

Humic Acid Improves Germination, Vegetative Growth, and Yield in Rice (*Oryza sativa* L.) Under Salinity Stress

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ABSTRACT

Salinity stress is a major constraint for rice growth and productivity. Rice cultivars with considerable salt tolerant capabilities have been developed and improving their performance in the salt-affected lands is crucial. Herein, the effects of humic acid (100 mg/l) as a stress-alleviator and a biostimulant on the germination, vegetative growth, and yield of Giza 179 rice cultivar under increased salinity (0.55, 3.40, 6.77, 8.00 mS/cm) were investigated. The humic acid-induced effects were also validated in salt-affected field. Salinity retarded Giza 179 germination-related traits which were associated with a significant decline in Gibberellic acid (GA₃) content and α -amylase activity. Also, salinity reduced the Giza 179 vegetative growth which was correlated with accumulation of shoot Na⁺, proline, and total soluble proteins (TSP), induction of membrane injury, and reduction of photosynthetic pigments. Humic acid significantly decreased most of the salinity-induced injury; however, it increased the salinity-induced synthesis of proline and TSP. The deteriorative effects of salinity during germination and vegetative stages were translated into significant reduction in grain yield and quality whereas the ameliorative effects of humic acid against salinity were reflected in better overall growth and yield. These results indicate the efficacy of humic acid in maintaining vigorous germination, growth, and yield in salt-affected lands.

Keywords: Gibberellic acid (GA₃); Humic acid; Osmolytes; Rice; Salinity; Yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is an economically important food crop worldwide, providing more than 50% of daily calories for nearly three billion people around the globe (Mishra and Chaturvedi, 2018). The endosperm of rice is rich in carbohydrates which account for approximately 90% of its total dry weight. Additionally, rice has high nutritional value due to its proteins, minerals, and vitamins content (Rathna Priya *et al.*, 2019; Das *et al.*, 2020). In the year 2022 alone, global production approached roughly 512.8 million tons - albeit down by approximately ~2.4% compared to production levels recorded in the previous year (FAO, 2022). Given that world population continues to rise rapidly each year it becomes increasingly critical to enhance rice farming practices so as to secure our future food supplies.

Salinity is a detrimental abiotic threat for rice growth and productivity. In fact, rice is classified as a salt sensitive crop because its growth and productivity are significantly reduced in salt-affected lands (Rahman *et al.*, 2017; Ullah *et al.*, 2022). Salinity induces ionic, osmotic, and oxidative stresses which negatively affect rice germination, growth, and productivity (Shrivastava and Kumar, 2015; Rahman *et al.*, 2017; Shahzad *et al.*, 2017; Shahid *et al.*, 2020). Such salinity-induced deteriorative effects are mainly attributed to accumulation of toxic levels of Na⁺ and Cl⁻ ions within rice plants (Parihar *et al.*, 2015; Kumar and Khare, 2016) which induces a set of consecutive physiological events including inhibition in

gibberellic acid (GA) synthesis and α -amylase activity, reduction of photosynthesis, distribution of the ionic homeostasis, generation of toxic levels of reactive oxygen species (ROS), and eventual reduction in crop yield (Hualong *et al.*, 2014; Hasanuzzaman *et al.*, 2021). In response to salinity stress, plants activate a wide array of defense mechanisms including synthesis of osmo-protectants, and regulation of Na⁺ and Cl⁻ accumulation to minimize ionic toxicity (Abdelrahman *et al.*, 2018; Chen *et al.*, 2021).

A wide array of bio-stimulant compounds has been utilized to mitigate the salinity-induced injury to crop growth, development, and productivity. Humic acid reduces the salinity stress and increases the yield of many crop plants (Jarošová *et al.*, 2016; Matuszak-Slamani *et al.*, 2017; Saidimoradi *et al.*, 2019). It positively affects multiple indices of plant growth including root and shoot growth, leaf photosynthetic pigments, yield, and its related traits (Osman *et al.*, 2017; El-Beltagi *et al.*, 2023). The general stimulative impact of humic materials on plant growth and productivity are associated with their positive impact on many physiological processes and biological membranes in salinity stressed plants (Ouni *et al.*, 2014). Such humic acid-induced biochemical and physiological changes include induction of mineral and nutrient uptake and assimilation, metabolic interconversion, increasing photosynthesis, antioxidant enzymes, and accumulation of grain protein (Trevisan *et al.*, 2010; Abbas *et al.*, 2022; Ampong *et al.*, 2022). It is worth mentioning that the effectiveness of humic acid in crop improvement can only be reliable after being tested on both the crop type and



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under the conditions of interest. Interestingly, humic acid treatments exert significant ameliorative effects under intense stress environments (Rose *et al.*, 2014; Olk *et al.*, 2018).

Giza 179 is a common rice variety in Egypt with a set of favourable agronomical traits such as higher flag leaf area, early heading and flowering, long panicle, high number of panicles/plants, large number of filled grains/panicle, high seedling growth vigour index, and high harvest index. It also has a relatively short growing season (~125 days) from planting to harvest. Altogether, these traits made Giza 179 one of the highest grain-yielding rice cultivar (~4.2 tons/acre) under continuous submergence conditions (Mohamed *et al.*, 2021; Gaballah *et al.*, 2022). Interestingly, yield trials by rice breeders indicated that Giza 179 exhibits relatively enhanced tolerance against salinity in salt-affected soil (Elmoghazy and Elshenawy, 2018). However, the Giza 179' growth responses and adaptive physiological mechanisms to salinity during germination, seedling- and vegetative growth as well as their link, if any, to the improved yield in the salt-affected lands are not fully understood. Further, the possible improvement of Giza 179 performance during these critical stages in non-saline and saline environments by grain soaking in humic acid has not been tested. Consequently, our hypothesis is that the improved yield of Giza 179 in salt-affected lands may be associated with improved tolerance against salinity during germination and vegetative growth. In addition, soaking Giza 179 grains in humic acid can reduce the salinity-elicited deleterious effects, accelerates germination, and maintains its active seedling- and vegetative growth under salt-affected conditions. Accordingly, the current study aimed at investigation of the effects of humic acid on the growth responses, physiological processes, yield, and grain quality of Giza 179 under saline conditions during germination, seedling- and vegetative growth, and yield. In addition, the humic acid-induced effects were validated in field-grown Giza 179 plants in two locations with varying soil salinity to extend the agronomic significance of the current study.

MATERIALS AND METHODS

Grain stock, experimental design, and treatments

The grains of Giza 179 (*Oryza sativa* L.) were provided by the Rice Research and Training Center, Sakha, Kafr El-Sheik, Egypt. Three main experiments were performed to investigate the ameliorative impacts of humic acid on the germination, vegetative, and yield stage of Giza 179 under salinity stress. These experiments included a laboratory germination experiment, a pot experiment, and a field experiment. To initiate any of these experiments, Giza 179 grains were treated with sodium hypochlorite (3.6%, v/v) for 15 minutes for surface sterilization and washed thrice with autoclaved distilled H₂O. The sterilized grains were divided into two sets. The first set was primed in 100 mg/l humic acid whereas the second set was drenched in distilled H₂O (control) for 72 h at 27 ± 2 °C in the dark. In the germination experiment, two sets of surface-sterilized grains were germinated in 32

plastic boxes, each containing 20 grains. There were 16 boxes for each grain set, and all boxes had two layers of sterile filter papers lining them. Boxes containing either distilled H₂O or humic acid-treated grains were further divided into four subsets (Figure 1). The boxes were incubated at the optimal temperature of 30 ± 2 °C for rice seedling growth. Every other day, the boxes received an equal volume of specific seawater dilutions [0% (tap water, control), 5%, 10%, and 12.5%] which have 0.55, 3.40, 6.77, and 8.00 mS/cm EC values, respectively. Samples were collected at 3- and 7-days post salinity treatment (DPT) and processed according to the downstream analysis. At 3 DPT, samples of seedlings were harvested, frozen immediately and grounded in liquid N, and kept at -80 °C for GA₃ content and α-amylase activity. At 7 DPT, seedling growth was assessed via monitoring plumule and seedling lengths.

In the pot experiment, the surface sterilized control and humic acid-treated grain sets were planted in 10 pots (25 cm in diameter; 7 kg soil; 5 pots for each set). The soil chemical features in 1:2.5 soil extracts including C/N ratio, organic matter, anions (HCO₃⁻, Cl⁻, SO₄²⁻), cations (Na⁺, K⁺, Ca²⁺, Mg²⁺) pH, and EC were recently reported (Abu-Ria *et al.*, 2023). Giza 179 plantlets were irrigated with tap water and maintained for 28 days in the greenhouse (the nursery stage). The 28-day-old plantlets with uniform morphological appearance were transplanted (15 plant/pot) into bigger pots (20 pots for each set, 30 cm in diameter, 10 kg soil with the same features) and kept in the greenhouse for full recovery and establishment of the rice plantlets. All pots were thinned (10 plants/pot). Pots with the recovered distilled H₂O- as well as those with the humic acid-treated plantlets were assigned into four subsets and salt stress was then induced by application of either tap water (control, EC 0.55) or saline water with increased EC values of 3.40, 6.77, 8.00 mS/cm (Figure 1). Both control and humic acid-treated plants were maintained in the greenhouse and received equal volumes of tap water or saline water every four days. At vegetative stage (45 days after transplanting), plant samples were harvested and either immediately put in liquid N then kept at -80 °C for TSP, or used for measuring growth parameters, dried in an electric oven to a constant weight at 65 °C, and used for analysis of Na⁺ ion and proline content. At maturity (92 days after transplanting), plant height and the yield indices including panicle weight and length, and 100 grain weight as well as grain quality were measured.

The field experiment was conducted to assess the impact of humic acid on Giza 179 yield in two locations with different salinity levels: location1(control; soil EC1.36 mS/cm, Sakha agricultural rice research station, Kafr El-Sheikh governorate), and location 2 (natural salt-affected land, soil EC 8.24 mS/cm, El-Sirw agricultural rice research station, Damietta governorate (Figure 2). Two grain sets of Giza 179 were surface sterilized and soaked either in distilled H₂O or in humic acid (100 mg/l) as indicated above. Distilled H₂O- and humic acid-treated Giza 179 grains were sown and grown in non-saline soil and maintained for 28 days (nursery stage). The 28-day-old plantlets from each set were then divided into two subsets, the first subsets were transplanted in location 1

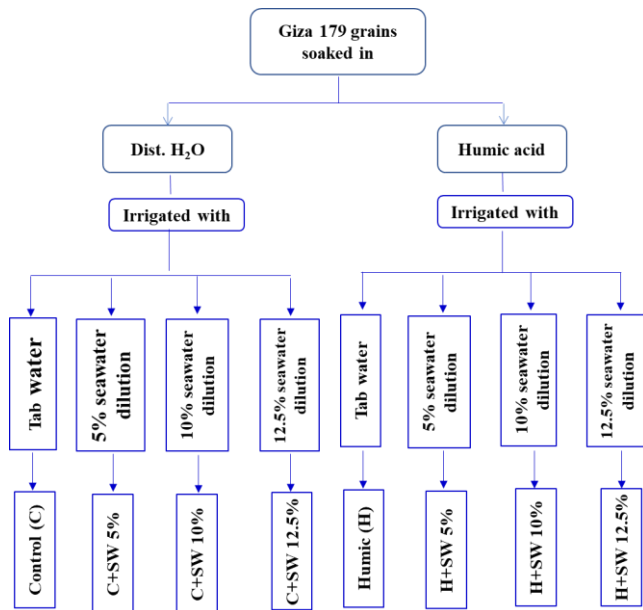


Figure (1): Experimental design demonstrating various treatments for grain germination and greenhouse experiments. .

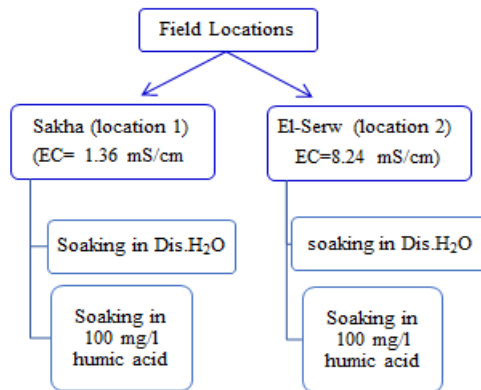


Figure (2): A visual overview of different grain germination treatments employed in field trials

(Sakha, control), whereas the second subsets were transplanted in location 2 (El-Sirw, natural salt-affected land) with a total of four treatments (Figure 2). At maturity (92 days after transplanting), plant height, yield and its related indices including number of filled and unfilled grain /panicle, 1000 grain weight, and grain yield /m² were measured. Also, grain quality traits such as grain protein, carbohydrates, and Na⁺ ion were determined.

Analysis of gibberellic acid (GA₃) content and α-amylase activity

Gibberellic acid (GA₃) was extracted as described by Shindy and Smith (1975). Briefly, known amounts of the frozen seedling tissues were extracted in methanol (80%, v/v) at 4 °C overnight then filtered. Using NaOH (1%), the extracts were adjusted to pH 8.6 and partitioned thrice using equal volumes of ethyl acetate. The ethyl acetate fractions were combined and evaporated. Using HCl (1%), the aqueous fraction was acidified to pH 2.8 and re-partitioned thrice using equal volumes of ethyl acetate. The acidic ethyl acetate fractions were combined, reduced to a known volume, and used for determination of GA₃ by HPLC analysis (Kelen *et al.*, 2004).

α-amylase was extracted in phosphate buffer (20 mM, pH 6.9) and its activity was monitored via determination of maltose released after starch hydrolysis using 3,5-dinitrosalysalic acid (DNSA) (Bernfeld, 1955). The absorption of the developed color at 560 nm was recorded using spectrophotometer (Shimadzu, model UV-160A). The activity was calculated and expressed as μg maltose min⁻¹ g⁻¹ FWT.

Growth measurements

Greenhouse-grown Giza 179 plants were carefully uprooted and washed thoroughly using distilled H₂O. The lengths of both shoot and root from the point of shoot/root attachment to the tip of the longest leaf and root, respectively were measured (Dabral *et al.*, 2019). The fresh weights of Giza 179 shoot and root were also recorded using a digital balance. Subsequently, both shoot and root were kept at 65 °C in an electric oven for a constant dry weight which was then recorded.

Photosynthetic pigments

Photosynthetic pigments were extracted in DMSO and determined spectrophotometrically at 480, 644, and 663 nm using spectrophotometer (Shimadzu, model UV-160A). The levels of the tested photosynthetic pigments were expressed as mg g⁻¹ DWT (Hiscox and Israelstam, 1979; Arnon, 1949).

Na⁺ ions content

The concentration of Na⁺ ions in the shoots, roots, and the harvested grains were determined using Flame photometer (PFP7, Jenway). Known weights of dry powdered tissues were placed in a mixture of HNO₃ (5 ml) and of HClO₄ (1ml) for complete digestion of plant tissues (Motsara and Roy, 2008). The concentration of Na⁺ ions was expressed as mmol g⁻¹ DWT.

Cell membrane injury

Electrolyte leakage (EL) was measured by incubation of fresh leaf discs in 30 ml deionized water in the dark at 28 °C for 24 hours. The EC of the solution containing leaf discs was measured before and after heating at 95 °C for 20 minutes (Shi *et al.*, 2006). EL was then calculated from the formula $EL\% = (EC_{\text{before heating}} / EC_{\text{after heating}}) \times 100$.

Proline content

Proline in known amounts of the powdered dry tissues was extracted using distilled H₂O according to Costa *et al.* (2011) and Meychik *et al.* (2013). Subsequently, 2 ml of acid ninhydrin, and 2 ml of glacial acetic acid were added to 2 ml of the extract and the mixture was heated in a boiling water bath for an hour, cooled to room temperature. The absorption of the developed color was recorded at 520 nm (Bates *et al.*, 1973). The cellular concentration of proline was calculated and expressed as mg g⁻¹ DWT.

Total soluble proteins

Total soluble proteins (TSP) in frozen plant samples were extracted using Tris-HCl buffer according to Scarponi and Perucci (1986), and then determined spectrophotometrically by mixing 20 μl of the extract with 980 μl of Coomassie reagent according to Bradford (1976). The optical density of the developed color at 595

nm was measured and the concentrations of TSP were calculated using bovine serum albumin standard curve and expressed as mg protein g^{-1} DWT.

RESULTS

Total carbohydrates

Known weights of dry powdered grains were mixed with 5 ml of HCl (2.5 N) and the mixture was heated for 3 hours in a boiling water bath then cooled down to 28 °C and neutralized using Na_2CO_3 . Subsequently, 4 ml of freshly prepared anthrone reagent were added to 1 ml carbohydrates extract and the mixture was heated for 8 minutes in a boiling water bath. The absorption of the resulted color at 630 nm was measured (Hedge and Hofreiter, 1962). TC were then estimated using the standard curve of authentic glucose and expressed as mg g^{-1} DWT.

Statistical analysis

The obtained data were subjected to one-way analysis of variance using CoStat Version 6.3. Data are displayed as means \pm SE and were compared using the Post Hoc Duncan's test at $p \leq 0.05$. Principal component analysis (PCA) and Pearson correlation coefficient were conducted by JMP Pro software.

Salinity and humic acid-induced responses during Giza 179 grains germination

The impact of both salinity and humic acid on GA_3 , α -amylase, and growth of Giza 179 rice variety are shown in Figure (3). Different salinity levels induced reduction with different magnitudes in the tested hormonal, enzymic and seedling growth parameters. Compared to unstressed plants, the salinity-induced decreases in GA_3 concentration were 4.06, 6.58, and 12.27% at 5, 10, and 12.5% seawater dilutions, respectively. The corresponding reduction values in α -amylase activity were 3.84, 7.86, and 9.78%. Similarly, increasing the levels of salinity progressively retarded the seedling growth. The 5, 10, and 12.5% seawater induced growth retardations of 9.83, 18.00, and 23.80% in plumule length corresponding to 11.58, 24.30, and 31.43% in seedling length, respectively. Grain soaking in humic acid significantly increased GA_3 , α -amylase, plumule length, and seedling length in humic acid-treated plants relative to their control peer plants in response to non-saline and saline treatments. The humic

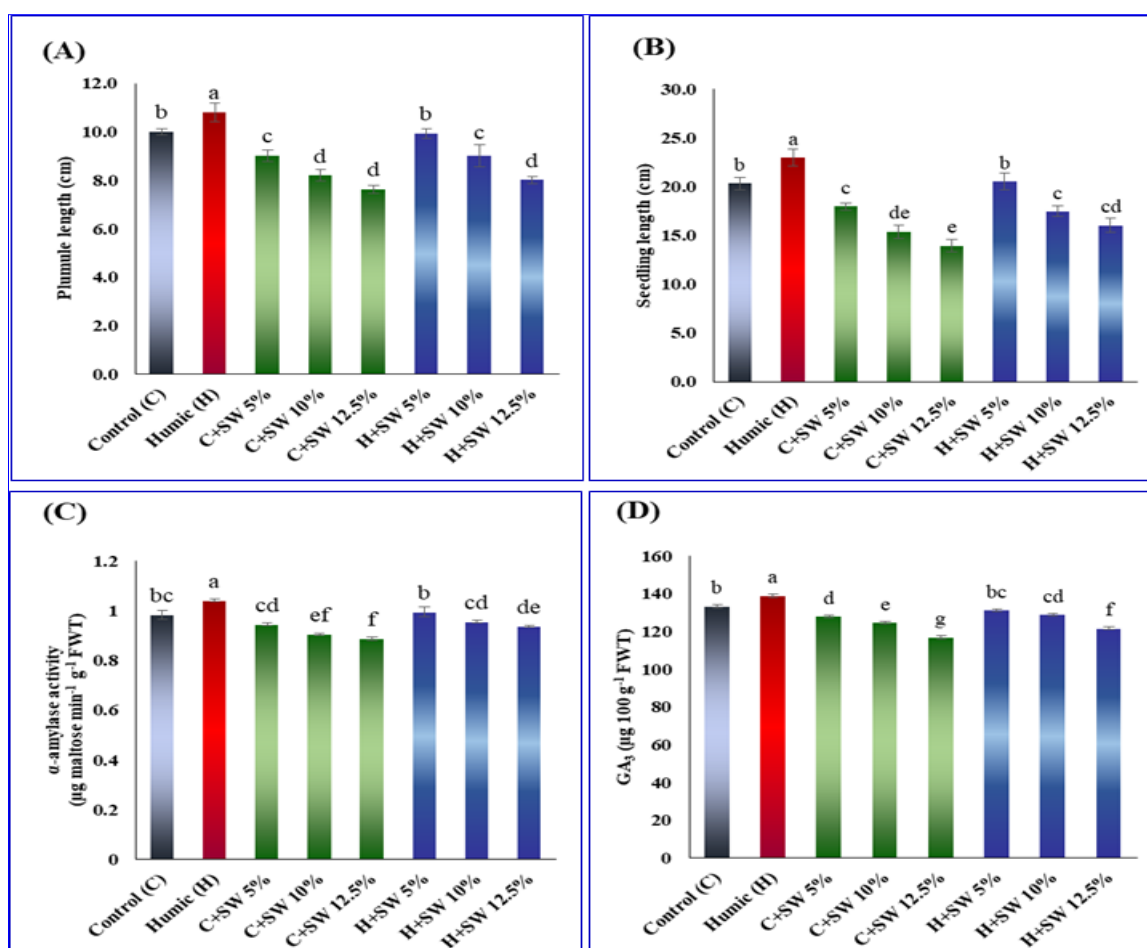


Figure (3): Effect of humic acid soaking on the growth indices of rice Giza 179 seedlings grown under increased levels of seawater. (A), plumule length; (B), seedling length; (C), α -amylase activity and (D) GA_3 content. Data represented as mean \pm SE. Bars with different letters are significant differences at $p \leq 0.05$, using Post Hoc Duncan's multiple range test.

acid-elicited increases in GA₃, α-amylase, plumule length, and seedling length in salinity-unstressed plants approached 4.21, 5.94, 8.00, and 13.14%, respectively.

On the other hand, the corresponding increments in salinity-treated plants at 5, 10 and 12.5% seawater dilutions approached 2.64, 3.40, and 4.00% in GA₃, 5.28, 5.51, and 5.67% in α-amylase, 10.17, 9.96, and 5.21% in plumule length, and 14.39, 13.56, and 15.06% in seedling length, respectively.

Salinity and humic acid-induced responses during Giza 179 vegetative Growth

At 45 days after transplanting, increasing salinity level reduced the lengths of both shoot and root, along with the fresh and dry mass of Giza 179 plants, in a dose-dependent way. The strongest retardation in these growth traits was observed at 12.5% seawater with a reduction of 16.03% in shoot length, 38.54% in root length, 50.62% in shoot fresh mass, 83.85% in root fresh mass, 56.52% in shoot dry mass, and 70.60% in root dry mass (Table 1). Soaking Giza 179 grains in humic acid significantly enhanced the tested growth traits under non-saline and saline treatments, relative to humic acid-untreated rice plants. In salinity unstressed plants, humic acid caused increases of 10.21% in shoot length, 22.69% in root length, 41.57% in shoot fresh mass, 20.71% in root fresh mass, 44.60% in shoot dry mass, and 73.12% in root dry mass compared to the control plants. In stressed plants at 5, 10 and 12.5% seawater dilutions, the humic acid-induced improvement in growth approached 7.69, 8.98, and 3.58% in shoot length, 25.35, 47.87, and 36.84% in root length, 33.38, 35.02, and 1.68% in shoot fresh mass, 11.86, 60.82, and 96.91% in root fresh mass, 99.56, 39.79, and 24.23% in shoot dry mass, and 75.94, 118.07, and 63.64% in root dry mass, respectively.

Changes in photosynthetic pigments

Increasing salinity levels significantly reduced the content of chlorophyll (Chl a and Chl b) and carotenoids pigments in Giza 179 leaves (Figure 4). Relative to unstressed plants, the salinity-stressed Giza 179 plants contained significantly lower levels of photosynthetic pigments with a reduction of 13.06, 28.32, and 21.49% in Chl a, 18.19, 31.91, and 21.90% in Chl b, and 16.31, 35.17, and 21.29% in carotenoids at 5, 10, and 12.5% seawater dilutions, respectively.

Humic acid-treated Giza 179 plants contained significantly greater levels of Chl a, Chl b, and carotenoids in non-saline and saline treatments, in comparison with humic acid-untreated plants. In the unstressed plants, humic acid resulted in increases of 16.00% in Chl a, 5.24% in Chl b, and 14.83% in carotenoids. Under salinity stress, humic acid-treated plants had 20.31, 25.48, and 1.43% higher in Chl a, 27.26, 37.99, and 9.05% in Chl b, and 23.04, 42.92, and 16.73% in carotenoids than the control plants at 5, 10, and 12.5% seawater dilutions in salinity-treated plants, respectively.

Changes in shoots and roots Na⁺ content

Raising salinity level progressively increased the accumulation of Na⁺ ions in Giza 179 shoots and roots (Figure 5A and 5B). The salinity-elicited increases in Na⁺ concentration in shoots approached 12.90, 22.93, and 27.23%

whereas their corresponding values in roots were 15.33, 15.92, and 24.77% at 5, 10 and 12.5% seawater dilutions, respectively. Soaking Giza 179 grains in humic acid significantly decreased the content of Na⁺ ions in both shoots and roots in non-saline and saline treatments. In unstressed plants, humic acid caused a reduction of 4.30% and 8.85% in Na⁺ ions content in Giza 179 shoots and roots, respectively relative to the humic acid-untreated plants. In stressed Giza 179 plants, humic acid reduced the Na⁺ ions content by 6.98, 6.41, and 1.13% in shoots and by 10.23, 5.60, and 8.04% in roots at 5, 10, and 12.5% seawater, respectively compared to the humic acid-untreated plants.

Cell membrane injury

To assess the salinity-induced injury in cell membrane in of Giza 179 leaves, electrolytes leakage (EL) was monitored and compared in the salinity-stressed and unstressed plants. Raising salinity levels gradually increased the electrolyte leakage (Figure 5C). Compared to Giza 179 unstressed plants, the salinity-induced increments in EL were 27.24, 54.24, and 96.32% at 5, 10, and 12.5% seawater, respectively. Compared to the humic acid-untreated plants, soaking Giza 179 grains in humic acid significantly decreased the salinity-elicited increases in EL by 21.53, 17.98, and 23.09% at 5, 10, and 12.5% seawater dilutions, respectively.

Changes in total soluble proteins and proline content

The changes in TSP and proline content under salinity and/or humic acid treatments are presented in Figure (6). Salinity stress resulted in significant increases in both analytes. At 5, 10, and 12.5% seawater, salinity stress resulted in increases of 23.20, 11.97, and 50.09% in TSP and 27.45, 3.92, and 41.18% in proline, respectively, compared to unstressed plants. Grain soaking in humic acid induced 3.87% increase in TSP in the unstressed Giza 179 plants relative to the control plants. In salt-stressed plants, humic acid treatment resulted in 4.19% and 7.98% increases in TSP in Giza 179 plants irrigated with 5% and 10% seawater concentrations, respectively. However, the humic acid treatment had no statistically significant effects on stressed Giza 179 plants under 12.5% seawater irrigation. For proline, soaking Giza 179 grains in humic acid had no statistically significant effects on the level of proline in salt-unstressed and 5% salt-treated Giza 179 plants. However, it significantly increased proline by 7.55% and 6.25% in salinity-stressed plants under 10% and 12.5% seawater irrigation, respectively.

Yield attributes

To test the consequences of the above growth and physiological response on Giza 179 productivity, the impact of salinity and humic acid on yield and its related traits at maturity were monitored and compared (Table 2). Increasing the magnitude of salinity stress progressively reduced plant height by 12.71, 21.23, and 27.94%, panicle length by 8.21, 20.60, and 23.82%, panicle weight by 53.33, 76.45, and 83.45%, and 100 grain weight by 19.45, 30.28, and 39.56% at 5, 10, and 12.5% seawater dilutions, respectively. Interestingly, humic acid successfully diminished the salinity-elicited reduction in plant height by 7.90, 6.49, and 4.11%, panicle length by 2.83, 6.90, and,

Table (1): Effects of soaking Giza 179 grains in humic acid (100 mg/L) on the vegetative growth, expressed as shoot and root length and shoot and root fresh and dry weight, (45 days from transplantation) indices under increased levels of seawater.

Treatments	Measured parameters					
	Shoot length (cm)	Root length (cm)	Shoot Fresh W (g)	Root Fresh W (g)	Shoot dry W (g)	Root dry W (g)
Control (C)	61.30 ± 0.77 ^b	30.91 ± 0.64 ^c	4.501 ± 0.223 ^b	1.117 ± 0.071 ^b	1.316 ± 0.025 ^c	0.235 ± 0.013 ^b
Humic (H)	67.56 ± 0.65 ^a	37.93 ± 0.23 ^a	6.372 ± 0.177 ^a	1.348 ± 0.069 ^a	1.903 ± 0.079 ^a	0.407 ± 0.011 ^a
C+SW 5%	57.20 ± 0.79 ^c	26.43 ± 0.71 ^d	3.421 ± 0.305 ^c	0.977 ± 0.027 ^c	0.783 ± 0.026 ^{de}	0.143 ± 0.004 ^c
C+SW 10%	52.03 ± 0.27 ^d	20.50 ± 0.76 ^e	2.640 ± 0.079 ^d	0.407 ± 0.021 ^e	0.623 ± 0.019 ^f	0.107 ± 0.004 ^d
C+SW 12.5%	51.47 ± 0.47 ^d	19.00 ± 0.53 ^e	2.223 ± 0.057 ^d	0.180 ± 0.007 ^f	0.572 ± 0.016 ^f	0.069 ± 0.002 ^e
H+SW 5%	61.60 ± 0.46 ^b	33.13 ± 0.74 ^b	4.563 ± 0.073 ^b	1.093 ± 0.051 ^{bc}	1.562 ± 0.064 ^b	0.252 ± 0.018 ^b
H+SW 10%	56.70 ± 1.01 ^c	30.31 ± 0.48 ^c	3.564 ± 0.252 ^c	0.655 ± 0.028 ^d	0.871 ± 0.060 ^d	0.233 ± 0.011 ^b
H+SW 12.5%	53.31 ± 0.61 ^d	26.00 ± 0.58 ^d	2.260 ± 0.053 ^d	0.355 ± 0.019 ^e	0.711 ± 0.039 ^{ef}	0.113 ± 0.003 ^d

SW, sea water concentration; W, weight; Data represented in mean ± SE; Means with different letter are significantly different at $p \leq 0.05$ level according to Duncan multiple range test.

Table (2): Effect of humic acid soaking (100 mg/L) on the yield attributes of rice Giza 179 plants grown under increased levels of seawater for 92 days after transplanting in greenhouse. Data represented as mean ± SE.

Treatments	Measured parameters			
	Plant height (cm)	Panicle length (cm)	Panicle weight (g)	100 grain Weight (g)
Control (C)	77.71 ± 0.83 ^b	18.26 ± 0.40 ^b	1.833 ± 0.097 ^b	2.257 ± 0.027 ^b
Humic (H)	82.99 ± 0.82 ^a	19.63 ± 0.14 ^a	2.433 ± 0.105 ^a	2.366 ± 0.014 ^a
C+SW 5%	67.84 ± 0.67 ^d	16.76 ± 0.40 ^c	0.855 ± 0.046 ^d	1.818 ± 0.037 ^d
C+SW 10%	61.21 ± 0.68 ^f	14.50 ± 0.18 ^e	0.432 ± 0.025 ^{ef}	1.574 ± 0.046 ^f
C+12.5%	56.00 ± 0.41 ^h	13.91 ± 0.44 ^e	0.303 ± 0.021 ^f	1.364 ± 0.013 ^g
H+SW 5%	73.20 ± 0.74 ^c	17.24 ± 0.22 ^c	1.057 ± 0.050 ^c	2.036 ± 0.033 ^c
H+SW 10%	65.19 ± 0.88 ^e	15.50 ± 0.32 ^d	0.521 ± 0.034 ^e	1.712 ± 0.025 ^e
H+SW 12.5%	58.30 ± 0.56 ^g	14.21 ± 0.31 ^e	0.365 ± 0.014 ^{ef}	1.516 ± 0.007 ^f

SW, sea water concentration; W, weight; Data represented in mean ± SE; Means with different letter are significantly different at $p \leq 0.05$ level according to Duncan multiple range test.

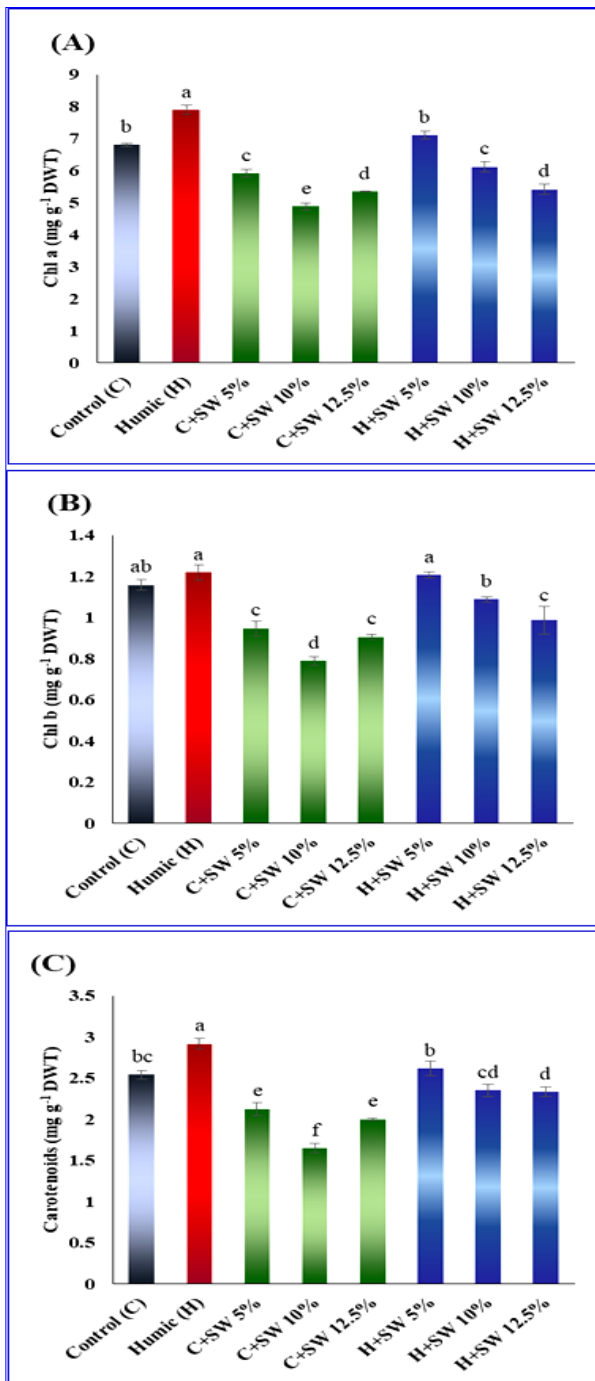


Figure 4: Effect of humic acid (100 mg/l) soaking on the growth indices of rice Giza 179 plants grown under increased levels of seawater. (A) chlorophyll a, (B) chlorophyll b, and (C) carotenoid content. Data represented as mean \pm SE. Bars with different letters are significant differences at $p \leq 0.05$, using Post Hoc Duncan's multiple range test.

2.16%, panicle weight by 23.62, 20.62, and 20.35%, and 100 grain weight by 12.00, 8.75, and 11.12% at 5, 10, and 12.5% seawater, respectively. In salt-unstressed plants humic acid results in an increase of 6.79% in plant height, 7.46% in panicle length, 32.75% in panicle weight, and 4.80% in 100 grain weight.

Grain quality

To evaluate the induced consequences of salinity stress and humic acid application on the quality of the harvested grains, if any, the total carbohydrates (TC),

TSP, and Na^+ content were analyzed (Figure 7). Increasing salinity stress progressively decreased TC and TSP, but increased Na^+ content in rice grains. Such salinity-induced decreases approached 10.08, 17.43, and 20.98% in TC and 16.76, 21.70, and 33.17% in TSP at 5, 10, and 12.5% seawater dilution, respectively. The corresponding salinity-induced increases in grain Na^+ concentration approached 21.55, 29.39, and 50.93%, respectively. Humic acid-treated plants contained higher concentrations of TC and TSP in the harvested grains of control and salt stressed plants. Under salt-unstressed treatment, the humic acid-induced increments in TC and TSP were 10.82% and 19.25%, respectively. Under salinity treatment, humic acid increased TC by 2.99, 3.57, and 6.00% compared to 35.04, 9.88, and 18.30% increases in TSP at 5, 10, and 12.5% seawater dilutions, respectively. In contrast, humic acid diminished grain Na^+ concentration in salinity-unstressed plants by 11.75% compared to 14.51, 12.11, and 12.98% decreases in the salt-stressed Giza 179 plants at 5, 10, and 12.5% seawater dilutions, respectively.

Field experiment

To compare the impacts of salinity as well as humic acid treatments on the growth, productivity and grain quality of Giza 179, uniform 28 days plantlets from distilled H_2O - and humic acid-soaked grains were transferred to two natural locations of different soil salinity. These field-grown plants were maintained until maturity. Compared to the salinity-unaffected soil (location 1, $\text{EC}=1.36$ mS/cm), high soil salinity (location 2, $\text{EC}=8.24$ mS/cm) significantly decreased plant height by 7.34%, number of filled grains /panicle by 16.89%, 1000 grain weight by 12.48%, and grain yield/ m^2 by 67.65%. On the other hand, high soil salinity (location 2) increased the number of un-filled grains /panicle by 32.14% (Table 3). Soaking Giza 179 grains in humic acid improved the performance of Giza 179 plants in both locations.

The humic acid resulted in 3.85% and 3.02% increases in plant height, 29.78% and 8.29% increases in the number of filled grains /panicle, 3.18% and 8.73% increases in 1000 grain weight, and 43.06% and 28.53% increases in grain yield/ m^2 in location 1 and location 2, respectively. Also, humic acid reduced the number of un-filled grains /panicle by 30.95% and 15.32% in location 1 and location 2, respectively. Regarding grain quality-related traits (Figure 8), salinity stress decreased TC by 3.02% and TSP by 8.60%, but increased Na^+ contents by 26.49%. On the other hand, humic acid increased TC by 2.09% and 1.44% and TSP by 51.60% and 33.73% in location 1 and location 2, respectively. The corresponding decreases in Na^+ concentration in humic acid-treated plants approached 20.43% and 19.15%, respectively.

Principal component analysis and Pearson correlations

The investigated 25 parameters throughout germination and greenhouse experiments were subjected to the principal component analysis (PCA) (Figure 9A) and Pearson correlations (Figure 9B). In PCA biplot,

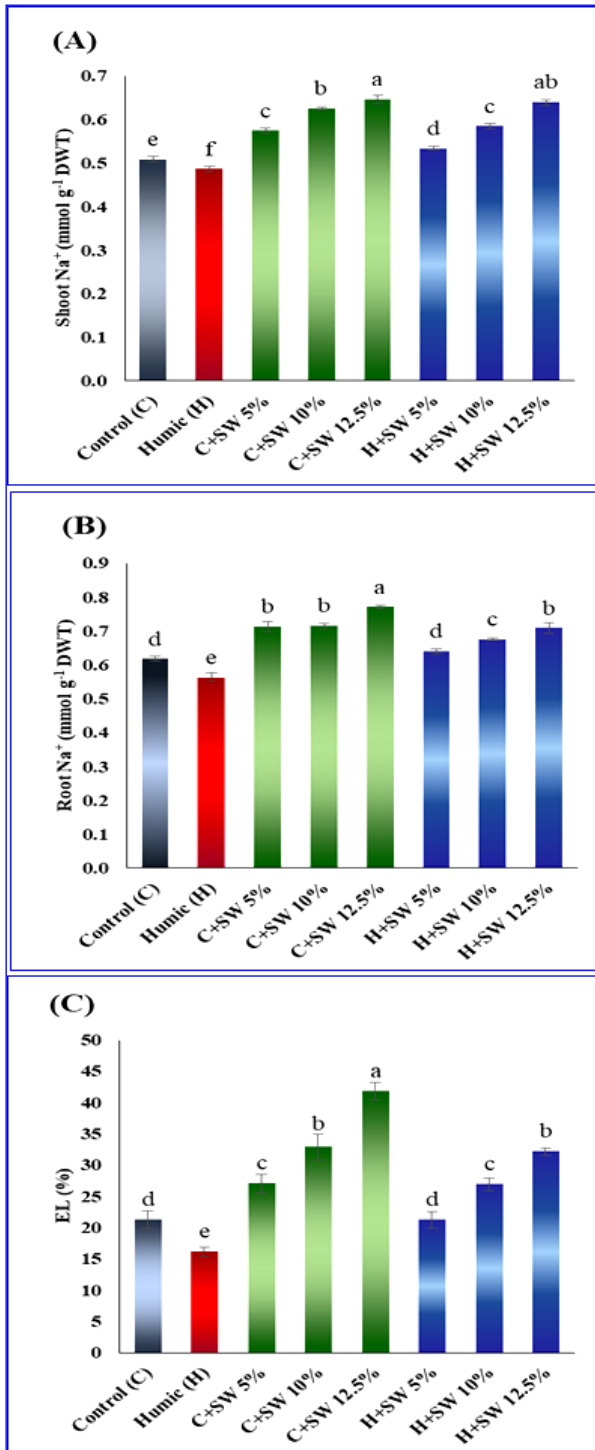


Figure 5: Effects of humic acid (100 mg/L) soaking on (A) shoot Na⁺, (B) root Na⁺, and (C) electrolyte leakage of Rice Giza 179 grown under increased levels of seawater for 45 days after transplanting in greenhouse. Data represented as mean ±SE. Bars with different letters indicate significant differences at $p \leq 0.05$, using Post Hoc Duncan's multiple range test.

the first principal component (88.6%) described the variation between the responses of Giza 179 plants in salt - unstressed and salt-stressed treatments, while the second principal component (7.02%) described the variation in salt stressed and salt - unstressed Giza 179 in salt - unstressed and salt-stressed treatments, while the second principal component (7.02%) described the variation in salt stressed and salt - unstressed Giza 179

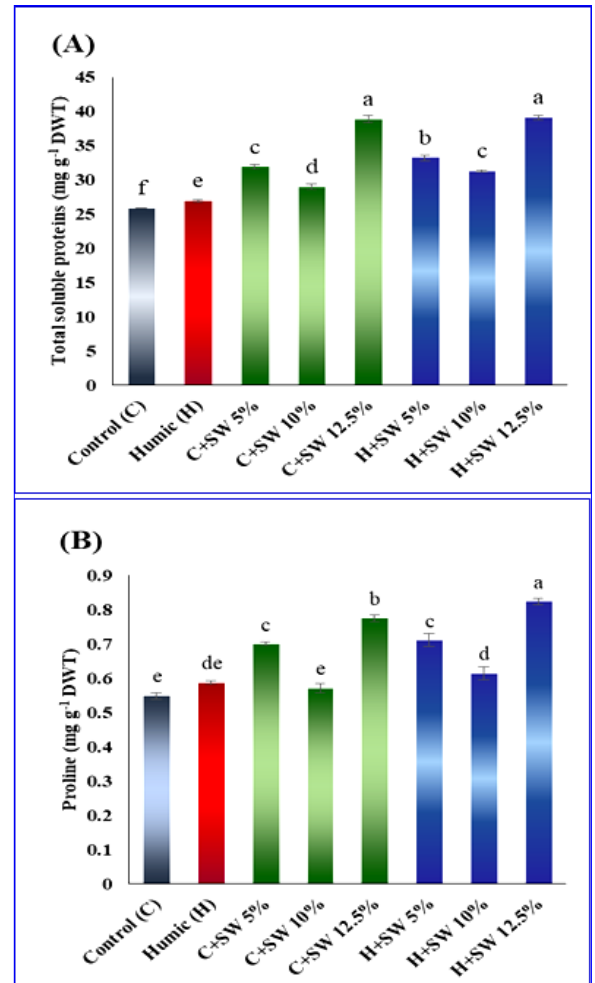


Figure 6: Effects of humic acid (100 mg/L) soaking on (A) total soluble proteins, and (B) proline of rice Giza 179 grown under increased levels of seawater for 45 days after transplanting in greenhouse. Data represented as mean ±SE. Bars with different letters indicate significant differences at $p \leq 0.05$, using Post Hoc Duncan's multiple range test.

plants in absence and presence of humic acid. Shoot, root, and grain Na⁺ contents as well as membrane injury index (EL percentage) were mostly associated with humic acid-untreated plants under high salt stress level (C+SW 12.5%), whereas TSP and proline showed strong association humic acid-treated plants under high salinity level (H+SW 12.5%). On the other hand, GA₃ content, α-amylase activity, seedling length, plumule length, growth indices of shoot and root (length, fresh weight, and dry weight), yield indices (plant height, panicle length, panicle weight, and 100 grain weight) and grain quality (grain total carbohydrates, and grain TSP exhibited strong association with humic acid treatment under non-saline conditions (humic). Chl a, Chl b, and carotenoids showed strong association with humic acid-treatment under non-saline conditions (Humic) as well as under the lowest salinity level (H+SW 5%). Heatmap correlations revealed positive relationships among GA₃ content, α-amylase activity, photosynthetic pigments, seedling length, plumule length, shoot and root growth indices, yield indices, and grain quality which all negatively correlated with the rest of investigated variables (Figure 9B).

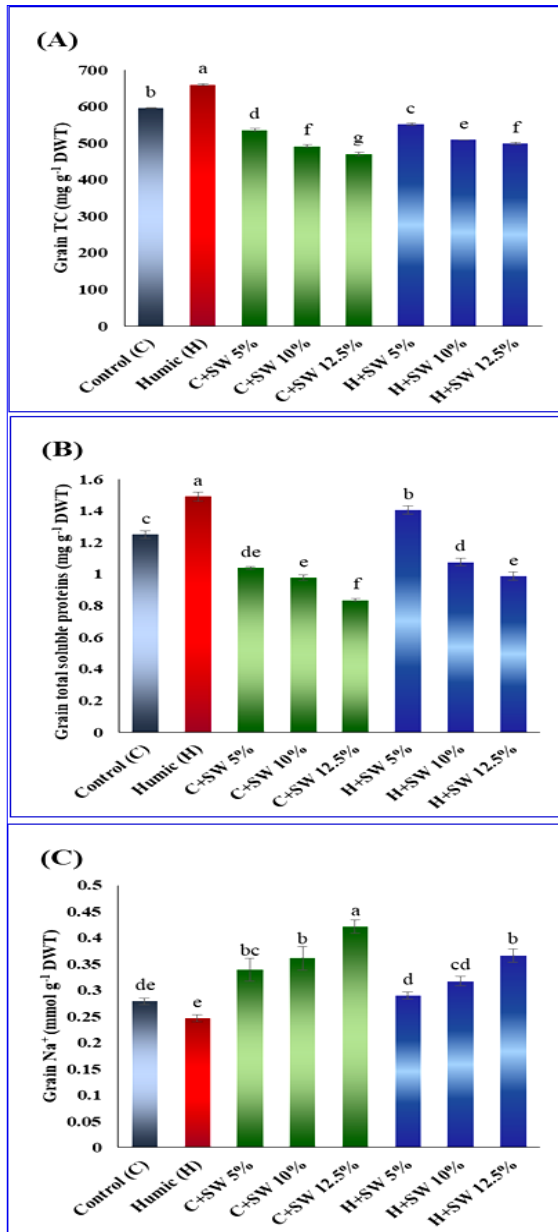


Figure (7): Effects of humic acid (100 mg/l) soaking on: (A), grain total carbohydrates (TC); (B), grain total soluble proteins, and (C), grain Na⁺ content of rice Giza 179 grown under increased levels of seawater for 92 days after transplanting in greenhouse. Data represented as mean \pm SE. Bars with different letters indicate significant differences at $p \leq 0.05$, using Post Hoc Duncan's multiple range test.

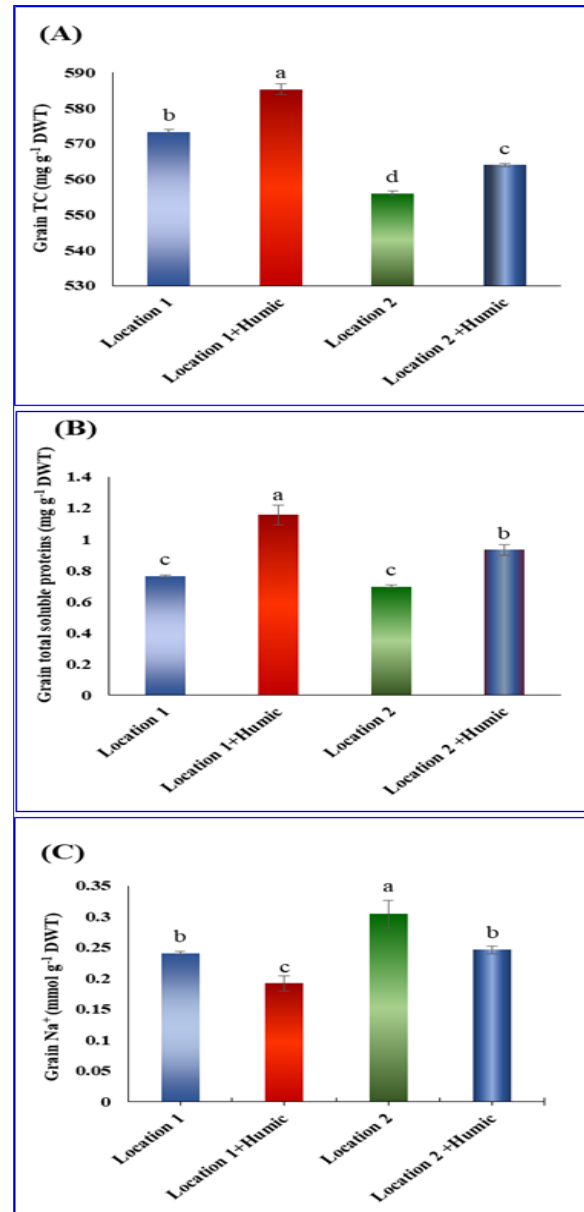


Figure (8): Effect of humic acid (100 mg/l) soaking on the growth indices of rice Giza 179 plants grown under different field location with different salinity content for 92 days of transplantation. (A), grain total carbohydrates (TC); (B), grain total soluble proteins and (C) grain Na⁺ of studied rice grain. Data represented as mean \pm SE. Bars with different letters are significant differences at $p \leq 0.05$, using Post Hoc Duncan's multiple range test.

Table (3): Effects of soaking Giza 179 grains in humic acid (100 mg/L) on the yield of rice plants grown in two locations varying in their soil salinity: location1 (Sakha agricultural rice research station, EC= 1.36 mS/cm) and location 2 (El-Sirw agricultural rice research station, EC=8.24 mS/cm).

Treatments	Measured parameters				
	Plant height (cm)	Grain count/panicle	Unfilled Grain	Weight of 1000 grains (g)	Grain yield/m ² (g)
Location 1	95.33 \pm 0.33 ^b	150.00 \pm 9.17 ^b	28.00 \pm 4.04 ^b	26.167 \pm 0.333 ^{ab}	3.027 \pm 0.048 ^b
Location 1+Humic	99.00 \pm 0.58 ^a	194.67 \pm 2.40 ^a	19.33 \pm 1.86 ^c	27.000 \pm 0.289 ^a	4.330 \pm 0.101 ^a
Location 2	88.33 \pm 0.88 ^d	124.67 \pm 0.88 ^c	37.00 \pm 1.15 ^a	22.900 \pm 0.700 ^c	0.979 \pm 0.055 ^d
Location 2+Humic	91.00 \pm 1.00 ^c	135.00 \pm 2.08 ^{bc}	31.33 \pm 1.33 ^{ab}	24.900 \pm 0.700 ^b	1.258 \pm 0.096 ^c

Data represented in mean values \pm SE of three replicates. Means with different letter are significantly different at $p \leq 0.05$ level according to Duncan multiple range test.

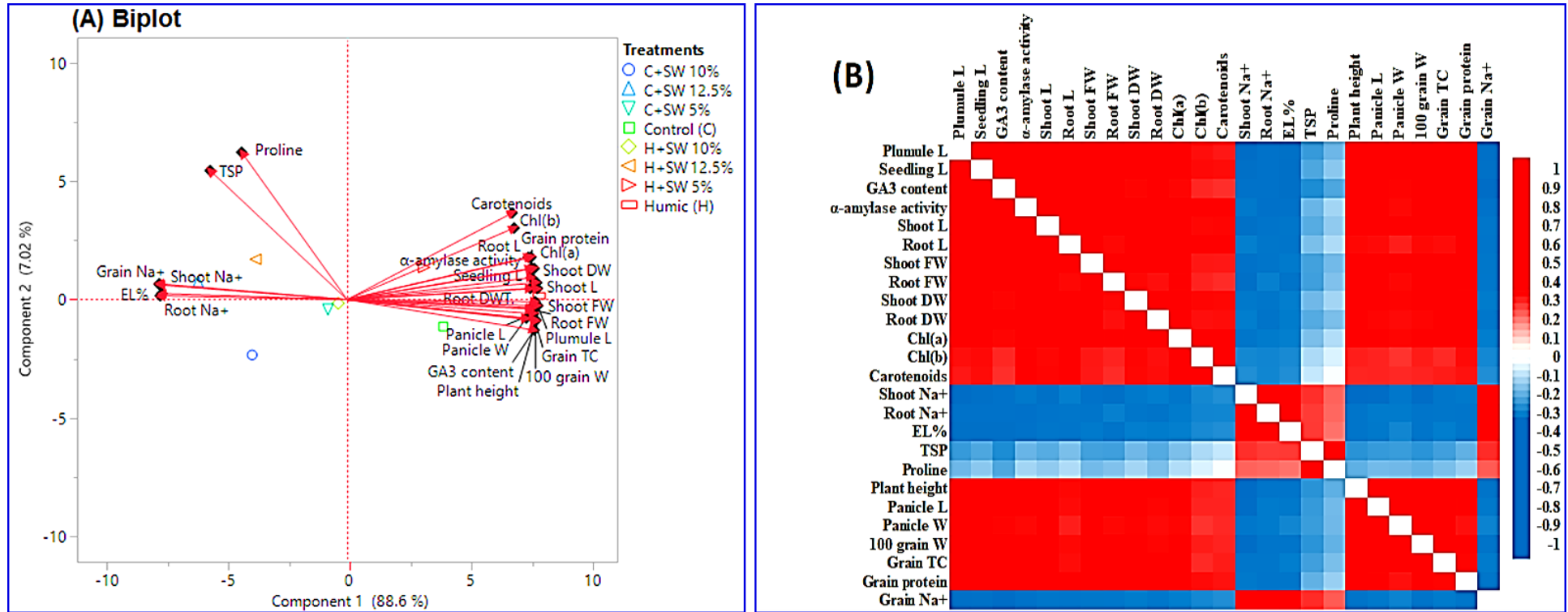


Figure (9): (A) Principal component analysis (PCA) and (B) Heatmap of Pearson correlation coefficient of the investigated variables throughout germination and greenhouse experiments in humic acid treated and non-treated rice Giza 179 plants grown under increased levels of seawater. In heatmap, red and blue colors represent positive and negative correlations, respectively, according to the color scale.

DISCUSSION

The current study aimed at assessing the possible stimulating influences of humic acid, as a biostimulant on Giza 179 growth and physiological adaptive responses during germination, seedling growth, vegetative growth, and yield under non-saline and increased salinity in saline irrigation (EC; 0.55, 3.40, 6.77, 8.00 mS/cm). Also, the impact of humic acid on grain quality of Giza 179 was also tested.

Our analysis revealed strong inhibitory impacts of salt stress on all Giza 179 germination and post germination-related traits. Such harmful effects of salt stress are ascribed to insufficient water absorption, toxic ion effects on the embryo, and cell membrane destruction (Farooq *et al.*, 2017; Rahman *et al.*, 2017). The observed retardation in Giza 179 growth in response to salinity was correlated with substantial decline in GA₃ content and α -amylase activity (Figure 3). These findings coincide with the salinity-elicited decreases in bioactive gibberellin content which suppresses rice grain germination via suppression of α -amylase activity through the downregulation of expression of α -amylase-encoding gene (Liu *et al.*, 2018). Interestingly, the heatmap correlation revealed a strong positive association between plumule and seedling lengths, GA₃ content, and α -amylase activity (Figure 9B). Soaking Giza 179 grains in humic acid significantly improved rice seedling growth under different salinity levels. Such humic acid-induced increases may be due to its promotive effects on GA₃ content, which induces α -amylase activity (Figure 3) and thus secures more energy from soluble sugars to support the embryo growth (Li *et al.*, 2019). Consistent with that, the upregulation of the expression of GA synthesis-related genes such as OsGA20ox2 and OsGA3ox1 genes in rice by humic acid has been reported (Sheteiwy *et al.*, 2017). Similar positive impacts of humic acid on both GA₃ synthesis and α -amylase activity have also been reported in *Zea mays* (Gao *et al.*, 2020) and *Coriandrum sativum* in non-saline and saline environments (Hassanein *et al.*, 2022).

During Giza 179 vegetative growth, increasing the magnitude of salinity in the irrigation water progressively decreased the shoot and root growth indices including length, fresh weight, and biomass (Table 1). These results coincide with the documented retardation in the growth of *Oryza sativa* (Singh *et al.*, 2018; Badawy *et al.*, 2021), *Triticum aestivum* (Ndiate *et al.*, 2022), *Pisum Sativum* (Ismail *et al.*, 2022) and *Cucumis sativus* (Ibraheem, 2015) under salt stress. The salinity-induced reduction in Giza 179 vegetative growth was associated with significant reduction in the concentration of chlorophyll pigments (Chl a and Chl b) and carotenoids (Figure 4). The heatmap correlation analysis supported these results, revealing positive relationships between shoot and root growth indices and levels of Chl a, Chl b, and carotenoids (Figure 9B). These results coincide with the salinity-induced decreases in the level of chlorophyll and carotenoid in

various *Oryza sativa* varieties (Cha-Um *et al.*, 2009; Abdallah *et al.*, 2016; Kumar *et al.*, 2021) and *Triticum aestivum* (Singh *et al.*, 2022). The salinity-induced reduction in the vegetative growth is due to its suppressive effects on the rice meristem activity which is also linked to the salinity-induced reduction in chlorophyll level (Kordrostami *et al.*, 2017). The reduction in the concentration of chlorophyll under salinity stress occurs because of the salinity-induced limitation of the precursors of chlorophyll biosynthesis as well as to the acceleration of chlorophyllase activity; two physio-logical processes that are linked to the buildup of toxic Na⁺ ions (Santos, 2004; Ashraf and Harris, 2013). The humic acid treatment enhanced Giza 179 vegetative growth which was correlated with significant increases in chlorophyll pigments and carotenoids. Similar positive impacts of humic acid on photosynthetic pigments in salinity-stressed plants have been found in *Capsicum annuum* (Akladiou and Mohamed, 2018), *Coriandrum sativum* (Hassanein *et al.*, 2022), *Sorghum bicolor* (Ali *et al.*, 2022), and *Phaseolus vulgaris* (El-Beltagi *et al.*, 2023). The beneficial impacts of humic acid on the biosynthesis of chlorophyll and carotenoid are ascribed to its role in induction of cell membrane permeability and stimulation of nutrients uptake (Gholami *et al.*, 2019). The PCA biplot revealed that the growth indices of shoot and root as well as the photosynthetic pigments (Chl a, Chl b, and carotenoids) were generally correlated with humic acid-treated Giza 179 plants under normal conditions (Humic) and the lowest level of seawater dilution (H+SW 5%; Figure 9A).

The observed salinity-elicited reduction in Giza 179 growth and photosynthetic pigments was correlated with a Na⁺ accumulation (Figure 5A and 5B). The excess Na⁺ in the cytoplasm disturbs ion homeostasis, interferes with K⁺ uptake, and disrupts cellular membranes, organelles, and photosynthetic machinery (Chakraborty *et al.*, 2018; Ketehouli *et al.*, 2019). Such salinity-induced Na⁺ build up thus stands behind the increased outflows of the electrolytes (Figure 5C). These results agree with the reported increases in membrane permeability and electrolytes leakages in *Oryza sativa* in response to salinity (Ueda *et al.*, 2013; Singh *et al.*, 2018). Therefore, salinity-induced Na⁺ influx in shoots and roots may be the primary cause of salinity-induced reductions in growth and photosynthetic pigments, as indicated by a negative relationship in heatmap (Figure 9B). Soaking Giza 179 grains in humic acid significantly decreased the Na⁺ content in both shoots and roots as well as electrolyte leakage. Similar humic acid suppressive impacts on Na⁺ ions content have been documented in shoots and roots *Urochondra setulosa* (Bano *et al.*, 2022) and *Triticum aestivum* (Abbas *et al.*, 2022) plants in response to salinity. Also, the humic acid-induced reduction in electrolyte leakage has been described in salinity stressed *Phaseolus vulgaris* (Taha and Osman, 2018) and *Zea mays* (Kaya *et al.*, 2018). The ameliorative effects of humic acid on electrolyte leakage in stressed plants are attributed to its role in

reducing the concentration of Na^+ in shoots and thus maintaining membrane integrity. The PCA biplot supported this view and revealed a strong association of shoots and roots Na^+ and EL percentage with humic acid-untreated plants under high salinity level (C+SW 12.5%; Figure 9A).

As a mechanism of survival against the Na^+ buildup in the cytoplasm, Giza 179 plants the accumulated significant amounts of TSP and proline in leaves in response to salinity stress (Figure 6). These results coincide with the reported salinity-induced accumulation of TSP and proline in *Tagetes minuta* plants (Moghaddam *et al.*, 2020). Plants have been shown to survive various stress conditions such as drought by accumulating more proline (Ibrahim and Alaraidh, 2010; Abbaspour *et al.*, 2020). The enhanced accumulation of these organic solutes under salinity stress coincides with their roles in maintaining membrane stability, cell osmotic potential, and stabilizing ionic homeostasis (Mushtaq *et al.*, 2020; El Moukhtari *et al.*, 2020). Humic acid-treated plants accumulated higher level of TSP and proline under non-saline conditions as well as saline irrigation. Similar stimulating impacts of humic acid on TSP and proline have been reported in salinity-stressed *Sorghum bicolor* (Ali *et al.*, 2020). These findings reflect the adaptability mechanisms of humic acid-treated plants against salinity-induced osmotic stress, as indicated by a strong association of TSP and proline with humic acid-treated plants in response to the heisted salinity level (H+SW 12.5%; Figure 9A).

The above suppressive effects of salinity as well as the promotive influence of humic acid on germination criteria, seedling growth parameters, and vegetative growth were all reflected into the yield indices (Table 2) and grain quality (Figure 7) under various treatments. Salinity stress significantly reduced mature plant height, yield attributes, and grain quality-related traits. Humic acid significantly reduced the salt-induced deteriorative effects in plant height as well as yield-related traits such as panicle length, panicle weight, 100 grain weight, grain total carbohydrates, and grain TSP. Humic acid also decreased the salinity-elicited accumulation of grain Na^+ . Such humic acid-promoting impacts on grain quality and yield were further validated in two locations with varying levels of soil salinity. Humic acid maintained its promotive effects and increased plant height and other yield-related traits including number of filled grains /panicle, 1000 grain weight, grain yield/m², grain total carbohydrates, and grain TSP. It also decreased the number of un-filled grains /panicle and grain Na^+ content in both non-saline and saline soils (Table 3 and Figure 8). The salinity-elicited reductions in rice grain yield and quality may be attributed to deficient photosynthates reallocation during grain filling (Kumar and Khare, 2016; Sangwongchai *et al.*, 2022). Similar promotive influences of humic acid on grain yield and its related attributes in salinity stressed *Oryza sativa* (Okasha *et al.*, 2019) and *Triticum aestivum* have been documented (Khedr *et al.*, 2022). Also, humic acid

increased spike length and weight and 1000 grain weight, as well as grain TC and TSP in *Triticum aestivum* in saline environment (Osman *et al.*, 2017). The humic acid-resulted increases in grain TC and grain TSP were attributed to the increased chlorophyll content in the humic acid-treated Giza 179 plants (Figure 4) as described previously (EL-Bassiony *et al.*, 2010; Osman *et al.*, 2017). PCA supported the above results and revealed a strong association of grain total carbohydrates, grain TSP, chlorophyll pigments (Chl a and Chl b) and carotenoids with humic acid-treated plants under salinity unstressed conditions (Humic) (Figure 9A).

CONCLUSION

Salinity stress is a predominant and determinant environmental factor that limits rice germination, growth, and yield worldwide. The findings of the current study indicated that, salinity stress adversely affects Giza 179 germination through decreasing GA_3 content and α -amylase activity. Likewise, salinity reduced Giza 179 vegetative growth and such reduction was correlated with reduced levels of photosynthetic pigments, buildup of Na^+ ions content in both roots and shoots, increasing the electrolyte leakage, and accumulation of proline and TSP. The salinity-elicited detrimental impacts in rice germination and growth were translated into significant reduction in both grain yield and grain quality. In contrast, humic acid ameliorated the salinity-induced damages on Giza 179 germination, seedling- and vegetative growth and yield. The humic acid improvement in germination and vegetative growth was associated with induction of various growth enhancing parameters such as GA_3 content, α -amylase activity, photosynthetic pigments, proline, and TSP, as well as suppression of growth-inhibiting parameters such as Na^+ concentration in roots and shoots and electrolyte leakage. Such humic acid effects were reflected into improved yield and grain indices. Our results suggest that soaking of Giza 179 grains in humic acid can be a promising approach for enhancing growth and yield in rice and may be other crops in salt-affected lands.

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حمض الهيوميك يحسن الإنبات والنمو الخضري وإنتاجية الحبوب في الأرز النامي تحت الإجهاد الملحي

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الملخص العربي

يعتبر إجهاد الملوحة أحد المعوقات الرئيسية لنمو وإنتاجية الأرز. تم إنتاج أصناف أرز ذات قدرات مقبولة على تحمل الملوحة، وما زال تحسين أدائها في الأراضي المتضررة من الملوحة أمر بالغ الأهمية. في البحث الحالي تم دراسة تأثيرات حمض الهيوميك (100 مجم / لتر) - كمحفز حيوي وكمخفف للتأثيرات الضارة للملوحة - على الإنبات والنمو الخضري وإنتاجية الحبوب في صنف أرز جيزة 179 تحت تركيزات متزايدة من الملوحة (0.55، 3.40، 6.77، 8.00 ms/cm). كذلك تم التحقق من التأثيرات التي يسببها حمض الهيوميك على جيزة 179 في حقول طبيعية متأثرة بالملوحة وذلك لزيادة الجانب التطبيقي للدراسة. أدت الملوحة إلى إعاقة الإنبات في جيزة 179 وارتبط ذلك بانخفاض معنوي في محتوى حمض الجبريليك (GA_3) ونشاط انزيم α -amylase. كما أدت الملوحة إلى خفض النمو الخضري في جيزة 179 وارتبط ذلك بتراكم أيونات الصوديوم ونقص تركيز كل من البرولين والبروتينات الذائبة الكلية وأصبغ التمثيل الضوئي، وزيادة تسرب الأملاح خلال الأغشية البلازمية. نجح حمض الهيوميك بشكل ملحوظ في تقليل الآثار الضارة الناجمة عن الملوحة، ولكنه سبب زيادة في تخليق البرولين والبروتينات الذائبة الكلية. أثناء مرحلة الإنتاج تم ترجمة التأثيرات الضارة الناجمة عن الملوحة أثناء الإنبات والمراحل الخضريّة إلى انخفاض معنوي في إنتاجية الحبوب وجودتها بينما انعكست التأثيرات الإيجابية لحمض الهيوميك ضد الملوحة على تحسن معنوي في النمو الكلي وإنتاجية الحبوب في جيزة 179. توضح نتائج البحث الحالي فعالية حمض الهيوميك في مساعدة نباتات صنف جيزة 179 على الحفاظ على إنبات قوي ونمو وإنتاجية عالية في الأراضي المتأثرة بالملوحة.