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Alleviating Salt Stress in Barley by Use of Plant Growth Stimulants and Potassium Sulfate

M. S. EL-Sharkawy[1], T. R. EL-Beshsbeshy[1], S. M. Hassan[2], E. K. Mahmoud[1], N. I. Abdelkader[1], R. M. Al-Shal[1], & A. M. Missaoui[3]

[1] Soil Science and Water Department, Faculty of Agriculture, Tanta University, Tanta, Egypt

[2] Department Crop and Soil Sciences and Center for Applied Isotope Studies, University of Georgia, USA

[3] Department of Crop and Soil Sciences and Institute of Plant Breeding Genetics and Genomics, University of Georgia, USA

Correspondence: A. M. Missaoui, Crop and Soil Sciences and Institute of Plant Breeding Genetics and Genomics, University of Georgia, USA. Tel: 1-706-542-8847. E-mail: cssamm@uga.edu

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Abstract

Salt impedes plant growth and yield. This study was conducted to explore the effect of plant growth stimulants (seaweed extract, humic acid) and potassium sulfate in alleviating salt stress in barley (*Hordeum vulgare* L.). Initially, 10 barley genotypes were germinated in a growth chamber at five salt levels (0, 0.5, 1.0, 1.5, and 2.0%). Increasing salt concentration reduced germination percent, the speed of germination, and seedling weight. One salt-tolerant genotype (Sharqiya Estate) and one salt-sensitive genotype (Red Sea) were selected and planted in greenhouse pots containing 2 kg of sand, then subjected to 10 and 15 dS m^{-1} salt levels using $CaCl_2.2H_2O$: NaCl (2:1) mixed with Hoagland solution. Four treatments consisting of (1) control (Hoagland solution), (2) seaweed extract at 4 Kg ha^{-1}, (3) humic acid at 28 L ha^{-1}, and (4) potassium sulfate at 300 Kg ha^{-1} were applied to each genotype under both salt levels. Seaweed extract resulted in higher shoot dry weight in the salt-sensitive genotype under both salt levels and maintained a low Na^+/K^+ ratio compared with humic acid and potassium sulfate. It also resulted in higher relative yield, relative water content, higher proline, and lower electrolyte leakage in the susceptible genotype at 10 dS m^{-1}, but the result was not different from humic acid and potassium sulfate treatments at 15 dS m^{-1}. Seaweed extract resulted in the highest catalase activity at 15 dS m^{-1} in both genotypes, with higher magnitude in the salt-tolerant genotype. These results suggest that seaweed extract has potential in improving barley growth under salt stress.

Keywords: biological growth stimulants, humic acid, salt stress, seaweed extract, potassium sulfate

1. Introduction

Nearly 0.9 million hectares of the irrigated areas in Egypt are affected by salinity (Doaa et al., 2012). The majority of salt-affected areas are located in the northern-central part of the Nile Delta and on its eastern and western sides. Sixty percent of the cultivated land of the Northern Delta region, 20% of the Southern Delta and Middle Egyptian regions, and 25% of the Upper Egypt region are salt-affected (El-Banna et al., 2004). Worldwide, salinity affects nearly 20% of the irrigated crop area (Munns & Tester, 2008), and the salt-affected area is expected to reach around 50% of the arable land worldwide in the near future (Mahajan & Tuteja, 2005). Salt stress is a major impediment to seed germination and seedling establishment, reducing plant growth and crop production in arid and semi-arid regions of the world. Plants grown on saline soils are exposed to various types of stresses, including water deficit caused by increased osmotic pressure, ion toxicity resulting from excess accumulation of ions, and nutritional imbalance caused by disturbances in the ion transport systems (Ellouzi et al., 2013). Relative to osmotic potential or the type of ions present in soil solution, plants exhibit different responses to salinity (Kiliç et al., 2008).

Barley is one of the most important cereal crops in Egypt. Since early history, it has occupied a very important position in the Egyptian cropping system, as it can grow across a wide range of conditions, including arid, poor, and saline soils (Abd El-Hady, 2007). Barley is also the fourth largest cereal crop in the world, with an annual production in excess of 143.95 million tons in addition to use as forage for livestock (Foreign Agricultural

Service/USDA, 2017). Barley has a wide range of ecological adaptation than any other cereals and is widespread in temperate, subtropical and artic regions, from sea level to the high altitudes of the Andes and Himalayas. Barley can tolerate about 5 g L^{-1} NaCl and can be considered a marginal halophyte (Glenn et al., 1999).

Growers in salt-affected areas have always strived to find cultural solutions to alleviate salt stress and increase crop production. Among the various practices, at least at the research level, are the application of biological stimulants and organic acids. Several studies have shown the beneficial effect of seaweed extracts in stimulating plant growth and stress tolerance (Fike et al., 2001; Rayirath et al., 2009; Godlewska et al., 2016; Martynenko et al., 2016). More than 15 million metric tons of seaweed products are used annually as nutrient supplements and bio-stimulants in agriculture and horticultural crop production (FAO, 2006). When applied to the rhizosphere or as a foliar spray, seaweed extracts exhibited positive effects on root growth (Finnie & Van Staden, 1985). Seaweed extracts have also been shown to increase shoot length and weight (Atzmon & Van Staden, 1994). Improved tolerance to biotic and abiotic stresses were also suggested (Norrie & Keathley, 2005; Eyras et al., 2008), as seaweed extract influenced cell metabolism and synthesis of antioxidant molecules that improve plant growth and stress tolerance (Cardozo et al., 2007). Chemical analysis of seaweed extracts revealed the presence of a wide variety of plant growth regulators, such as auxins and cytokinins (Zhang & Ervin, 2004; Zhang & Ervin, 2008). In addition to enhancing stress tolerance of treated plants by increasing the concentration of bioactive molecules, such as antioxidants (Rayirath et al., 2009; Fan et al., 2011), seaweed extracts increased nutrient availability (Aziz et al., 2011). Reports have also shown that extracts of the brown seaweed (*Ascophyllum nodosum*) induced gibberellic acid (GA_3)-independent amylase activity in barley and enhanced germination and seedling vigor (Rayorath et al., 2008). Foliar application of seaweed extracts on spring barley grown hydroponically across a 6-week period using trace elements with/without micro- and macro-nutrients resulted in 56-63% more growth compared with the control (Steveni et al., 1992). Grain yield per plant was increased by nearly 50% in winter barley grown in pots in a growth chamber and supplied with various seaweed concentrates as a foliar spray and soil drench two weeks after seedling emergence and as a seed dip (1:250) prior to planting (Featonby & Van Staden, 1987). Mercer et al. (2010) found that barley seedlings grown at 15 °C emerged significantly faster following treatment with various seaweed extracts from *Palmaria palmata*, *Delesseria sanguinea*, *Porphyra* sp., *Laminaria* sp., and *Ulva lactuca* compared with untreated seedlings.

Some studies have also reported that humic and fulvic acids were beneficial for crop production and might increase the uptake of mineral nutrients by plants (Maggioni et al., 1987; De Kreij & Başar, 1995; Mackowiak et al., 2001). Humic acid chelates mineral nutrients, such as Na, K, Mg, Zn, Ca, Fe, and Cu, and helps overcome nutrient deficiencies (Khalesro et al., 2015). Humic compounds were shown to have a positive effect on photosynthesis, respiration, root system formation, biomass yield, and seed germination (Garcia et al., 1992; Liu & Cooper, 2002). Other benefits of humic substances include alleviation of abiotic stresses, such as temperature extremes, pH, and salinity, in addition to the enhancement of nutrient uptake and the reduction of intake of toxic metals from soil (Ayuso et al., 1996; Liu & Cooper, 2002; Masciandaro et al., 2002; Asik et al., 2009).

Potassium (K) is an essential macro-nutrient that affects most biochemical and physiological processes involved in plant growth and metabolism. It also enables plants to withstand various biotic and abiotic stresses, including those caused by diseases, pests, drought, salinity, cold and frost, and water-logging (Wang et al., 2013). The application of K^+ mitigates the adverse effects of salinity through its roles in stomatal regulation, osmotic adjustment and maintenance of the membrane ion-charge balance, cellular-energy status, and protein synthesis (Dawood et al., 2014).

The effect of seaweed extracts in alleviating salt stress in barley has not been investigated. Having been derived from a renewable resource, the bioactive extracts from seaweed could be useful on a large scale to improve barley productivity and sustainability of agricultural systems if proven effective in overcoming salt stress. The primary objective of this investigation was to explore the effect of different growth-enhancing substances, viz., seaweed extract, humic acid, and potassium sulfate, on barley growth under salt stress.

2. Materials and Methods

2.1 Seed Germination Experiment

Seed of 10 barley genotypes (Red Sea C1 3694, Alexandria C1 3734, Kafr El-sheikh 1532, Kafr El-Sheikh 1693, Damietta 1898, Sharqiya Estate, 538, Giza 2000, Beheira 1927, and Matruh Egyptian Dry land barley) originating from Egypt were germinated on August 4th, 2015 in 100-mm Petri plates containing a Whatman no. 2 filter paper imbibed with saline solutions. NaCl was added to deionized water in concentrations of 0, 0.5, 1.0, 1.5 and 2.0% (wt/wt), resulting in salt concentrations of 0, 58.6, 171.1, 256.7, and 342.2 mM. Ten seeds from each barley genotype were placed in a Petri plate and 4.5 mL of an appropriate salt solution was added to submerge

the seeds. The experiment was arranged in a completely randomized design with three replications for each salt concentration. The germination experiment was conducted in a dark growth chamber at 25 °C for 14 days. Seed germination percentage (GP) was calculated as GP = 100 (G/TS), where G = number of germinated seeds and TS = total number of seeds. Speed of germination (SG) was determined using the following formula described by (Maguire, 1962):

$$SG = 1/4(n_1) + 1/6(n_2 - n_1) \quad (1)$$

Where, n_1 and n_2 represent the number of seeds that germinated on day 7 and day 14 after the start of the germination test. Three seedlings were randomly chosen from each plate for dry weight (DW) determination.

2.2 Whole-Plant Response to Salt Stress in the Greenhouse Study

Two barley genotypes were selected on the basis of the results of the germination test described above. One genotype (Sharqiya Estate, PI 57753) was salt tolerant, and the other (Red Sea, C1 3694) was susceptible. Six seeds of each genotype were planted in a greenhouse on August 28, 2015 in each pot containing 2 kg sand and lined with plastic bags (Table 1). Following seed germination, the seedlings within each pot were thinned to keep four plants per pot. The plants were gradually subjected to two levels of salt concentrations starting four weeks after planting. Calcium chloride ($CaCl_2 \cdot 2H_2O$) and sodium chloride (NaCl) were mixed in a 2:1 proportion ($CaCl_2$:NaCl) and added to Hoagland solution to make two saline nutrient solutions of 10 and 15 dS m^{-1} electrical conductivity. Moisture in the pots was kept at field capacity. The treatments consisted of (1) control (Hoagland solution), (2) a commercially available seaweed extract from *Ascophyllum nodosum* (Organic Approach, Lancaster, PA) at 4 Kg ha^{-1}, (3) humic acid (KELP4LESS, Idaho Falls, ID) at 28 L ha^{-1}, and (4) potassium sulfate at 300 kg ha^{-1}. Humic acid and seaweed extract were applied once, one week after the start of salt treatments, whereas potassium sulfate was added in two split-applications. The first application was one week following salt application and the second at the boot stage. All treatments were irrigated with Hoagland solution. Plants were harvested 90 days after planting. Shoot and root fresh weights and respective dry weights were determined using a digital scale with 0.001 g sensitivity. In addition, root length in cm and number of tillers were measured. Relative yield was determined according to (Isla & Aragüés, 2009) by dividing the actual yield in each saline treatment by the highest yield observed. Relative water content (RWC) of shoots was measured according to (Turner, 1981) using the equation:

$$RWC = (FW - DW)/(TW - DW) \quad (2)$$

Where, FW = fresh weight, TW = turgor weight, and DW = dry weight. Dry weight was estimated by drying the samples in a convection oven at 80 °C for 48 h. Turgor weight was determined by floating the shoots on water at room temperature for 48 h.

2.2.1 Salt Stress Response

Free proline content was determined according to the methods of (Bates et al., 1973), where 100 mg of plant material was homogenized in 2 ml of 3% aqueous sulfosalicylic acid. The homogenate was centrifuged at 13,000 × g for 10 min, then 1 ml of supernatant was placed in a reaction test tube and reacted with 1 ml of acid-ninhydrin and 1 ml glacial acetic acid. The test tubes were heated in boiling water in a warm bath for 1 h and the reaction was terminated by placing the test tubes in an ice bath. The reaction mixture was extracted with 2 ml of toluene and mixed vigorously by vortex. The toluene layer separated at room temperature and the absorbance of chromophore containing toluene was measured at 520 nm using a spectrophotometer (Varian Cary 50 UV-Vis spectrophotometer, Agilent Technologies, Santa Clara, CA), using pure toluene as the blank. Standard curves were prepared for each assay using standard proline in 3% sulfosalicylic acid solution. The proline content was expressed as micromoles per gram of fresh weight of plant materials.

Table 1. Physical characteristics and chemical composition of the soil used to study response of two barley genotypes evaluated under three salt levels (0, 10 and 15 dS m^{-1} EC) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate

Sand	100.0%	Mineral concentration (mg L^{-1})								
Silt	0.0%	Ca	Mg	Na	K	Cl	NH_4-N	NO_3-N	P	Fe
Clay	0.0%	35.17	5.94	< 1.79	2.55	8.70	< 6.40	< 0.17	0.97	5.46
Soil Type	Sand									
EC†	0.011 dS·m^{-1}	Zn	B	Mn	Cu	Mo	Cd	Cr	Ni	Pb
pH	6.68	0.33	0.775	0.88	0.29	< 0.04	< 0.04	0.04	< 0.04	0.36
CEC‡	0.21 meq·100g^{-1}									
OM§	0.01%									

Note. † EC, Electrical conductivity in dS m^{-1}; ‡ CEC, Cation exchange capacity in meq·100 g^{-1} soil; § OM, Organic matter.

Electrolyte leakage was determined as described by (Lutts et al., 1996). Fresh leaves (200 mg) from the barley plants were placed in test tubes containing 10 ml of distilled deionized water. The tubes were incubated at 25 °C on a rotary shaker for 24 hours and, subsequently, the electrical conductivity of the solution (Lt) was determined. Samples were then autoclaved at 120 °C for 20 min and the final electrical conductivity (L0) was obtained after equilibration at 25 °C. Measurements of electrical conductivity were made using H1993310 conducti-meter (HANA Instruments, Romania). The electrolyte leakage (EL) was expressed as EL (%) = (Lt/L0) × 100.

2.2.2 Antioxidant Enzyme Analysis

One gram of each leaf sample was homogenized with 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 2% (w/v) polyvinylpyrrolidone (PVP). The entire extraction procedure was carried out at 4 °C. The homogenate was centrifuged at 10,000 g for 15 min at 4 °C and the supernatant was collected and used for assaying enzyme activity.

Catalase (CAT, EC 1.11.1.6) activity was measured according to (Bergmeyer & Gawehn, 1970) as the rate of H_2O_2 disappearance at 240 nm by adding 100 µl leaf crude extract to the solution mixture containing 50 mM sodium phosphate buffer (pH 7.0) and 2% H_2O_2. The activity was expressed as units (µmol H_2O_2 consumed per min) per gram fresh weight.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed spectrophotometrically as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm according to the method of (Beauchamp & Fridovich, 1971). The reaction mixture (3 ml) consisted of 50 mM Na-phosphate buffer (pH 7.8), 13 mM L-methionine, 75 µM NBT, 10 µM EDTA, 2.0 µM riboflavin and 0.3 ml enzyme extract. The test tubes containing reaction mixtures were weighed for 10 min under 4,000 lx at 35 °C. One-unit SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the rate of NBT reduction measured at 560 nm.

2.3 Statistical Analysis

Analysis of variance (ANOVA) was conducted on the data from the seed germination study and the greenhouse pot experiment using PROC GLM of SAS 9.4 (SAS Institute Inc., Cary, NC). Replications were considered random and all other variables were considered fixed effects. Means of all variables were separated using Fisher's protected LSD test.

3. Results and Discussion

3.1 Barley Germination under Salt

Increasing salt concentrations affected all the traits measured after one week and two weeks of germination, as indicated by the significant ($p < 0.01$) main effect of salinity (Table 2). The germination percentage decreased in all genotypes with increasing salt concentration (Table 3). The magnitude of decrease in seed germination was small at the lower salt levels (0.5 and 1%). It varied from 0 to 23.3% at the 0.5% salt level and from 0 to 36.67% at the 1% salt level compared with the no-salt control. Increasing the salt concentration to 1.5% resulted in a decrease in germination, ranging from 13.33 to 76.67% compared to the no-salt control. At 2% salt level, the magnitude of decrease in germination varied from 13.33 to 96.67%. This trend was also observed in other crops exposed to salt stress (Epstein et al., 1980; El-Madidi et al., 2004; Abbas et al., 2012).

Table 2. Mean squares for germination percent, speed of germination, and seedling dry weight, measured 2 weeks after planting of 10 barley genotypes in response to five different salt concentrations (0, 0.5, 1.0, 1.5, and 2.0%)

Source	DF	G%[†]	SG [‡]	DW[§]
Genotypes	9	1712.96**	4.04**	366.93**
Salt conc.	4	10876.67**	26.80**	5374.98**
Salt conc. × Genotypes	36	589.63**	0.71**	186.38**
Error	134	331.21	0.52	99.87
LSD[¶]		11.14	3.21	5.98

Note. ** Significant at 0.01 probability level; [†] G%, seed germination%; [‡] SG, speed of seed germination (% d^{-1}); [§] DW, seedling dry weight (mg); [¶] LSD, Least significant difference at $\alpha = 0.05$.

Table 3. Percent seed germination measured at two weeks after planting of 10 barley genotypes exposed to five different salt concentrations (0, 0.5, 1.0, 1.5, and 2.0%)

Genotypes	Salt concentrations (%)				
	Control	0.5	1	1.5	2
538 (PI 531877)	93.33	70	83.33	80	70
Alexandria C1 3734	100	100	93.33	46.67	16.67
Beheira 1927 (PI 531926)	90	83.33	83.33	73.33	53.33
Damietta 1898 (PI 531923)	100	93.33	100	80	53.33
Giza 2000 (PI 642787)	90	90	73.33	56.67	33.33
Kafr El-sheikh 1532 (PI 531907)	93.33	90	80	66.67	46.67
Kafr El-Sheikh 1693 (PI 531918)	100	90	76.67	80	60
Matruh Egyptian Dry land Barley (PI 542707)	96.67	100	86.67	83.33	63.33
Red Sea C1 3694	96.67	80	60	20	0
Sharqiya Estate (PI 57753)	100	100	93.33	93.33	73.33
LSD[†]	9.7	32.84	32.81	26.51	25.33

Note. [†] LSD, Least significant difference at $\alpha = 0.05$.

The genotype Sharqiya Estate exhibited the highest tolerance to increasing salt concentrations, whereas Red Sea C1 3694 was the most susceptible of the 10 genotypes tested. Germination percentage of Sharqiya Estate decreased from 100% under no-salt to 73.33% at 2% NaCl after 2 weeks, whereas Red Sea C1 3694, grown under 2% NaCl, showed no germination after 2 weeks.

The increase in salt levels had a significant effect ($p < 0.01$) on the speed of seed germination and seedling dry weight (Table 2). Seedlings vigor (dry weight) was not affected by increasing salt levels to 0.5 and 1% (Table 4). Increased salt level of 1.5% reduced seedling weight by 13 to 80% compared with the no-salt control. Under 2% salt level, seedling weight decreased by 32 to 100% across the 10 genotypes. Sharqiya Estate maintained the largest seedling weight under 2% NaCl (37.3 mg), whereas no Red Sea C1 3694 seedlings survived at this salt concentration (Table 4). The speed of germination was also reduced with increasing salt concentrations, with a lesser effect of the concentrations of 0.5 and 1%. The magnitude of the decrease in germination speed varied from 0 to 0.78% d^{-1} at 0.5% salt level with the genotype Red sea displaying the largest decrease (Table 4). It varied from 0.25 to 2.44% d^{-1} at the 1% salt concentration with the genotype Red Sea exhibiting the highest decrease and the genotype 538 the lowest (Table 4). The reduction in germination speed at the 1.5% salt level ranged from 0.28 to 3.69% d^{-1} and from 1.36 to 4.02% d^{-1} (Table 4). The genotype Sharqiya Estate was the most tolerant genotype and showed the least reduction in the speed of germination (1.36% d^{-1} under 2% NaCl compared with the no-salt control. Red Sea C1 3694 was the least tolerant of salt stress, and expressed the highest reduction in the speed of germination (4.02% d^{-1}) under 2% NaCl (Table 4). The tolerance level of the genotype Sharqiya at 2% NaCl (342.2 mM) was higher than levels reported in wheat and other cereals (Munns et al., 2006) and *Pisum sativum* L. (Shahid et al., 2012). Salt tolerant barley seeds may have the ability to accumulate sodium, which would result in an increase in osmotic potential and therefore absorb more water and germinate faster under low water potential conditions (Zhang et al., 2010).

There was a significant interaction ($p < 0.01$) between genotypes and salt concentrations for germination percent, speed of germination and seedling dry weight (Table 2). When there is a significant interaction between two variables (genotypes and salinity levels), then you must tell us which level of salinity is best for which genotype. Red Sea C1 3694, Alexandria C1 3734, and Giza 2000 were the highest three genotypes in the magnitude of reduction of germination at the 1% salt level, with 77%, 53%, and 33%, respectively. They were also the three highest genotypes in the magnitude of reduction in germination at the 2% salt level, with 96%, 83%, and 57%, respectively. Red Sea C1 3694, Alexandria C1 3734, and Giza 2000 ranked in the same order (1st, 2nd, and 3rd, respectively) in the magnitude of reduction in speed of germination at the 1%, 1.5%, and 2% salt levels compared with no-salt control. Giza 2000 and 538 ranked second and third highest following Red Sea in their magnitude of decrease in seedling dry weight at 1.5% salt level while at the 2% salt level, they ranked among the lowest decreases (7th and 9th). The change in ranking for the seedlings dry weight in some genotypes might be associated with a differential response of the genotypes to the absence of light as the germination was conducted in a dark growth chamber (Tables 3 and 4).

Table 4. Average speed of germination and seedlings dry weight measured at two weeks after planting of 10 barley genotypes under five different salt concentrations (0, 0.5, 1.0, 1.5, and 2.0%)

Genotype	Seedling dry weight (mg)					Speed of germination (% d^{-1})				
	Control	0.5	1	1.5	2	Control	0.5	1	1.5	2
538	50.3	54.7	55.0	36.3	34.0	3.8	3.41	3.55	3.24	2.16
Alexandria C1 3734	43.7	49.7	44.3	35.3	19.0	4.16	4.16	2.88	1.69	0.61
Beheira 1927	50.0	54.3	50.0	40.7	26.7	3.66	3.55	3.30	2.88	1.61
Damietta 1898	56.0	64.3	52.0	40.7	19.7	4.08	3.88	3.91	3.24	1.89
Giza 2000	56.7	60.0	54.3	40.7	34.3	3.74	3.74	2.88	2.36	1.30
Kafr El-sheikh 1532	44.3	60.0	47.3	48.7	19.0	3.80	3.74	3.25	2.61	1.61
Kafr El-Sheikh 1693	52.0	47.0	58.3	45.0	29.3	4.16	3.83	3.19	3.33	2.41
Matruh Egyptian Dry land Barley	54.0	67.3	67.0	48.7	34.7	4.02	4.16	3.61	3.38	1.72
Red Sea C1 3694	57.7	70.3	53.3	11.7	0.0	4.02	3.24	1.58	0.33	0
Sharqiya Estate	57.7	59.3	50.0	48.3	37.3	4.16	4.16	3.38	3.88	2.80
LSD†	11.27	12.11	13.86	15.21	16.19	0.43	0.61	1.1	0.66	0.89

Note. † LSD, Least significant difference at $\alpha = 0.05$.

Seedling dry weight was significantly correlated with the germination percent ($r = 0.56$, $p < 0.01$) and with the speed of germination ($r = 0.61$, $p < 0.01$). The speed of germination was also highly correlated with the germination percent ($r = 0.91$, $p < 0.01$) (Table 5). Seedling vigor, following seed germination, is most likely attributable to relative coleoptile elongation and ability to mobilize energy reserves in the seed. Under increasing salt levels, the seeds with higher ability to germinate and mobilize reserves will eventually result in higher seedling vigor compared with seeds whose germination process is compromised by salt stress.

Table 5. Correlation between germination percent (G%), seedling dry weight (DW) and speed of germination (SG) measured at two weeks after planting of 10 barley genotypes under five different salt concentrations (0, 0.5, 1.0, 1.5, and 2.0%)

	G%†	DW (mg)‡
G%†	1.00	0.56**
DW (mg)	0.56**	1.00
SG (%d^{-1})	0.91**	0.61**

Note. **Significant at 0.01 probability level; † G%, seed germination%; ‡ SG, speed of seed germination (% d^{-1}); § DW, seedling dry weight (mg).

3.2 Greenhouse Experiment

3.2.1 Soil Properties

An overall change in mineral composition in the soil was observed relative to the no-salt control (Table 6) The electrical conductivity (EC) increased 3 to 4 folds with increasing salinity levels under the 10 dS m^{-1} salt level and by 6 to 7 folds under the 15 dS m^{-1} salt level, regardless of growth enhancing treatment applied (Table 6). There were significant differences ($p < 0.01$) in soil pH between genotypes, with the salt-tolerant genotypes resulting in a higher pH (Table 6). The difference in pH does not seem to be related to salt concentration nor to the application of growth-enhancing treatments, as none of these factors showed a significant main effect or interaction (Table 7). There were significant effects ($p < 0.01$) of salt concentration on Na, Ca contents and EC (Table 6). Sodium, Ca, and Zn increased with increasing salinity levels relative to the control, whereas Mg, K, P and Fe decreased. Na and Ca were also higher in the soil where the salt-tolerant genotype was grown, indicating possible higher exclusion of these ions by the salt-tolerant barley genotype. Seaweed extract resulted in higher Ca accumulations in the soil compared with the other growth-stimulant treatments in both salt-sensitive and salt-tolerant genotypes (Table 6), suggesting that seaweed extract might potentially improve the quality of salt-affected soils. The growth enhancing treatments resulted in significant differences ($p < 0.01$) in K and P in the soil at the end of the experiment (Table 7). The seaweed extract treatment resulted in lower residual K and P in the soil across the two genotypes and the three salt-levels, suggesting that seaweed extract enhanced plant uptake of the two nutrients.

Table 6. Soil characteristics after harvesting the plants of two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} EC) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate

Genotypes	Salt level	Treatments	pH	EC†	Na	Ca	Mg	K	P	Fe	Zn	Mn	CEC‡
Red Sea C1 3694	0 dS m^{-1}	Control	7.1 ±0.19	0.2 ±0.04	2.9 ±0.70	20.1 ±7.40	9.2 ±0.79	10.4 ±3.79	24.0 ±3.78	35.9 ±10.26	0.3 ±0.02	0.2 ±0.06	0.2 ±0.01
	10 dS m^{-1}	Seaweed extract	7.0 ±0.14	0.9 ±0.04	10.6 ±0.93	39.4 ±4.30	3.9 ±1.82	7.3 ±4.14	22.4 ±1.11	25.1 ±5.60	0.5 ±0.03	0.2 ±0.01	0.1 ±0.03
		Humic acid	7.1 ±0.25	0.8 ±0.09	8.9 ±0.57	35.9 ±2.82	3.2 ±0.64	8.2 ±2.89	23.1 ±1.98	24.9 ±4.82	0.3 ±0.07	0.2 ±0.03	0.2 ±0.03
		Potassium sulfate	7.2 ±0.33	0.7 ±0.16	11.0 ±3.04	38.8 ±6.01	3.0 ±0.67	14.4 ±4.49	26.0 ±1.15	33.5 ±4.77	0.6 ±0.07	0.2 ±0.06	0.2 ±0.01
	15 dS m^{-1}	Seaweed extract	7.3 ±0.16	1.3 ±0.04	10.9 ±2.47	76.2 ±13.52	3.7 ±0.23	12.2 ±3.83	28.2 ±4.55	26.5 ±3.00	0.1 ±0.01	0.2 ±0.02	0.2 ±0.01
		Humic acid	7.2 ±0.21	1.3 ±0.06	16.5 ±2.00	61.6 ±3.94	4.5 ±2.29	10.0 ±3.21	24.2 ±2.14	28.9 ±9.85	0.1 ±0.01	0.5 ±0.06	0.2 ±0.01
		Potassium sulfate	7.0 ±0.16	1.2 ±0.19	17.5 ±1.76	60.7 ±9.50	3.2 ±0.60	17.9 ±9.66	29.5 ±5.11	37.2 ±13.23	0.2 ±0.03	0.2 ±0.09	0.2 ±0.01
Sharqiya Estate	0 dS m^{-1}	Control	7.8 ±0.41	0.2 ±0.06	1.3 ±1.33	14.0 ±2.63	4.1 ±0.62	12.7 ±5.20	22.5 ±3.25	28.8 ±5.00	0.2 ±0.03	0.2 ±0.07	0.2 ±0.04
	10 dS m^{-1}	Seaweed extract	7.4 ±0.04	0.9 ±0.04	10.7 ±1.94	48.2 ±3.65	3.3 ±0.15	10.2 ±1.00	27.1 ±2.07	36.1 ±11.73	0.02 ±0.002	0.2 ±0.01	0.3 ±0.13
		Humic acid	7.4 ±0.09	0.9 ±0.07	9.9 ±0.38	45.7 ±1.94	3.3 ±0.27	11.9 ±2.38	28.9 ±0.34	48.5 ±14.78	0.1 ±0.04	0.2 ±0.07	0.2 ±0.02
		Potassium sulfate	7.6 ±0.07	0.9 ±0.07	14.9 ±2.73	57.3 ±15.80	4.2 ±1.07	16.5 ±3.23	39.3 ±10.63	39.5 ±10.63	0.1 ±0.03	0.5 ±0.01	0.2 ±0.01
	15 dS m^{-1}	Seaweed extract	7.4 ±0.08	1.2 ±0.11	16.6 ±3.42	68.6 ±13.79	4.3 ±1.90	10.5 ±2.42	30.3 ±8.34	45.0 ±11.25	0.3 ±0.03	0.3 ±0.05	0.1 ±0.03
		Humic Acid	7.5 ±0.08	1.3 ±0.09	26.0 ±1.49	63.1 ±11.02	4.1 ±1.68	12.4 ±2.75	29.5 ±5.77	19.3 ±5.36	0.003 ±0.00	0.3 ±0.07	0.2 ±0.01
		Potassium sulfate	7.5 ±0.20	1.2 ±0.07	16.7 ±1.68	72.3 ±4.70	4.2 ±1.04	24.3 ±3.98	36.2 ±3.82	26.0 ±9.74	0.02 ±0.00	0.3 ±0.01	0.2 ±0.04

Note. Mineral elements concentration in mg kg^{-1}; † EC: Electrical conductivity in dS m^{-1}; ‡ CEC: Cation exchange capacity in meq 100 g^{-1} soil.

Table 7. Mean squares for various soil properties and mineral concentrations (mg kg^{-1}) measured after the evaluation of two barley genotypes grown under three salt levels (0, 10, and 15 dS m^{-1} EC) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate

Source	DF‡	pH	EC§	Na	Ca	Mg	K	P	Fe	Zn	Mn	CEC¶
Genotypes	1	1.63**	0.005	1.02	138.93	10.1	62.4	182.1*	86.66	0.35*	0.01	0.01*
Salt Conc.	2	0.008	1.50**	676.1**	4730.2**	2.42	87.7	31.83	152.97	0.15	0.04	0.001
Treatments†	3	0.012	0.03	1.09	151.81	0.07	254.3**	145.9**	43.37	0.08	0.04	0.001
Genotypes × Salt Conc.	2	0.02	0.01	5.01	248.9	0.07	0.73	24.22	461.45	0.36*	0.04	0.01
Genotypes × Treat	2	0.02	0.01	64.83	161.69	1.32	10.33	33.07	227.29	0.04	0.06	0.001
Salt Conc. × Treat	2	0.07	0.003	48.35	80.99	0.73	15.38	15.91	238.77	0.007	0.06	0.001
Genotypes × Salt Conc. × Treat	2	0.03	0.003	57.73	20.43	0.62	15.12	7.29	315.34	0.11	0.02	0.01*
Error	26	0.03	0.009	19.28	67.54	5.54	26.52	24.07	256.63	0.081	0.03	0.003
LSD#		0.19	0.06	2.79	5.21	2.06	3.27	2.06	2.06	2.06	0.12	0.03

Note. *, ** Significant at 0.05 and 0.01 probability level; † Treatments: growth-enhancing substances (seaweed extract, humic acid, potassium sulfate); ‡ DF: Degrees of freedom; §EC: Electrical conductivity in dS m^{-1}; ¶ CEC: Cation exchange capacity in meq100 g^{-1} soil; # LSD: Least significant difference at $\alpha = 0.05$.

3.2.2 Plant Biomass

Plant-shoot dry weights were reduced under both salt concentrations in both genotypes, with a greater magnitude in the susceptible genotype Red Sea, compared with the control (Table 8). There were significant differences ($p < 0.01$) in shoot dry weight, number of tillers, and relative yield between the two barley genotypes in their response to salt levels and growth-stimulant treatment applications (Table 9). Similar observations were reported in barley plants exposed to salt stress levels of 0.75 and 13 dS m^{-1} with and without potassium application ((Endris & Mohammad, 2007). The lowest shoot dry weight (2.96 g) in Red Sea genotype resulted from humic acid treatment at the 10 dS m^{-1} salt level. At the 15 dS m^{-1}, potassium sulfate recorded the lowest shoot dry weight (2.73 g pot^{-1}). In the susceptible genotype, seaweed extract resulted in the highest improvement in shoot dry weight under both salt concentrations (3.86 and 4.19 g at 10 and 15 dS m^{-1}, respectively). Khan et al. (2009) reported that seaweed extracts stimulated abiotic stress tolerance in plants and enhanced plant performance and that the beneficial effects might be related to cytokinin activity.

In the salt-tolerant genotype Sharqiya Estate, the application of potassium sulfate resulted in the highest shoot dry weight (4.07 g) under 10 dS m^{-1} salt level, followed by seaweed extract (3.96 g) and humic acid (3.14 g). At the 15 dS m^{-1} salt level, the application of potassium sulfate resulted in the largest increase in shoot dry weight (4.29 g), followed by seaweed extract and humic acid with 3.43 and 3.25 g pot^{-1} (Table 7). Relative yield followed the same trend as shoot dry weight. Seaweed extract enhanced plant relative yield in the salt-sensitive Red Sea genotype under both 10 and 15 dS m^{-1}, recording 69.43% and 75.23%, respectively. Humic acid was more effective under 15 dS m^{-1} (62.67 g pot^{-1}) compared with 10 dS m^{-1} (53.25 g pot^{-1}). Potassium sulfate was more effective than humic acid and seaweed extract in Sharqiya Estate genotype under both 10 and 15 dS m^{-1} salt concentrations, with values for relative yields of 75.10% and 79.15%, respectively.

Under no-salt conditions, Red Sea genotype exhibited higher root dry weight and root length than Sharqiya Estate (Table 8). Under both salt levels, the salt-tolerant genotype Sharqiya showed higher root biomass than the salt-susceptible genotype under the seaweed extract and potassium sulfate treatments (Table 8). In the salt-susceptible genotype, Red Sea, the application of humic acid had the largest effect on root dry weight and root length, with 3.2 g and 21.81 cm, respectively, at 15 dS m^{-1}. In the tolerant genotype, humic acid had a lower effect on root weight compared with seaweed extract and potassium sulfate applications at both salt levels (Table 8). The application of potassium sulfate recorded a slightly higher effect on root dry weight under 10 dS m^{-1} than seaweed extract (3.63 and 3.32 mg pot^{-1}), whereas at 15 dS m^{-1}, seaweed extract resulted in a higher root weight (3.89 and 3.41 g pot^{-1}). Seaweed extract was reported to promote root growth and development, most likely as result of enhancing the endogenous auxins as well as by other compounds in the extracts (Metting et al., 1990; Jeannin et al., 1991).

Table 8. Shoot dry weight (g), number of tillers, root dry weight (g), root length (cm), and relative yield (%) of two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate)

Genotypes	Salt level	Treatments	Shoot dry weight (g)	Number of tillers	Root dry weight (g)	Root length (cm)	Relative yield (%)
Red Sea C1 3694	0 dS m^{-1}	Control	5.02 ± 0.56	5.11 ± 1.07	5.24±4.40	22.42 ± 4.32	90.27 ± 10.07
	10 dS m^{-1}	Seaweed extract	3.86 ± 0.54	3.22 ± 0.69	1.17 ± 0.08	16.82 ± 1.57	69.43 ± 9.79
		Humic acid	2.96 ± 0.30	3.33 ± 0.67	1.29 ±0.37	17.60 ± 2.06	53.25 ± 5.32
		Potassium sulfate	3.12 ± 0.48	3.44 ± 0.84	1.55 ± 0.44	19.89 ± 1.50	55.11 ± 4.44
	15 dS m^{-1}	Seaweed extract	4.19 ± 0.10	3.67 ± 0.88	1.76 ± 0.51	16.80 ± 1.71	75.23 ± 1.84
		Humic acid	3.49 ± 0.62	3.56 ± 0.84	3.20 ± 1.75	21.81 ± 3.26	62.67 ± 10.99
		Potassium sulfate	2.73 ± 0.61	2.45 ± 0.19	1.55 ± 0.19	19.10 ± 1.86	49.02 ± 10.91
Sharqiya Estate	0 dS m^{-1}	control	4.84 ± 0.70	5.22 ± 0.51	3.87± 3.55	21.68 ± 6.77	89.21 ± 12.85
	10 dS m^{-1}	Seaweed extract	3.96 ± 0.70	3.67 ± 1.15	3.32 ± 0.53	17.34 ± 0.67	72.97 ± 12.95
		Humic acid	3.14 ± 0.56	3.89 ± 1.02	2.88 ± 1.50	17.73 ± 2.58	57.86 ± 10.37
		Potassium sulfate	4.07 ± 0.34	6.44 ± 1.39	3.63 ± 1.49	15.93 ± 0.70	75.10 ± 6.25
	15 dS m^{-1}	Seaweed extract	3.43 ± 1.22	4.78 ± 2.34	3.89 ± 2.04	18.22 ± 1.70	63.30 ± 22.56
		Humic acid	3.25 ± 0.40	5.00 ± 1.76	2.17 ± 0.62	17.01 ± 2.45	59.90 ± 7.46
		Potassium sulfate	4.29 ± 0.47	7.44 ± 0.69	3.41 ± 1.16	17.91 ± 1.82	79.17 ± 8.66

Table 9. Mean squares for shoot dry weight (g), number of tillers, root dry weight (g), root length (cm), and relative yield (%) of two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate)

Source	Degrees of Freedom	Shoot dry weight	Number of tillers	Root dry weight	Root Length	Relative yield
Genotypes	1	5.10**	20.81**	5.47	13.13	1553.39**
Salt Concentration	2	0.53	2.09	1.16	7.66	162.19
Treatments†	3	0.23	4.50 *	0.1	4.96	70.19
Genotypes × Salt Conc.	2	0.08	3.16	2.06	0.4	25.6
Genotypes × Treatments	3	2.58**	9.72**	3.16	11.77	787.77**
Salt Conc. × Treatments	6	2.03**	0.53	0.48	1.541	617.37**
Genotypes×Salt Conc. × Treatments	6	0.52	0.38	1.57	12.1	158.48
Error	26	0.36	1.27	3.53	8.13	110.37
LSD ‡		0.34	0.71	1.19	1.81	6.66

Note. *, **Significant at 0.05 and 0.01 probability level; †Treatments: Seaweed extract, humic acid, potassium sulfate; ‡ LSD: Least significant difference at $\alpha = 0.05$.

3.2.3 Plant Tissue Chemical Characteristics

Analysis of variance showed a significant difference ($p < 0.05$) between barley genotypes in Na^+/K^+ ratio, Ca, Mg, Fe, and B (Table 10). Salt levels significantly affected Na^+/K^+ ratio and the concentration of Ca, P, Fe, and B in plant tissue. Regardless of the growth-stimulant treatment, the Na^+/K^+ ratio increased with increasing salt levels (Table 10). In the salt-sensitive genotype, Na^+/K^+ ratio was more than twice that of the non-salt treatment (3.28) at both 10 dS m^{-1} (6.79) and 15 dS m^{-1} (7.2). In the salt-tolerant genotype, Na^+/K^+ ratio increased across all the growth-stimulant treatments to 5.0 at 10 dS m^{-1} and to 5.7 at 15 dS m^{-1} compared with 4.21 in the non-salt control (Table 11). The lower Na^+/K^+ ratio in the salt-tolerant genotype was a possible indication of higher Na exclusion in this genotype. Na^+/K^+ ratio is an important consideration in salt tolerance and salt-stress alleviation because reducing Na^+ uptake and transport from roots to shoots and increasing retention of K^+ in the cytosol are considered key factors in conferring salt tolerance in plants (Garthwaite et al., 2005; Zepeda-Jazo et al., 2008). The application of seaweed extract on the susceptible genotype resulted in the lowest Na^+/K^+ ratio under both

salt levels (5.54 at 10 dS m^{-1} and 6.67 at 15 dS m^{-1}) compared with humic acid and potassium sulfate. This may suggest a positive effect of seaweed extract on the the response to salt stress conditions of the salt-sensitive genotype by reducing Na accumulation in plant tissue. In the salt-tolerant genotype, the difference in Na^+/K^+ ratio was relatively small between the three treatments at the 10 dS m^{-1} salt level (5.24 for seaweed extract, 5.11 for humic acid, and 4.82 for potassium sulfate). At the higher salt level, humic acid and potassium sulfate resulted in lower Na^+/K^+ ratios than seaweed extract (Table 11). The high content of Ca at 10 and 15 dS m^{-1}, under all the treatments was possibly attributable to the fact that calcium was one of the components of the saline solution. Magnesium, P, Fe, and B concentrations were reduced in both genotypes with increasing salt levels and Mo was only slightly increased (Table 11). The growth-stimulant treatment effects were significant for Ca ($p < 0.01$), Fe ($p < 0.05$), and Mo ($p < 0.01$). There were significant interactions between genotypes and salt levels for Ca, Fe, and Mo ($p < 0.05$). Under 10 dS m^{-1}, Red Sea genotype had a higher concentration of Ca, Fe and Mo than those under 15 dS m^{-1}. The genotype Sharqiya Estate had a higher concentration of Ca, Fe and Mo under 15 dS m^{-1} than under 10 dS m^{-1}.

There was also significant interaction between genotypes and growth-stimulant treatments for Ca, Mg, Fe, and Mo ($p < 0.05$) (Table10). The application of humic acid to Red Sea genotype resulted in a higher concentration of Mg and Fe than under 10 dS m^{-1}. In the genotype Sharqiya Estate, the application of humic acid resulted in a higher concentration of Mg, Ca and under 15 dS m^{-1} and increased Mo concentration in the plant tissue under both salt levels. The application of potassium sulfate recorded the highest concentration of Mo in Red Sea genotype and Ca and Fe in Sharqiya Estate under 10 dS m^{-1}. The application of Seaweed extract enhanced Mg concentration under both salt levels in Sharqiya Estate. Magnesium is one of the main building blocks of Chlorophyll. Improving Mg concentration may lead to enhanced photosynthesis, as Mg plays a major role in the photosynthesis process.

Table 10. Mean squares for chemical composition of two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate)

Source	DF†	Na/K	Ca	Mg	P	Fe	Zn	Mn	Cu	B	Mo
			---------- mg L^{-1} ----------								
Genotypes	1	8.82*	69416.45*	34917.56*	397.84	16454.35**	4.41	193.61	0.08	6.36**	935.20
Salt Conc.	2	3.14*	186317.41**	6184.51	1652.96*	2574.04**	0.05	2.50	3.49*	3.04*	343.11
Treatments‡	3	1.22	82524.03**	1843.99	297.25	395.04*	0.08	3.68	0.16	0.80	5737.28*
Genotypes × Salt Conc.	2	0.21	80463.95*	611.33	192.65	812.73**	0.28	6.92	1.12	1.40	805.42
Genotypes × Treatments	3	7.28	11902.99	1148.47	9.51	417.36*	0.09	3.65	1.04	0.03	873.38
Salt Conc. × Treatments	6	2.42	994.26	704.23	146.92	96.14	0.13	2.45	0.66	0.44	427.49
Germpl × Salt Conc. × Treatments	6	0.23	3939.75	758.58	8.07	91.59	0.05	0.79	0.74	0.17	1036.28
Error	26	2.46	16288.004	10798.12	410.58	119.41	1.841	78.37	0.81	0.94	1811.62
LSD¶		0.99	80.96	65.92	12.85	6.93	0.86	5.62	0.58	0.62	27.00

Note. *, ** Significant at 0.05 and 0.01 probability level; † DF: Degrees of freedom; ‡ Treatments: seaweed extract, humic acid, and potassium sulfate; ¶ LSD: Least significant difference at $\alpha = 0.05$.

Table 11. Average mineral composition (mg kg^{-1}) of plant tissue of two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate)

Genotypes	Salt level	Treatments	Na/K	Ca	Mg	P	Fe	Zn	Mn	Cu	B	Mo
Red Sea C1 3694	0 dS m^{-1}	Control	3.28	406.7	252.22	125.48	102.03	1.56	4.45	2.1	6.3	181.8
	10 dS m^{-1}	Seaweed extract	5.54	736.7	183.19	123.86	96.11	1.53	6.18	1.1	4.0	181.2
		Humic acid	8.30	659.9	188.44	128.96	98.24	1.91	7.24	1.3	3.3	241.3
		Potassium sulfate	6.54	620.0	165.67	112.87	78.80	1.89	7.47	1.5	3.1	236.1
	15 dS m^{-1}	Seaweed extract	6.67	628.2	148.98	102.79	79.96	1.75	7.35	1.3	2.7	209.3
		Humic acid	7.82	736.7	160.07	104.90	62.25	1.53	7.59	2.0	2.2	209.5
		Potassium sulfate	7.22	713.3	124.88	103.46	51.69	1.73	6.37	1.3	2.6	230.0
Sharqiya Estate	0 dS m^{-1}	control	4.21	355.7	346.33	142.40	58.37	1.26	3.48	1.2	4.0	206.7
	10 dS m^{-1}	Seaweed extract	5.24	491.7	184.96	125.14	40.18	1.49	7.02	1.1	3.2	190.6
		Humic acid	5.11	681. 4	130.52	125.40	38.05	1.79	6.56	1.1	2.3	236.4
		Potassium sulfate	4.82	721.8	129.01	110.10	44.46	1.66	8.09	1.2	2.7	218.1
	15 dS m^{-1}	Seaweed extract	7.15	761.5	149.64	110.96	35.57	1.90	7.76	3.1	2.6	201.6
		Humic acid	5.05	889.6	128.31	114.34	33.77	2.08	8.31	1.61	2.6	261.7
		Potassium sulfate	5.19	869.7	130.55	108.56	31.70	1.71	8.07	1.6	2.5	228.6

Note. Mineral Elements concentrations in mg kg^{-1}.

3.2.4 Physiological Effects

1) Relative Water Content (RWC)

RWC is a ratio of the amount of water in the leaf tissue at sampling to that present when fully turgid. Elevated salt concentrations tend to decrease RWC and increase electrolytes leakage (EL) in plant species exposed to salt stress (Wang et al., 2013). RWC in both genotypes was lower under the salt treatments compared with the no-salt control, regardless of the growth-stimulant compound applied and decreased with increasing salt levels (Table 13). The magnitude of RWC decrease was higher in the salt susceptible genotype. In the salt-sensitive genotype Red Sea, the application of seaweed extract resulted in the highest RWC under 10 dS m^{-1} (15.3%) (Table 12), suggesting that seaweed extract may have improved root growth and enhanced soil water-holding capacity (Khan et al., 2009). At the 15 dS m^{-1}, there was no difference between growth-stimulant treatments (Table 13). In the salt-tolerant genotype Sharqiya Estate, humic acid resulted in the highest RWC under 10 dS m^{-1} and was only 19% lower than that for the non-salt control (62.19 vs. 76.43). It also resulted in the highest RWC under 15 dS m^{-1} (51.75 compared with 50.52 and 43.75 for seaweed extract and potassium sulfate, respectively).

2) Proline

Increasing the salt levels resulted in a significant increase ($p < 0.05$) in proline content in plant tissue under all the growth enhancing treatments compared with no-salt control (Table 12, 13). In the salt-susceptible genotype Red Sea, the application of seaweed extract resulted in the highest accumulation of proline in plant tissue compared with humic acid and potassium sulfate (0.40, 0.27, and 0.13, respectively). At the 15 dS m^{-1} salt level, there was no significant difference among the three growth-stimulant treatments. In the salt-tolerant genotype, Sharqiya Estate, the application of humic acid resulted in the highest accumulation of proline under 10 dS m^{-1} with 0.76 μmol g^{-1} fresh weight (FW), followed by seaweed extract (0.48 μmol g^{-1} FW). At the 15 dS m^{-1} salt level, there was no difference in proline accumulation between growth-stimulant treatments.

Proline plays an important role in reducing the damaging effects of salt and in accelerating the repair processes following stress. Proline concentrations were shown to gradually increase in shoots of barley exposed to increasing salt levels (El-Tayeb, 2005). The higher proline concentrations in the salt-tolerant genotype Sharqiya Estate at both salt levels compared to the salt-sensitive Red Sea, suggested that the tolerant barley genotype is able to adjust better to salt stress. Proline is a metabolite that enhances salt tolerance through osmotic adjustment (Ueda et al., 2007; Shelden et al., 2016). Reports have shown that proline acts as an osmo-protectant and is associated with the mechanism of tolerance under salt stress (Yu, Lei, & Shaozheng, 2000). Besides being an osmolyte, proline confers enzyme protection and increases membrane stability (Hayat et al., 2012).

3) Electrolyte Leakage

Electrolyte leakage might be correlated with various physiological and biochemical parameters conditioning plant responses to environmental stresses that result in decreases in leaf water (Maheswari et al., 1999), stomatal resistance, osmotic potential, and leaf-rolling index (Premachandra et al., 1989). Electrolyte leakage in the salt-sensitive genotype Red Sea was slightly lower than that in the tolerant genotype under both salt concentrations (Tables 12 and 13). Application of seaweed extract reduced electrolyte leakage in both salt-sensitive and salt-tolerant genotypes under 10 dS m^{-1}. At the higher salt level, the application of potassium sulfate resulted in lower electrolyte leakage compared with seaweed extract and humic acid in both genotypes, which could be a result of change in nutritional status of the leaf, as suggested by (Bajji et al., 2002). Electrolyte leakage has been associated with K^+ loss from plant cells, triggered by reactive oxygen species and mediated by cation conductance of the plasma membrane (Demidchik et al., 2014).

Table 12. Relative water content (RWC), proline, and electrical leakage (EL) in two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate

Genotypes	Salt level	Treatments	RWC (%)	Proline (μmol g^{-1} FW)	EL (%)
Red Sea C1 3694	0 dS m^{-1}	Control	82.75 ± 0.99	0.057 ±0.04	66.11±3.25
	10 dS m^{-1}	Seaweed extract	53.51 ± 5.78	0.402 ± 0.28	75.67±1.12
		Humic Acid	48.74 ± 3.06	0.267 ± 0.15	78.12±1.15
		Potassium sulfate	46.61 ± 0.01	0.126 ± 0.07	77.46±1.41
	15 dS m^{-1}	Seaweed extract	45.99 ± 1.81	0.195 ± 0.08	72.39±0.39
		Humic acid	49.19 ± 2.66	0.282 ± 0.07	78.55±2.04
		Potassium sulfate	48.04 ± 3.60	0.241 ± 0.14	73.87±0.75
Sharqiya Estate	0 dS m^{-1}	Control	76.43 ± 1.57	0.038 ± 0.01	75.50±5.69
	10 dS m^{-1}	Seaweed extract	52.99 ± 3.57	0.480 ± 0.48	78.42±1.66
		Humic acid	62.19 ± 1.77	0.756 ± 0.62	80.76±3.03
		Potassium sulfate	49.83 ± 0.02	0.183 ± 0.06	89.90±5.62
	15 dS m^{-1}	Seaweed extract	50.52 ± 4.44	0.254 ± 0.09	83.45±0.76
		Humic acid	51.75 ± 0.99	0.200 ± 0.14	86.11±0.07
		Potassium sulfate	43.75 ± 1.17	0.321 ± 0.30	78.55±0.36

Table 13. Mean squares for relative water content (RWC), electrolyte leakage (EL) and proline in plant tissue two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate

Source	Degrees of Freedom	RWC (%)	Proline (μmol g^{-1} FW)	EL (%)
Genotypes	1	12.65	0.06	539.74**
Salt Concentration	2	50.30*	0.13*	13.36
Treatments†	3	33.63	0.08	36.48*
Genotypes × Salt Conc.	2	44.55	0.08	7.71
Genotypes × Treatments	3	88.76*	0.02	9.02
Salt Conc. × Treatments	6	45.19	0.14*	90.84**
Genotypes × Salt Conc. × Treatments	6	17.5	0.08	54.35**
Error	26	39.24	0.062	6.70
LSD‡		3.974	0.16	1.70

Note. *, ** Significant at 0.05 and 0.01 probability level; † Treatments: seaweed extract, humic acid, and potassium sulfate; ‡ LSD: Least significant difference at α = 0.05.

3.2.5 Antioxidant Enzymes

The response to salt tolerance is usually associated with increased oxidative stress because of increased accumulation of reactive oxygen species (ROS), particularly O^{2-} and H_2O_2 in chloroplasts, mitochondria, and peroxisomes (Abedi & Pakniyat, 2010). As a result, the stimulation of antioxidant enzyme activity is an adaptation strategy that plants use to overcome oxidative stress (Foyer & Noctor, 2003). Our results showed a significant difference ($p < 0.01$) between the barley genotypes for catalase and SOD activities (Table 14). There were also significant differences ($p < 0.01$) in the effect of salt concentrations on both catalase and SOD as well as a significant response ($p < 0.01$) to the different growth-stimulant treatments (Table 14). The salt-sensitive genotype Red Sea showed a much lower catalase activity under all growth-stimulant treatments and both salt levels compared with the salt-tolerant genotype Sharqiya Estate (Figure 1). Application of seaweed extract resulted in the highest catalase activity at the 15 dS m^{-1} in both genotypes, with higher magnitude in the salt-tolerant genotype (Figure 1). The application of humic acid resulted in the highest catalase activity in the salt-tolerant genotype at 10 dS m^{-1} and the lowest activity in the salt-sensitive genotype at the same salt level. Potassium sulfate resulted in a similar increase in catalase activity to seaweed extract in the salt-tolerant genotype (Figure 1).

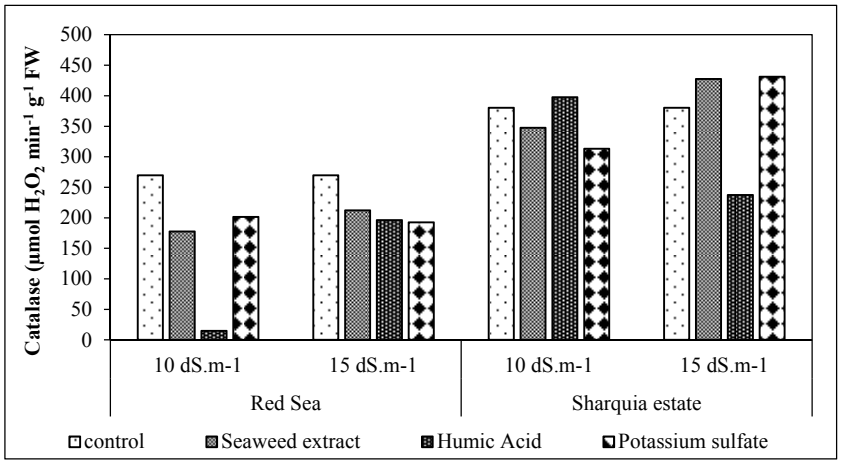

Figure 1. Effect of growth enhancing materials on catalase (µmol H_2O_2 min^{-1} g^{-1} FW) activity in one salt sensitive (Red Sea) and one salt tolerant (Sharqiya Estate) barley genotypes grown under two levels of salt concentrations (10 and 15 dS m^{-1})

The application of seaweed extract has been reported to enhance K^+ uptake under abiotic stress (Khan et al., 2009). Seaweed extract is thought to contain betaines, including gamma-aminobutyric acid betaine, 6-aminovaleric acid betaine, and glycine betaine, which play an important role in enhancing chlorophyll and antioxidant enzymes (Blunden et al., 1986). Potassium plays a key role in plants through the activation of enzymes by stabilizing the pH between 7 and 8 and changing the conformation of enzymes by binding to enzyme surface (Marschner, 2002). Tripathi et al. (2009) reported that proteins, such as thioredoxin, glutaredoxin, and cyclophilin, facilitated the regeneration of the catalytically active form of peroxiredoxins that played an important role in reducing the formation of reactive oxygen species in plants under biotic and abiotic stress.

There was no interaction between the main factors for catalase, but there were significant interactions ($p < 0.01$) of growth-stimulant treatments with genotypes and salt concentrations for SOD (Table 14). The levels of SOD were mostly higher in the salt-sensitive barley genotype under both salt levels (Figure 2) than those in the salt-tolerant genotype. The application of seaweed extract and potassium sulfate resulted in higher increases in SOD in the salt-sensitive genotype compared with humic acid at 10 dS m^{-1} (Figure 2).

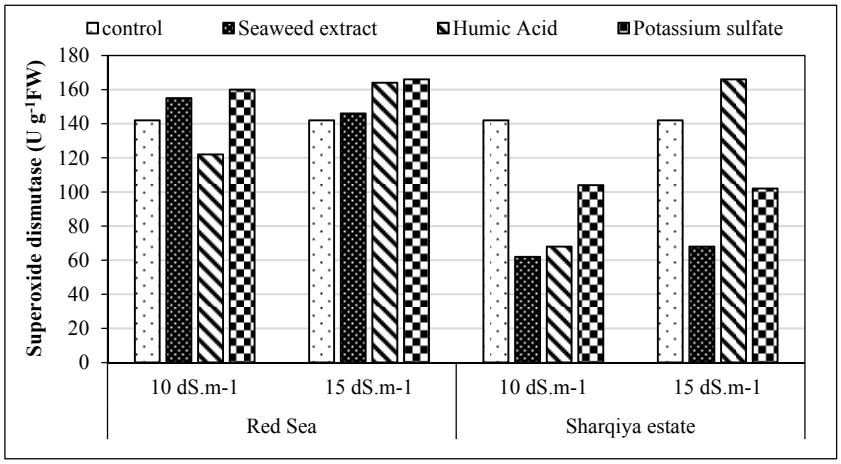

Figure 2. Effect of growth enhancing materials on Superoxide dismutase (U g^{-1}FW) activity of one salt sensitive (Red Sea) and one salt tolerant (Sharqiya Estate) barley genotypes grown under two levels of salt concentrations (10 and 15 dS m^{-1})

At the 15 dS m^{-1}, humic acid and potassium sulfate resulted in higher increases in SOD than seaweed extract. In the salt-tolerant genotype, none of the growth-stimulant treatments resulted in increases in SOD compared with the control. Only humic acid application resulted in a significant increase in SOD at the 15 dS m^{-1} (Figure 2). Considering the difference between genotypes in their SOD content, the growth-stimulant treatments and salt concentrations for SOD suggest that the mechanism of salt stress alleviation is possibly dependent on the genetic background.

Table 14. Mean squares for catalase (mmol H_2O_2 mn^{-1} g^{-1} fresh weight) and super oxide dismutase (SOD, U g^{-1} FW) in plant tissue of two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid) and potassium sulfate

Source	Degrees of Freedom	Catalase (mmol H_2O_2 mn^{-1} g^{-1} FW)	SOD (U g^{-1} FW)
Genotypes	1	292327.98**	17647.35**
Salt Concentration	2	76985.90**	4970.25**
Treatments†	3	33516.69**	2283.25**
Genotypes × Salt Conc.	2	12598.57	992.25
Genotypes × Treatments	3	2460.15	2673.25**
Salt Conc. × Treatments	6	0347.85	4874.25**
Genotypes × Salt Conc. × Treatments	6	3258.14	788.25
Error	26	4175.17	281.43
LSD‡		40.99	10.64

Note. ** Significant at 0.01 probability level; † Treatments: seaweed extract, humic acid, and potassium sulfate; ‡ LSD: Least significant difference at $\alpha = 0.05$.

3.3 Correlation between Physiological and Phenotypic Responses

Across genotypes and growth enhancing treatments, there was a significant positive correlation ($r = 0.55$, $p < 0.01$) between proline content in the plant tissue and relative water content. Proline content was also negatively correlated ($r = -0.36$, $p < 0.01$) with electrolyte leakage. Proline is a well-known osmolyte that plays a major role in plant response to stress conditions by maintaining cell turgor and stabilizing membranes thus preventing electrolyte leakage, in addition to modulating reactive oxygen species levels and preventing oxidative damage (Hayat et al., 2012). This explains the negative correlation observed in this study between proline and electrolyte leakage and the positive correlation with relative water content. There was no correlation between proline content and shoot and root dry weights (Table 15).

Table 15. Correlation between shoot dry weight (g), root dry weight(g), relative Yield (%), relative water content, electrolyte leakage (%), Proline (μmol g^{-1} FW), catalase (mmol H_2O_2 mn^{-1} g^{-1} FW) and SOD (U g^{-1} FW) of two barley genotypes evaluated under three salt levels (0, 10, and 15 dS m^{-1} electrical conductivity) and three treatments of growth-enhancing substances (seaweed extract, humic acid), and potassium sulfate

	Root dry weight	Relative yield (g)	RWC†	Electrolyte leakage	Proline	Catalase	SOD‡
Shoot dry weight	0.19	1.00**	-0.23	0.22	-0.01	-0.2	0.03
Root dry weight	1.00	0.19	-0.25	-0.06	-0.09	0.40**	-0.17
Relative yield	-	1.00	-0.23	-0.22	-0.01	-0.2	-0.03
RWC	-	-	1.00	-0.36*	0.55**	-0.13	-0.23
Electrolyte leakage	-	-	-	1.00	0.06	0.25	-0.29*
Proline	-	-	-	-	1.00	0.07	-0.31*
Catalase	-	-	-	-	-	1.00	-0.42**

Note. *, ** Significant at 0.05 and 0.01 probability level; † RWC: Relative water content; ‡ SOD: Sodium oxide dismutase enzyme.

Considering the low Na^+/K^+ ratio in the salt tolerant genotype (5.4) compared to the salt sensitive (7.2), this may suggest that Na exclusion is playing an important role in salt tolerance of this barley genotype. SOD showed a negative correlation with electrolyte leakage ($r = -0.29$, $p < 0.05$) corroborating its role in minimizing membrane damage, but was not correlated with shoot and root dry weight. SOD was also negatively correlated with catalase ($r = -0.42$, $p < 0.01$), but catalase levels were significantly correlated with root dry weight ($r = 0.4$, $p < 0.01$). Considering the higher levels of catalase in the salt tolerant genotype Sharqiya Estate under all growth enhancing substances and both salt levels, this may suggest that catalase plays a more important role in alleviating salt tolerance in this genotype than SOD. A study on the effect of salt stress on anti-oxidative enzymes in leaves and roots of salt-tolerant and salt-sensitive maize genotypes showed that catalase and guaiacol peroxidase enzymes had a greater role in scavenging H_2O_2 in both leaves and roots, compared to SOD and ascorbate peroxidase (de Azevedo Neto et al., 2006).

4. Conclusion

Barley genotypes exhibited significant differences in germination in response to salt concentrations. Sharqiya Estate was the most tolerant genotype and Red Sea was the most susceptible one. Application of seaweed extract, humic acid, and potassium sulfate under two salt levels (10 and 15 dS m^{-1}) resulted in differential responses in shoot dry weight, number of tillers, root dry weight and root length between genotypes, with seaweed extract improving growth of the susceptible genotype under both salt levels, whereas potassium sulfate was most effective in the tolerant genotype. Overall, seaweed extract was more effective in maintaining a low Na^+/K^+ ratio in the susceptible genotype compared with humic acid and potassium sulfate, whereas it did not show a large effect on the tolerant genotype. Seaweed extract also resulted in the highest RWC and the highest proline concentration, whereas humic acid recorded the lowest electrolyte leakage in the susceptible genotype at 10 dS m^{-1} salt level. Application of seaweed extract resulted in the highest catalase activity at the 15 dS m^{-1} in both genotypes, with a higher magnitude in the salt-tolerant genotype. Seaweed extract seemed to have an overall positive effect in alleviating salt stress in the salt-sensitive barley genotype. Being derived from a renewable resource, the bioactive extracts from seaweed could be useful in overcoming salt stress and improving barley productivity.

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Abbreviations

CAT, Catalase activity; EL, Electrolyte leakage; ROS, Reactive Oxygen Species; RWC, Relative water content; SOD, Superoxide dismutase.