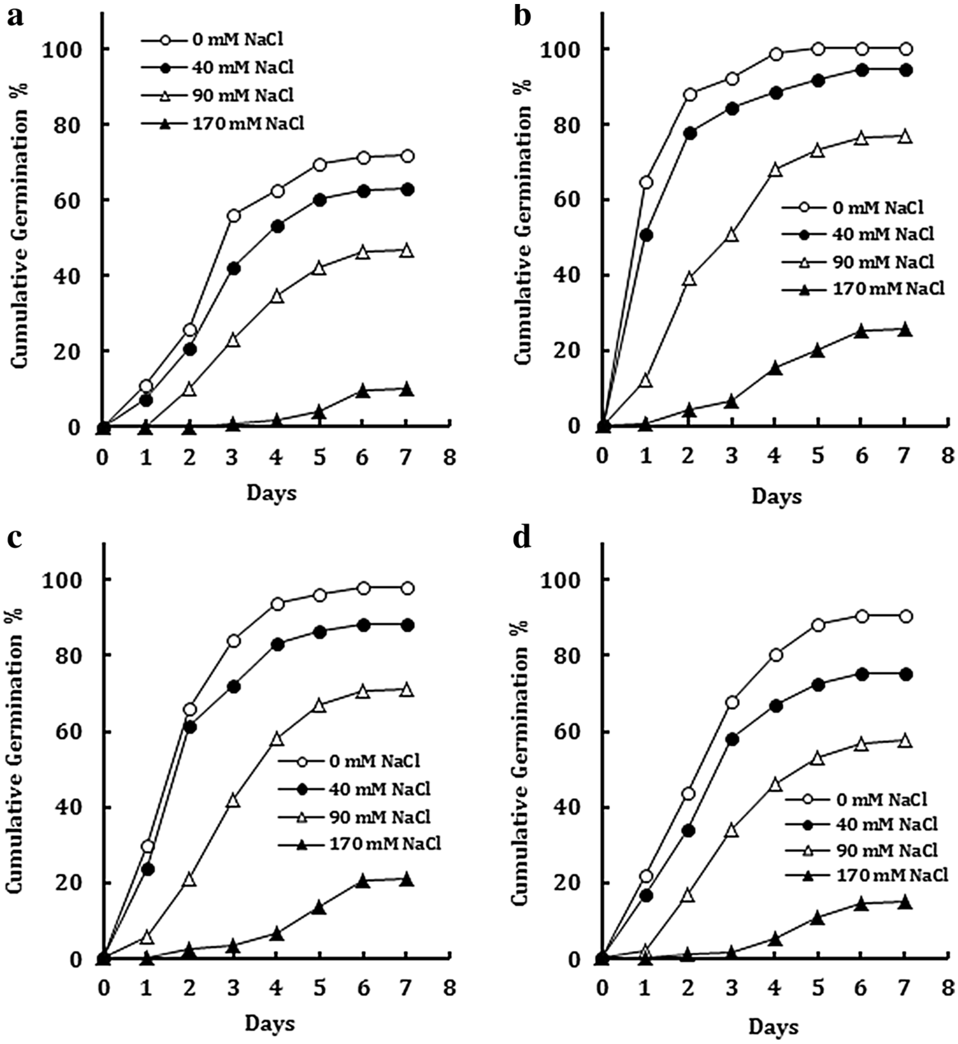

Fig. 4 Time course of germination of *O. sativa* L. seeds either non-amended (NA, **a**) or amended with the aqueous extract (AE, **b**), ethanolic extract (EE, **c**) of *D. dichotoma* or nutrient solution (NS, **d**) in response to salt stress. Values are means of four replicates ± SE

62% and 32%, respectively (Fig. 5b). By contrast, salinity substantially reduced germination parameters, particularly speed of germination, with more evident and progressive reduction in non-amended and nutrient-amended seeds compared with a relatively moderate reduction in algal-amended seeds. Increasing salinity from 0 to 170 mM NaCl reduced germination by an average of 85% in both non-amended and nutrient-amended seeds and by an average of 77% in algal-amended seeds. Across the same range of salinity, the reduction in Timson index amounted to 92% in both non-amended and nutrient-amended seeds and 87% in algal-amended seeds.

Uniformity of germination was least (the highest CV_t) in the aqueous algal extract, followed by both ethanolic extract and nutrient solution, but it was highest (the lowest CV_t) in non-amended seeds. Increasing salinity beyond a threshold of 40 mM NaCl and up to 170 mM NaCl doubled germination uniformity (halved CV_t), irrespective of amendment regime. However, within the salinity threshold, CV_t showed a 13% increase only in the aqueous algal extract, but the changes were non-significant in the other three amendment regimes (Fig. 5c).

The response of germination synchrony to treatments had two distinct phases around a critical concentration of 90 mM NaCl (Fig. 5d). Within the moderate salinity range (< 90 mM NaCl), germination synchrony was higher (lower $\overline{E}$ under the favorable conditions of algal extracts than under the less favorable conditions of no amendment and nutrient amendment. Associated with this trend was a progressive lowering of germination synchrony (an increase in $\overline{E}$ of 65% and 24% in the aqueous and ethanolic algal extracts, respectively) as the salt level increased to 90 mM NaCl, compared with no effect on the less synchronized seeds of no amendment and nutrient amendment treatments. The reverse was evident within the high salinity range (beyond 90 mM NaCl), where germination synchrony of no amendment and nutrient amendment treatments experienced a progressive increase (40% decrease in $\overline{E}$ as salt level exceeded 90 mM

Table 2 Two-way ANOVA showing the effect of amendment and salinity and their interaction on germination of *O. sativa* L. seeds

Source of variation	df	*F*	*P*	*F*	*P*
		Germinability (deg.)		Synchronization index ($\overline{E}$)	
Amendment	3	215.5	0.000	3.868	0.015
Salinity	3	1170	0.000	30.73	0.000
Amend. × salinity	9	8.856	0.000	14.99	0.000
		Timson index		Radicle length	
Amendment	3	1128	0.000	1657	0.000
Salinity	3	6007	0.000	1399	0.000
Amend. × salinity	9	66.11	0.000	50.27	0.000
		Coefficient of variation in germination time (CV_t)		Plumule length	
Amendment	3	49.71	0.000	2712	0.000
Salinity	3	159.6	0.000	2174	0.000
Amend. × salinity	9	1.599	0.143	72.01	0.000

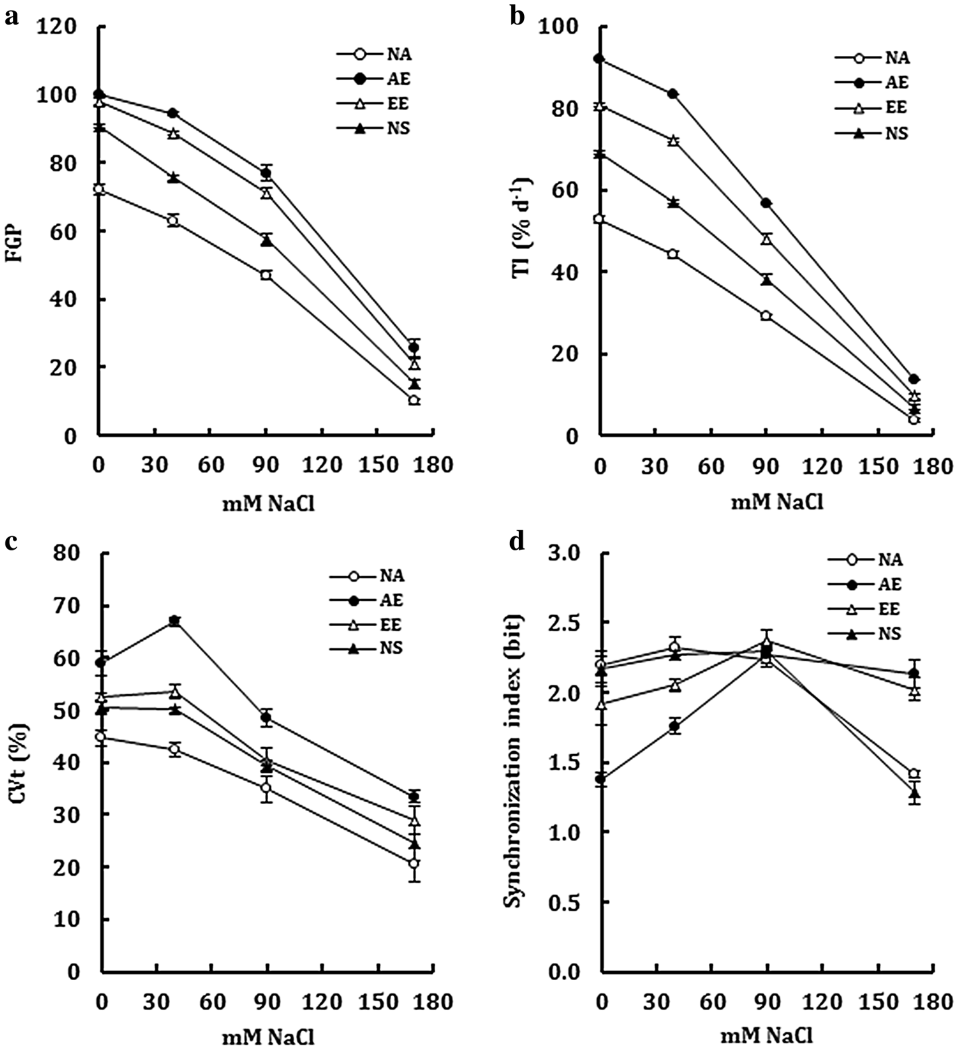

Fig. 5 Final germination percentage (**a**), Timson index (**b**), coefficient of variation in germination time (CV_t, **c**) and synchronization index ($\overline{E}$, **d**) of *O. sativa* L. seeds in response to salt stress and amendment either with the aqueous extract (AE), ethanolic extract (EE) of *D. dichotoma* or nutrient solution (NS). Each value is the mean of four replicates ± SE

NaCl up to 170 mM NaCl) versus a non-significant effect in the two algal extracts. Consequently, germination synchrony, opposite to its behavior at low salinity, was higher (lower $\overline{E}$) in the two algal extracts than in the no amendment and nutrient amendment treatments at the topmost salinity level (170 mM NaCl). The contrasting behavior of germination synchrony toward amendment and salinity led to clustering of germination synchrony of the different treatment combinations around a comparable minimum (maximum $\overline{E}$) at 90 mM NaCl irrespective of the diverse values at both sides of this point.

The different amendment regimes led to shortening of the lag period of germination (T_{10}), with the greatest effect being of the aqueous algal extract and the least effect being of nutrients. By contrast, increasing salinity beyond a certain level (40 mM NaCl in both no amendment and nutrient amendment, and 90 mM NaCl in the two algal extracts) up to 170 mM NaCl substantially increased T_{10} (Fig. 6a).

Embryo growth followed a pattern similar to germination in response to algal amendment and salinity stress, with comparable magnitude for radicle and plumule growth. Amendment of germinating rice seeds with algal extracts improved embryo growth under salt stress to a greater extent than that of a nutrient amendment. Increasing salinity from 0 to 170 mM NaCl reduced lengths of radicles and plumules by an average of 63% in the two algal extracts, by 78% in nutrient-amended seeds and further by 83% in non-amended seeds (Fig. 6b, c).

Recovery from Salt Stress

Recovery from salt stress was monitored at 90 and 170 mM NaCl. Upon withdrawal of salt stress, seeds promptly recovered with preservation of the beneficial effect of the previous amendment treatment. However, the final recovery percentage and the speed of recovery were significantly lower than those of the control (non-treated) seeds (Figs. 1 and 7). The final recovery percentage and the speed of recovery from salt stress were highest from the aqueous algal extract, followed by the ethanolic extract and the nutrient solution but were least in non-amended seeds. Likewise, recovery was higher from the high (170 mM NaCl) salt pre-treatment than from the moderate salinity (90 mM NaCl), particularly in non-amended seeds (Table 3). Uniformity of recovery was not significantly affected by pre-treatments but was markedly lower (with about twice the CV_t) than that of the control seeds. Synchrony of recovery was not significantly affected by the amendment pre-treatment but was significantly reduced ($\overline{E}$ increased by an average of 56% in the two algal amendments) as salinity pre-treatment increased from 90 to 170 mM NaCl, with a non-significant effect recorded in non-amended and nutrient-amended seeds. Synchrony of recovery was generally greater (with 17% lower $\overline{E}$) than that of the

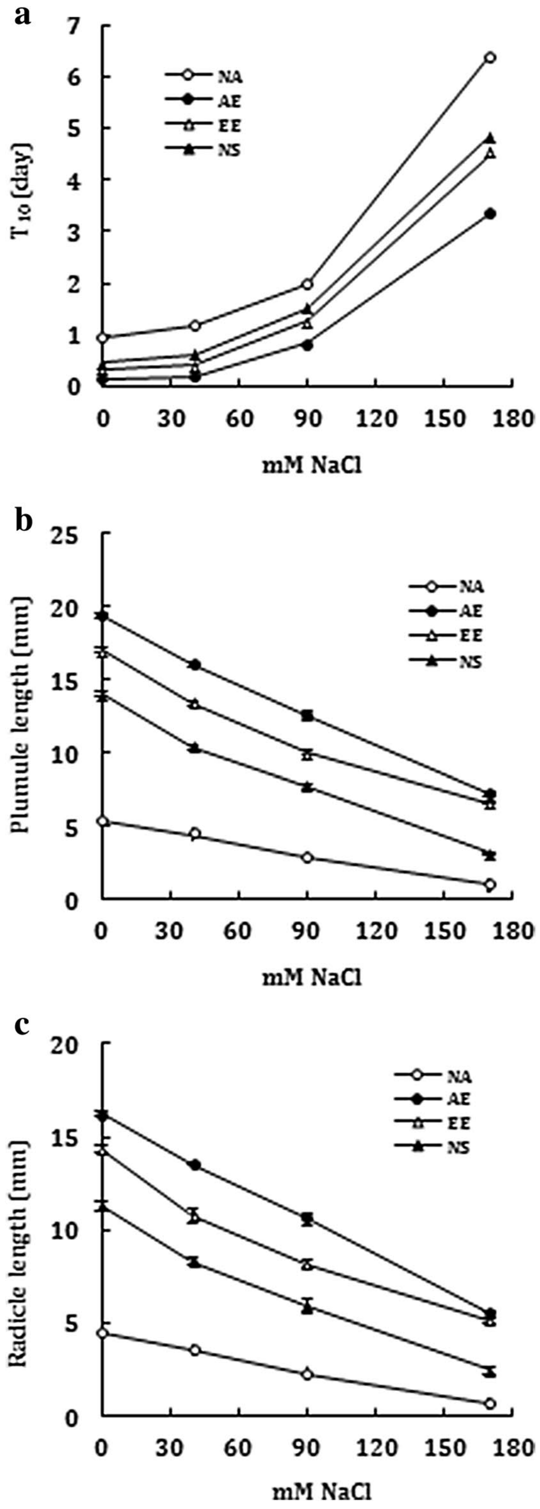

Fig. 6 Time to 10% germination (T_{10}, **a**), lengths of plumule (**b**) and radicle (**c**) of *O. sativa* L. embryo under salt stress and different amendments: the aqueous extract (AE), ethanolic extract (EE) of *D. dichotoma* or nutrient solution (NS). Embryo lengths are means of four replicates ± SE

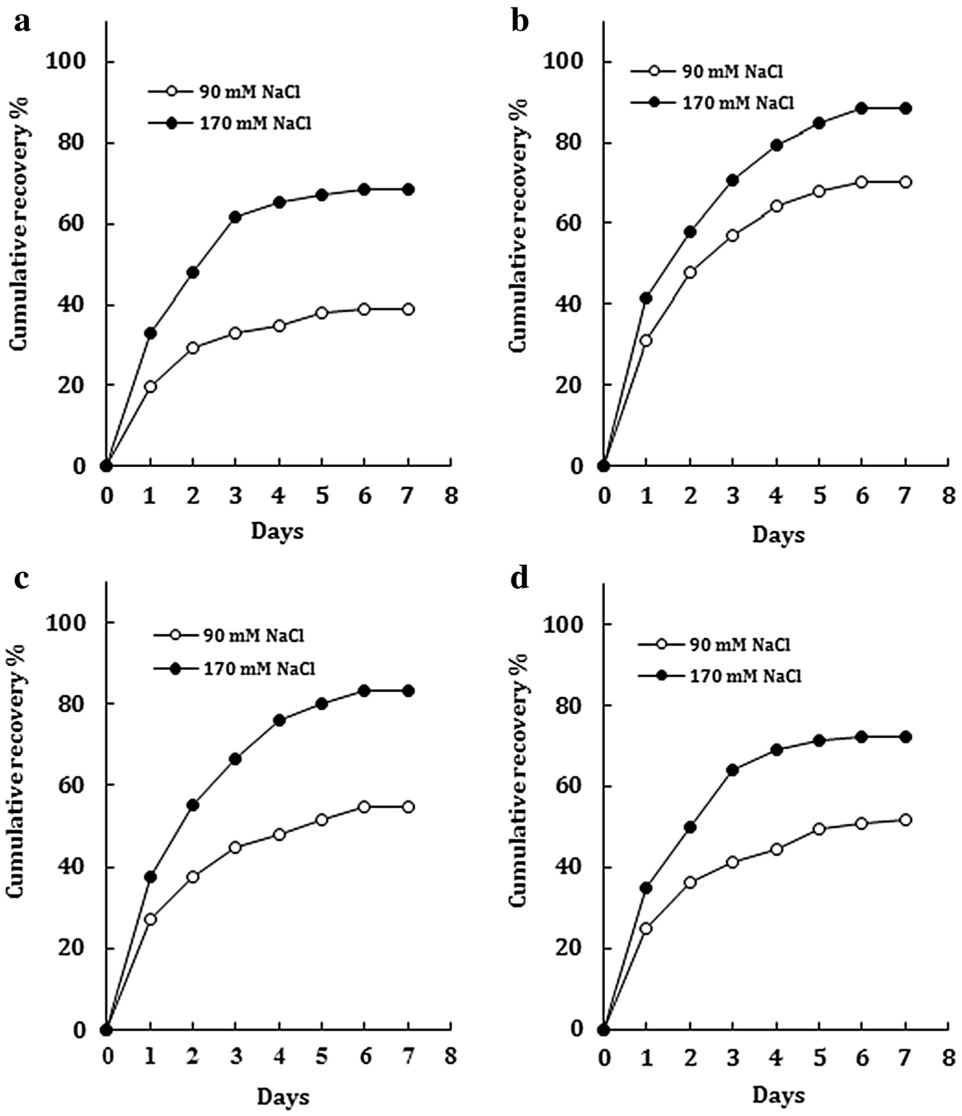

Fig. 7 Time course of germination recovery of *O. sativa* L. seeds from salt stress. Salt-treated seeds were either non-pre-amended (NA, **a**) or pre-amended with the aqueous extract (AE, **b**), ethanolic extract (EE, **c**) of *D. dichotoma* or nutrient solution (NS, **d**). Values are means of four replicates ± SE

control seeds (Fig. 3 and Table 3). The lengths of radicles and plumules of the recovered embryos were lowest from non-amended seeds; but they increased by an average of 300%, 240% and 150% under pre-amendment with aqueous algal extract, ethanolic algal extract, and nutrients, respectively. Increasing the level of salinity pre-treatment from 90 to 170 mM NaCl reduced radicle length of the recovered embryos by 59%, 43% and 31% under no amendment, nutrient pre-amendment, and algal pre-amendment, respectively, but with an average reduction of 40% in plumule length, irrespective of the amendment pre-treatment (Table 3).

Discussion

The relatively low germination of the control seeds of *O. sativa* cv. Sakha 101 is unusual for a well-domesticated crop, since most crop species exhibit prompt germination and approach 100% germination under favorable conditions (Desheva 2016). Seed dormancy is more common, to varying extents and under different causes, in wild plants, as a strategy to secure long-term survival under unfavorable conditions in the wild. Wild plants follow a strategy of cautious germination of the seed bank to avoid eradication resulting from reckless germination under illusive favorable conditions (Finch-Savage and Leubner-Metzger 2006; Shaykhi et al. 2015). A considerable proportion of the seed population of *O. sativa* cv. Sakha 101, thus, seems to retain the wild trait of enforced or conditional dormancy (Nerson 2007) rather than being non-viable, because algal extracts

Table 3 Germination recovery from salinity of *O. sativa* L. seeds amended with the aqueous extract, ethanolic extract of *D. dichotoma* Hudson or nutrient solution

Amendment	No amendment		Aqueous algal extract		Ethanolic algal extract		Nutrient solution	
mM NaCl	90	170	90	170	90	170	90	170
Percent recovery	39 ± 2.8^{a}	68 ± 2.2^{c}	70 ± 1.8^{c}	89 ± 0.8^{d}	55 ± 4.4^{b}	83 ± 4.0^{d}	52 ± 2.4^{b}	72 ± 1.4^{c}
Timson index (% day^{-1})	33 ± 2.3^{a}	59 ± 2.0^{bcd}	58 ± 2.4^{bcd}	56 ± 17^{bcd}	45 ± 3.7^{abc}	69 ± 3.5^{d}	43 ± 1.5^{ab}	62 ± 1.5^{cd}
T_{10} (day)	0.52	0.30	0.34	0.25	0.39	0.26	0.40	0.29
CV_t (%)	68 ± 1.7^{a}	61 ± 2.8^{a}	66 ± 5.8^{a}	66 ± 1.3^{a}	69 ± 6.0^{a}	64 ± 1.4^{a}	71 ± 2.8^{a}	61 ± 1.5^{a}
$\overline{E}$ (bit)	1.6 ± 0.1^{b}	1.8 ± 0.0^{bcd}	1.3 ± 0.1^{a}	1.9 ± 0.1^{cd}	1.2 ± 0.1^{a}	2.0 ± 0.1^{d}	1.8 ± 0.1^{bcd}	1.7 ± 0.1^{bc}
Plumule length (mm)	4.5 ± 0.2^{g}	2.8 ± 0.1^{h}	17 ± 0.1^{a}	9.9 ± 0.1^{d}	14 ± 0.3^{b}	8.8 ± 0.1^{e}	12 ± 0.3^{c}	6.7 ± 0.4^{f}
Radicle length (mm)	3.3 ± 0.1^{e}	1.4 ± 0.1^{f}	12 ± 0.5^{a}	7.9 ± 0.4^{c}	9.9 ± 0.4^{b}	7.0 ± 0.4^{c}	7.6 ± 0.2^{c}	4.4 ± 0.1^{d}

Each value is the mean of four replicates ± SE

Means with the same letter are non-significantly different at $P \leq 0.05$

T_{10} was calculated using the time course of germination curves; therefore, they are not followed by SE

had the ability to restore full germination capacity of seeds with enhanced speed, brief lag, and better embryo extension. The beneficial effects of the *D. dichotoma* extract can be related to its high content of plant macronutrients and micronutrients in addition to the wide variety of bioactive compounds which might play a marked role in breaking the enforced dormancy of rice seeds. The chemical composition of *D. dichotoma* collected from the same site was quantified as: macronutrients (% DW): N 3.1, P 0.17, K 3.8, Ca 2.5 and Mg 0.06; and micronutrients (μg g^{-1} DW): Fe 23, Mn 85, Cu 22, Ni 24, Zn 6 and Co 7; in addition to the growth hormones (μg g^{-1} DW): cytokinins 260, auxins 45, gibberellins 12 and abscisic acid 24, and other bioactive compounds (mg g^{-1} 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). Microalgae (Ronga et al. 2019) as well as extracts of brown algae (Jithesh et al. 2019) can be used as plant biostimulants to improve growth and to afford tolerance against abiotic stress. Priming of rice seeds with the alga *Turbinaria ornata*-based biogenic ZnO nanoparticles at 10 mg L^{-1} enhanced seed germination and seedling growth (Itroutwar et al. 2019). Treatment of salt-stressed *Arabidopsis thaliana* with extracts of the brown alga *Ascophyllum nodosum* improved plant growth via modulation of the expression of a number of genes involved in stress responses, carbohydrate metabolism and phenylpropanoid metabolism (Jithesh et al. 2019).

The beneficial influence of the algal extract in enhancement of rice germination was associated with less uniform but more synchronized germination. These effects were independent of the temperature of extraction. The information about the effect of beneficial treatments, in general, and algal amendment in particular on uniformity, synchrony and lag of germination is scarce. However, amendment of the psammophytic grass *Elymus farctus* seeds with a balanced nutrient supply had no appreciable effect on uniformity, synchrony, and lag of germination (El-Katony et al. 2015).

The dose–response relationship between the concentration of algal extract and rice germination approached saturation beyond a relatively low optimal concentration of 20 g L^{-1} without toxic effect at higher concentrations. Extracts of the brown algae *Sargassum vulgare*, *Colpomenia sinuosa* and *Padina pavonica* led to maximum germination of fenugreek (*Trigonella foenum-graecum*) seeds at a concentration of 5% (w/v) but higher concentrations decreased the germination rate below the control (El-Sheekh et al. 2016). Priming of rice seeds with the alga *Turbinaria ornata*-based biogenic ZnO nanoparticles at 10 mg L^{-1} enhanced seed germination and seedling growth (Itroutwar et al. 2019) and extracts of *Kappaphycus alvarezii* and *Gracilaria edulis* at 5% improved the germination and seedling vigor of maize (Layek et al. 2016). The enhanced germination and embryo growth of rice seeds treated with polysaccharides from the alga *Grateloupia filicina* under salt stress was attributed to increase in proline and antioxidant enzyme activities in addition to restriction of Na^+ uptake via upregulation of the Na^+/H^+ antiporter gene (Liu et al. 2019). For *Arabidopsis thaliana*, a beneficial effect of the brown alga *Ascophyllum nodosum* extracts has been reported under salt stress (Jithesh et al. 2019) versus a toxic effect of *Ulva intestinalis* extracts (Ghaderiardakani et al. 2019). Such a toxic effect was attributed to the high levels of Al^{3+} in *Ulva intestinalis* extracts. Generally, uptake of Al^{3+} by algae to toxic levels is not likely in light of the very low levels of Al^{3+} in the sea water and the well-established beneficial role of Al^{3+} in higher plants (Watanabe et al. 2006). The consistent advantage of the BLE over the RTE of *D. dichotoma* might be attributable to extraction of greater concentrations of thermostable bioactive compounds at higher temperatures, which seem to benefit radicle growth more than plumule growth.

The ranking of efficiency of the different amendments in relief of the toxic effect of salinity on rice germination in the order: aqueous algal extract > ethanolic extract > nutrients suggests that the protective effect of the algal amendment against the hazards of salinity on rice germination involves more than a nutritional component; and that the superiority of the aqueous algal extract might be attributable to the presence of high concentrations of polar bioactive components. However, the significant improvement in germination with nutrient amendment over non-amended seeds suggests also that the nutritional requirements of the germinating seeds are considerable but, at the same time, limited. The beneficial effect of pre-treatment with *Sargassum latifolium* and *Ulva lactuca* extracts in alleviating the damage of drought stress on *Triticum aestivum* during the vegetative stage was attributed not only to the provision of micronutrients essential for growth but also to activation of the antioxidative system and supplementation with growth-promoting hormones (Hashem et al. 2019). Application of the brown alga *Ecklonia maxima* extract at a concentration of 1% as a spray improved the growth and yield of soybean without any negative effect on the nutritive value of seeds (Kocira et al. 2019).

The inhibitory effect of salt stress on magnitude and speed of rice germination was associated with a considerable lag of germination, which coincides with the postulation of Hilhorst and Toorop (1997) about restoration of the wild trait of dormancy by crop species under stress conditions. The sharp reduction in germination parameters of rice under the relatively moderate salt level of 170 mM NaCl signifies that rice is salt-sensitive at germination, which is in accordance with the characterization of rice as a natrophobic species (Broadley et al. 2012). The marked salt sensitivity of rice has been documented (Yamamoto et al. 2003) even when compared with barnyard grass, which is one of the commonly occurring weeds of rice.

The high germination efficiency, in terms of the high magnitude and speed of germination, along the brief germination lag under the favorable conditions of algal amendment and low salinity, was associated with reduced uniformity of germination and vice versa, the retarded germination efficiency under the less favorable conditions of high salinity combined with absence of algal amendment was associated with high uniformity of germination. Thus, it seems that the limited germination events available under the impact of abiotic stress were concentrated within a short time period, in contrast to the surplus germination events under favorable conditions which were scattered across prolonged time. The effect of salinity on germination uniformity seems, however, to be species-specific, ranging from a non-significant effect on *Physalis peruviana* (De Souza et al. 2014) at 12 dS m^{-1} (about 120 mM NaCl), to a significant reduction in *Moringa oleifera* at 40 mM NaCl (Elhag and Abdalla 2012).

The present results suggest that moderate salinity reduces germination synchrony of the vigorously germinating and highly synchronized rice seeds amended with algal extracts, with no further reduction at high salinity. This was in contrast to the less vigorously germinating, poorly synchronized seeds of no amendment and nutrient amendment, where germination synchrony was not affected by moderate salinity but considerably increased at high salinity. In this context, increasing NaCl salinity has been reported to reduce germination synchrony of *Senna spectabilis* seeds (Jeller and Perez 2001). Nevertheless, higher germination synchrony in non-amended above nutrient-amended seeds of the psammophytic grass *Elymus farctus*, with an increase in synchrony by salinity only in non-amended seeds has been found (El-Katony et al. 2015). One of the aspects of the beneficial role of algal amendment on rice germination is, thus, the hastening of germination with a shorter lag. On the other hand, the adverse effect of salinity was manifested as a delay in germination onset with retarded embryo growth, particularly in non-amended seeds. The comparable reduction in radicle and plumule growth of rice in response to salinity is in contrast to that reported for *Elymus farctus* where radicle was less salt-affected than plumule (El-Katony et al. 2015) and for rice where germination and radicle growth were less affected by drought than plumule growth (Pirdashti et al. 2003).

During the amendment × salinity experiment, algal amendment exerted a beneficial effect on rice germination in contrast to the adverse effect of salinity; but during recovery from salt stress, both treatments (algal amendment and salinity) exerted a positive effect. The consistently lower speed and final percent recovery, along with the impeded embryo growth of the recovered seeds compared with those of the control seeds, might point to a toxic ion effect on seed viability, which was particularly evident in the low salt and no amendment pre-treatment. Thus, it seems that salt ions can seep into seeds along with water at moderate salinity and hence damage the embryo (Othman et al. 2006); but at the high salinity, the osmotic effect is more likely, with restricted imbibition and low intake of salt ions and hence less damage to the embryo. As a rule, restoration of germination efficiency after the release of salt stress points to an osmotic effect, whereas low recovery means specific ion toxicity (Rasool et al. 2016).

Conclusion

Amendment of rice seeds with an aqueous extract of the brown alga *D. dichotoma* at a concentration of 20 g dry biomass/L increased seed germination from 84 to 100% with enhanced speed and synchrony but reduced uniformity of germination. Algal extracts also relieved the toxic effect of

NaCl salinity on rice germination. Approaching full germination of rice seeds with algal amendment might point to the efficiency of algal extracts to break the enforced dormancy of the used seed lot. The efficiency of aqueous and ethanolic algal extracts exceeded that of nutrient amendment, which can be related to the high content of algal material in bioactive compounds such as growth hormones, vitamins, flavonoids, and fucoidan (Ward et al. 2017). The lower speed and magnitude of recovery of germination from salt stress compared with the control (non-treated seeds) suggested that rice, as a natrophobic and salt-sensitive species, suffered from the toxic ion effect of salt stress on the embryo more than from the osmotic effect. Thus, the beneficial role of *D. dichotoma* extracts in enhancement of rice germination and relief of the impact of salinity on seed germination can be attributed to the algal content of plant growth hormones, vitamins, flavonoids, and the unique algal constituent fucoidan.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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