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Effect of *Bacillus subtilis* on Some Physiological and Biochemical Processes in Barley (*Hordeum vulgare* L.) Plant Grown under Salt Stress

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THE GOAL of this study was to explore the effect of salinity on some growth parameters, changes in some physiological and biochemical reactions, particularly those associated with nitrogen metabolism, in barley plant (*Hordeum vulgare* L.). Also, to determine the effect of plant growth-promoting bacteria (PGPB), *Bacillus subtilis*, to alleviate the inhibitory effect of salt on plant growth and development. Addition of 100mM NaCl to hydroponic growth cultures significantly suppressed the growth of barley plants. This was accompanied with a significant increase of osmoregulatory components including sucrose, trehalose and proline. There was a significant accumulation of ethylene in salt stressed barley plants. Moreover, salinity stress resulted in a significant decline of nitrate content and nitrate assimilating enzymes activity; nitrate reductase (NR) and glutamine synthetase (GS) while ammonia content was significantly increased in the roots. Inoculation of plants with *Bacillus subtilis* mainly improved the growth of salt stressed barley plants via protecting the cellular membranes integrity and increasing NR and GS activities as well as supplying a growth hormone indole-3-acetic acid/indole acetic acid (IAA) to the cultures and reducing the generation of ethylene under salt stress through the secretion of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which might increase nutrient uptake and growth. Hence, *B. subtilis* could promote the growth of barley plants under salinity through secretion of extra amount of IAA.

Key words: *Bacillus subtilis*, Barley, Ethylene, Salinity, Trehalose.

Abbreviations: f.m. and d.m.: Fresh and dry biomasses, GS: Glutamine synthetase, NR: Nitrate reductase.

Introduction

Barley (*Hordeum vulgare* L.) is an extremely adaptable cereal grain and positioned the fifth among all yields for dry matter construction in the world. Furthermore, it is an essential nutrient for protein. Although barley is considered as salt tolerant among crop plants, its growth and improvement are extremely affected by osmotic plus ionic stresses in salty soils (Mahmood, 2011). Soil salinity is a major destructive environmental stress, leading to reduction of crop quality and productivity (He et al., 2018). The reduction in growth biomarkers of plants subjected to salinity stress was related to the decrease of osmotic potential of the cells (Xu et al., 2016) and induction of lipoperoxidation damage of plasma membranes (Munns &Tester, 2008). Moreover, the induction of senescence hormone, as ethylene and disturbance of growth hormones (Greenberg et al., 2008; Bhattacharyya & Jha, 2012) may lead to decline of elongation and growth. Salinity induces several physiological

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and biochemical changes including disorder of cellular membranes, reduction of water and nutrient uptake, generation of reactive oxygen species (ROS), suppression of photosynthetic pigments and photosynthesis (Acosta-Motos et al., 2017). It is clearly shown that higher plants have developed complex adaptive mechanisms to shift off the inhibitory effect of biotic and abiotic stresses *via* biosynthesis of compatible osmolytes such as glucose, sucrose, trehalose, proline and quaternary ammonium compounds (Li et al., 2015). Sucrose and trehalose are non-reducing sugars existing in an extensive variety of organisms, these sugars might act as energy and carbon supply and as protective molecules against abiotic stresses (Mark et al., 2012). In addition, proline accumulation in salt-stressed plants may be considered as a defense mechanism causing osmotic adjustment (Szabados & Savour, 2010), energy and nitrogen source (Verslues & Sharma, 2010) and ROS scavenger (Yavuz, 2006). On the other hand, Ashraf & Aktar (2004) stated that the data do not always show a positive correlation between proline accumulation and salt stress. Meloni et al. (2004) mentioned that the accumulation of proline in *Prosopsis alba* was insignificantly affected by salinity stress. Many authors have reported that salinity stress exerts inhibitory effects on nitrogen metabolism *via* suppression of NO_3^- uptake and activities of N-assimilating enzymes (Hossain et al., 2012; Dai et al., 2015; Ashraf et al., 2018). Ehlting et al. (2007) mentioned that the decrease of NO_3^- accumulation might be related to the reduction of water absorption. Balliu et al. (2015) suggested that the disturbance of plasma membranes-associated NO_3^- transporters could result in an inhibition of NO_3^- uptake. Oukarroum et al. (2015) reported that the increase of ROS generation under salinity stress resulted in oxidative damage of nitrogen assimilating enzyme proteins and suppression of enzymes activity.

Biofertilizers prepared by combining PGPB with manures could enhance growth-promoting effects and bio-control of plants (Chen et al., 2011). *Bacillus* spp. is one of PGPB established to be an effective bio-control agents. The manner of action of PGPB is stimulating plant growth via (i) The ability to accelerate seed germination (Lugtenberg et al., 2002); (ii) Secretion of plant growth regulators; (iii) Producing siderophores; (iv) Producing unstable organic compounds; (v) Uptake of nutrients by plants; (vi) Construction of protective enzymes for example chitinase, glucanase, and ACC-deaminase for the inhibition of plant diseases and (vii) Abiotic stress tolerance in plants (Kumar & Verma, 2017). Plant growth promoting rhizobacteria ,which have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, enable plant growth and improvement by reducing ethylene levels through deaminating the precursor ACC and transform it into 2-oxobutyrate besides ammonium (Glick, 2014). Currently, microbial species displaying ACC deaminase activity have existed in many kinds such as *Achromobacter, Acinetobacter, Azospirillum, Agrobacterium, Alcaligenes, Bacillus, Ralstonia, Burkholderia, Pseudomonas, Enterobacter, Rhizobium* and *Serratia* etc. (Kang et al., 2010). It has been reported that inoculation of definite PGPB strains helps in improvement the growth of several glycophytes subjected to salt stress because they induce the level of osmoprotectants biosynthesis (Ramezani et al., 2011), promote the selectivity of Na^+, K^+ and Ca^{+2} and sustain high K^+/Na^+ ratio (Hamdia et al., 2004). Moreover they enhance the production of non-enzymatic antioxidants like phenolics (Sharma & Sharma, 2017).

The aim of this study is to shed light on the role of PGPB *Bacillus subtilis* in amelioration the growing of barley plants under NaCl stress.

Materials and Methods

Barley (*Hordeum vulgare* L. cv. Minorimugi) grains were kindly supplied by Niigata, Japan. *Bacillus subtilis* was used as an example of plant growth-promoting bacteria (PGPB) and obtained from Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt.

Characterization of B. subtilis for PGPB traits

Production of indole-3-acetic acid

Its production was detected as described by Rahman et al. (2010).

ACC-deaminase activity

Its assay was done on the basis of the ability of *B. subtilis* to use 1-aminocyclopropane-1-carboxylate (ACC) as a sole N source according to the method described by Ali et al. (2014).

Production of ammonia

B. subtilis was tested for ammonia production in peptone water as described by Cappuccino & Sherman (1992).

Preparation of bacterial inoculum

B. subtilis cultures were prepared 48hrs. prior to inoculation. The densities of bacterial suspensions used for the experiment were adjusted to an absorbance of 1 at 600nm using PBS which was corresponding to an approximate concentration of 10^9 colony-forming unit (CFU) ml^{-1} (Nabti et al., 2014).

Bacterial inoculation

The sterilized barley grains (positive control) were immersed in the bacterial inoculum as mentioned by Mohamed & Gomaa (2012) to enable the adherence of bacteria to the grains for 2hrs. at room temperature. For the non-inoculated treatment (negative control), the sterilized grains were germinated directly without immersion.

Experimental conditions

Air-dried barley grains were grown in plastic containers filled with 0.1mM $CaSO_4$ solution and placed in a growth chamber at Faculty of Agriculture, Niigata University, Japan, under complete darkness (temperature 25°C±2). After 2 days, light was turned on (16L/8D, light intensity of 200µ mol m^{-2} s^{-1}). At the sixth day from the beginning of the experiment, germinated grains were transferred to hydroponic cultures. Supplementation with salt (100mM NaCl) was done to the hydroponic cultures only for plants of stress conditions. The cultures were divided into four treatments as following; *set I*, sterilized non-inoculated grains and supplied with hydroponic solution described as negative control (C); *set II*, sterilized non-inoculated grains and supplied with hydroponic solution supplemented with 100mM NaCl solution (C+NaCl); *set III*, inoculated grains in bacterial suspension of *Bacillus subtilis* and supplied with hydroponic solution described as (*B. subtilis*) and *set IV*, inoculated grains in bacterial suspension of *Bacillus subtilis* and supplied with hydroponic solution supplemented with 100mM NaCl solution (*B. subtilis*+NaCl). Harvesting was done after 10 days.

Growth parameters

Shoots and roots of homologous seedlings (three triplicates) from each treatment were weighed for the determination of fresh biomass and measurement of plant length. The freeze-dry biomass was determined subsequently after drying the samples in a lyophilizer and stored at -30°C.

Ethylene measurement

The estimation of ethylene concentration was carried out using GC- 17A gas chromatograph that was equipped with Porapak-N (HP-GL-science, 80-100 mesh, 1mm) and a hydrogen flame ionization detector (Cheng et al., 2012). The ethylene concentration in the gas samples was determined by calibrating the peak area to a standard curve produced with known ethylene concentration. Ethylene production was estimated as nmol ethylene $100g^{-1}$ f.m.

Determination of glutamine synthetase activity (GS, EC 6.3.1.2)

Extraction

According to the method of Pérez-Soba et al. (1994).

Enzyme assay

Glutamine synthetase (GS) activity was assayed according to the method of Nievola et al. (2001). The absorbance was measured in a spectrophotometer (UV/visible; Ultrospec 3300 pro, GE Healthcare UK Ltd.) at 540nm with reference to a standard curve prepared from known concentrations of L-glutamic acid γ-monohydroxamate. The GS activity was expressed as µmol glutamyl hydroxamate min^{-1} g^{-1} d.m.

Determination of nitrate reductase activity (NR, EC 1.6.6.1)

Extraction

According to the method of Abdel-Latif (2005), the NR activity was assayed in a reaction mixture containing 50mM Hepes-KOH (pH 7.7), 5mM KNO_3, 1mM EDTA, 0.2mM β-NADH, 5µM leupeptin and 10µM FAD (Dailey et al., 1982). The reaction began by adding 100µL of crude extract to 450µL of the reaction buffer in each tube. After 15min, the reaction was stopped by adding 50µL of 1M zinc acetate solution, and centrifugation at 14,000g for 10min at 4°C. Equal volumes of 1% (w/v) sulfanilamide in 3N HCl and 0.02% (w/v) N-naphthylethylenediamine dihydrochloride were added to the supernatant. The tubes were kept at room temperature for 20min, then the absorbance was read at 540nm using spectrophotometer (UV/visible; Ultrospec 3300 pro, GE Healthcare UK Ltd.) with reference to a standard curve prepared from known concentrations of $NaNO_2$. The NR activity was expressed in µmol NO_2 h^{-1} g^{-1} d.m.

Determination of nitrate, trehalose, sucrose proline and ammonia

Extraction according to the method of Chow & Landhausser (2004)

Determination of nitrate

Nitrate was determined by capillary electrophoresis (Quanta 4000E, Millipore Corp., MA, USA), as described by Kawachi et al. (2002)

Determination of trehalose and sucrose

Preparation of derivatization legends was done according to the method of Ruiz-Matute et al. (2011) and the determination was done using gas chromatography/ Mass Spectrometry (GC/ MS Shimazu GC-2014, GLS Cat. No. 1010-18642, Niigata, Japan). Data were collected and processed using Chromato-PRO software (Que´ro et al., 2012). Trehalose content was expressed as µmol $100g^{-1}$ d.m., however sucrose content was expressed as µmol g^{-1} d.m.

Determination of proline and ammonia using ultra-high-pressure liquid chromatography (UPLC) analysis

Chemicals and reagents: Waters AccQ Fluor Reagent Kit was used as a derivatization reagent for proline and ammonia analysis. The kit consists of three vials. Vial 1; borate buffer to the proper pH environment for the derivatization, vial 2A; 6-aminoquinolyl-N-hydroxysuccinimidyl-carbamate (AQC) reagent, vial 2B; the diluent acetonitrile to reconstitute the reagent for derivatization. One ml of vial 2B diluent was added to vial 2A reagent powder and heated at 55°C using dry heater for 7 min with continuously stirring by vortex until the solution became clear.

Method: Determination of proline and ammonia content was performed according to the method of Nagumo et al. (2009). Empower 2 Chromatography Data Software (Waters, Milford, MA, USA) was used for data acquisition, processing and for regulating the UPLC system. Proline content was calculated as µmol $100g^{-1}$ d.m., however ammonia content was calculated as µmol g^{-1} d.m.

Statistical analysis

All treatments were replicated three times and results are given as mean. Data for all attributes were statistically analyzed by analysis of variance (ANOVA) test and the mean values were matched with least significance difference (LSD) following a Japanese online program (js-STAR) version 9.0.6j Programing by Satoshi Tanaka and nappa (Hiroyuki Nakano).

Results

Qualitative estimation of bacterial products

Ammonia, IAA and ACC deaminase enzyme were qualitatively estimated in bacterial culture of *B. subtilis*. The results present in Table 1 show that *B.subtilis* has the ability to secrete IAA, ammonia and ACC-deaminase in culture medium.

Changes in fresh and dry biomasses and plant length

Supplementation of the nutrient medium with 100 mM NaCl resulted in a significant decrease in fresh mass (f.m) and dry mass (d.m) in shoots and roots of barley plants, compared to control. This was accompanied with a significant decrease of plant length (Table 2). The decrease in f.m. of 100 mM NaCl-stressed shoots and roots was 43% and 36%**,** respectively**,** compared to non-salinized control. The corresponding values for d.m. were 34% and 12%, respectively. While the decline of plant length reached 23% of the non- salinized control. It is noteworthy that co-inoculation of barley grains with *B. subtilis* significantly increased f.m., d.m. and plant length of the non-salinized and salinized barley plants, compared to controls (Table 2). The increase of f.m. in shoots and roots of *B.subtilis*- inoculated plants was 16% and 21% respectively, compared to the non-salinized control. Whereas, the increase of f.m. in shoots and roots of inoculated barley plants with *B. subtilis* in presence of NaCl was 28% and 46%, respectively, compared to bacterial untreated plants. The increase of plant length of *B.subtilis*-inoculated barely plants in presence of NaCl was 47%, compared to those of the non-inoculated plants (Table 2).

TABLE 1.Qualitative estimation of ACC deaminase, IAA and ammonia of *B.subtilis*

Bacterial strains	**Bacterial characterization**		
	ACC-deaminase activity	**IAA production**	**Ammonia production**
Bacillus subtilis	+	+	++

+ Means that these compounds were produced by *B.subtilis*

TABLE 2. Changes in fresh and dry biomasses (f.m. and d.m.) (mg plant^{-1}) and plant length (cm) of *B.subtilis*-inoculated barley plants in absence and presence of 100mM NaCl

Treatment	f.m.		d.m.		Plant length
	mg plant^{-1}				cm
	Shoot	Root	Shoot	Root	
C	258.4^{a}	77.3^{ad}	20.2^{a}	6.0^{a}	22.0^{a}
C+NaCl	147.6^{b}	49.6^{b}	13.4^{b}	5.3^{b}	16.9^{b}
B.subtilis	301.0^{c}	93.2^{c}	22.5^{c}	6.9^{cd}	31.5^{c}
B.subtilis+NaCl	189.1^{d}	72.5^{d}	16.5^{d}	6.9^{d}	24.9^{d}

LSD: Means indexed by the same superscript are not significantly different at P≤0.05.

Changes in sucrose content

As compared to control, inoculation with *B. subtilis* insignificantly changed sucrose content in the shoots and roots of 10-d old barley plants, compared to control. Additionally, supplementation of the growth medium with NaCl in absence or presence of *B. subtilis* resulted in a significant increase of sucrose accumulation in shoots and roots of barley plants, compared to those of the controls in absence of NaCl (Table 3). The sucrose content in shoots and roots of NaCl-stressed barley plants was 1.5- and 2.6-fold, respectively, compared to the non-salinized control. Moreover, the sucrose content in shoots and roots of *B. subtilis*- was 2.4- and 3.8-fold of those of the non-salinized control. It is noteworthy that inoculation with *B. subtilis* in presence of NaCl significantly increased sucrose content in shoots and roots of barley plants, compared to those of the non inoculated ones. The sucrose content of shoots and roots of salinized *B. subtilis*-inoculated plants was 1.6- and 1.5-fold, respectively, compared to NaCl- salinized plants.

Changes in trehalose content

Results in Table 3 show that trehalose content in shoots and roots of *B. subtilis*-treated barley plants was insignificantly changed in absence of salt stress. Addition of NaCl with the growth medium significantly increased trehalose accumulation in the shoots and roots, in comparison to unstressed plants. The trehalose content in shoots and roots of NaCl-stressed plants was 1.5-and 4.1-fold, respectively, compared to unstressed control. It is interesting to demonstrate that *B. subtilis* application resulted in a significant increase in trehalose content in shoots and roots of salt-stressed barley plants, compared to those grown under salt treatment only. The trehalose content in shoots and roots of *B. subtilis*- inoculated NaCl-stressed plants was 1.5- and 1.1-fold , respectively of those in the non-inoculated. In addition, inoculation with *B.subtilis* induced accumulation of trehalose in NaCl barley plants, compared to the non-salinized ones. The trehalose content in shoots and roots of *B.subtilis* inoculated-salinized barley was 2.6- and 3.5-fold, respectively compared to those inoculated in absence of NaCl (Table 3).

Changes in proline content

There was a significant increase of proline content in *B. subtilis*-inoculated shoots and roots of barley plants, in comparison to controls. In absence of NaCl, the proline content in shoots and roots of *B. subtilis* inoculated -plants was 2.4-and 2.2-fold, compared to control (Table 3). It is clearly noted that supplementation of the nutrient medium with NaCl significantly enhanced the accumulation of proline in only roots of barley plants compared to those in non-salinized control. The proline content in shoots and roots of NaCl-treated plants was 1.2-and 8.9-fold , respectively of unstressed non-inoculated plants. It is noteworthy that proline content of *B. subtilis*-inoculated plants was significantly increased in response to NaCl stress, compared to those of non-inoculated or inoculated ones. The proline content in shoots and roots of *B.subtilis* inoculated-NaCl-stressed plants was 3.4-and 1.9-fold, respectively of NaCl- stressed control. In addition, the proline content in shoots and roots of NaCl-stressed-*B.subtilis* inoculated plants was 1.7-and 7.7-fold, respectively of the unstressed-inoculated ones.

Changes in ethylene content

It is clearly shown that the, inoculation of barley grains with *B.subtilis* resulted in a significant decrease of ethylene concentration in the shoots and roots, compared to control plants. The ethylene content in shoots and roots from *B.subtilis*- treated barley plants was 67% and 54%, respectively, compared to control ones (Fig.

1). On the other hand, addition of 100mM NaCl with growth media significantly induced ethylene accumulation in shoots and roots of barley plants, compared to the non-salinized control. The ethylene content in salt stressed shoots and roots was 2.9- and 3.7- fold, respectively of the control. (Fig. 1). Inoculation of barley grains with *B.subtilis* as PGPB and grown in the salinized nutrient medium significantly suppressed the ethylene content in the shoots and roots, but the attained values were markedly higher than those of the inoculated- non-salinized plants. The percentage of decline of ethylene content in shoots and roots of *B. subtilis*-inoculated plants in presence of NaCl was 73% and 50%, respectively, compared to the NaCl-stressed control.

Changes in nitrate content

Salinization of the growth media of *B. subtilis*-inoculated or non-inoculated grains significantly decreased the content of nitrate in shoots and roots of hydroponically grown barely plants, compared to the non-salinized controls; and this suppression in the *B. subtilis*-inoculated plants was markedly lower than those in the non-inoculated ones (Table 4). The decrease of nitrate content in shoots and roots of NaCl- stressed plants was 58% and 77%, respectively, in comparison with control. Whereas, this reduction in shoots and roots of salinized plants from *B. subtilis*-inoculated was 24% and 55%, in comparison to those grown in absence of NaCl, respectively.

Changes in ammonia content

It is clearly demonstrated that ammonia content in shoots of *B. subtilis*-inoculated or non-inoculated barley plants was insignificantly changed in response to the presence of 100mM NaCl with the growth media (Table 4). On the other hand, there was a significant increase of ammonia content in roots of NaCl-stressed inoculated or non-inoculated plants, compared to controls. The increase of ammonia content in roots of NaCl-salinized-non-inoculated and -inoculated barley plants was 47% and 35%, respectively, compared to controls.

Changes in nitrate reductase activity

It was obviously demonstrated that, there was an insignificant change of nitrate reductase (NR) activity in shoots and roots of *B. subtilis*-inoculated barley plants, compared to control under non-salinized conditions (Table 5). Supplementation of the culture medium with NaCl significantly decreased NR activity in the non-inoculated shoots; the suppression of NR activity in salt-stressed shoots was 47%, compared to the non-salinized control. Inoculation with *B. subtilis* significantly increased NR activity in shoots and roots of NaCl-salinized barley plants, in comparison to the non-inoculated salinized ones. The increase of NR activity in shoots and roots of *B. subtilis*-inoculated-NaCl-stressed plants was 23% and 9% respectively, compared to the non-inoculated-salt stressed ones.

Changes in glutamine synthetase activity

Application of NaCl to the hydroponic growth media of non-inoculated barley grains significantly decreased glutamine synthetas (GS) activity in the shoots and roots, compared to the non-salinized controls. The decrease of GS activity is obvious in roots more than in shoot; the decline of GS activity in shoots and roots of NaCl-stressed 10-d barley plants was 12% and 53%, respectively, compared to the non-stressed control (Table 5). It is interesting to demonstrate that GS activities in shoots and roots of barley plants from *B. subtilis*- inoculated grains and subjected to NaCl was significantly higher than those of the non-inoculated plants. The increases of GS activity in shoots and roots of *B. subtilis*- inoculated stressed plants were 13% and 21%, respectively, compared to those subjected to NaCl stress.

TABLE 3. Changes in sucrose (µmol g^{-1}d.m.), trehalose (µmol 100 g^{-1}d.m.) and proline (µmol 100 g^{-1} d.m.) content in shoots and roots of *B. subtilis*- inoculated barley plants in absence and presence of 100 mMNaCl

Treatment	Sucrose µmol g^{-1} d.m.		Trehalose µmol 100g^{-1}d.m.		Proline µmol 100g^{-1} d.m.	
	Shoot	Root	Shoot	Root	Shoot	Root
C	14.42^{ac}	12.38^{ac}	40.57^{ac}	95.29^{ac}	12.99^{ab}	2.81^{ac}
C+NaCl	22.15^{b}	32.23^{b}	60.27^{b}	393.22^{bd}	15.69^{b}	25.14^{b}
B. subtilis	13.78^{c}	9.59^{c}	34.11^{c}	124.49^{c}	30.76^{c}	6.27^{c}
B. subtilis+NaCl	34.41^{d}	47.52^{d}	89.38^{d}	438.48^{d}	52.87^{d}	48.29^{d}

LSD: Means indexed by the same superscript are not significantly different at P≤0.05.

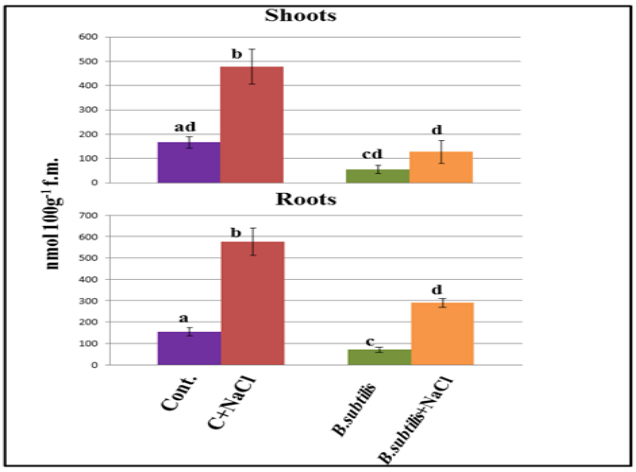

Fig. 1. Changes in ethylene content (µmol 100g^{-1} f.m.) in shoots and roots of *B. subtilis*- inoculated barley plants in absence and presence of 100mM NaCl

TABLE 4. Changes in nitrate (µmol g^{-1}d.m.), and ammonia (µmol g^{-1}d.m.), content in shoots and roots of *B. subtilis*- inoculated barley plants in absence and presence of 100mM NaCl

Treatment	Nitrate µmolg^{-1}d.m.		Ammonia µmolg^{-1}d.m.	
	Shoot	Root	Shoot	Root
C	1411.53^{ac}	680.13^{ac}	14.30^{a}	13.71^{a}
C+NaCl	596.31^{b}	157.69^{b}	13.46^{a}	20.09^{b}
B.subtilis	1450.67^{c}	684.97^{c}	12.94^{a}	14.89^{c}
B.subtilis+NaCl	1069.59^{d}	304.20^{d}	13.52^{a}	18.55^{d}

LSD: Means indexed by the same superscript are not significantly different at P≤0.05.

TABLE 5. Changes in nitrate reductase (NR) (µmol NO_2 h^{-1} g^{-1}d.m.), and glutamine synthetase (GS) (µmol glutamyl hydroxamate min^{-1} g^{-1}d.m.), content in shoots and roots of *B. subtilis*- inoculated barley plants in absence and presence of 100mM NaCl

Treatment	Nitrate reductase µmol NO_2 h^{-1} g^{-1} d.m.		Glutamine synthetase µmol glutamyl hydroxamate min^{-1} g^{-1} d.m.	
	Shoot	Root	Shoot	Root
C	21.91^{ac}	5.48^{ab}	13.73^{acd}	5.73^{a}
C+NaCl	11.68^{b}	5.86^{b}	12.07^{b}	2.68^{b}
B.subtilis	20.64^{c}	4.97^{c}	14.91^{ce}	4.67^{c}
B.subtilis+NaCl	14.32^{d}	6.37^{d}	13.60^{de}	3.24^{d}

LSD: Means indexed by the same superscript are not significantly different at P≤0.05.

Discussion

As shown in the results, supplementation of the growth medium with 100mM NaCl significantly decreased the plant length and fresh and dry biomassas of barley plants. Many authors have reported that the application of NaCl significantly decreased the growth of several glycophytes including maize (Abbasi et al., 2012), tomato (Ezzat et al., 2015) and bean cultivars (Ismail et al., 2017). Gill & Tuteja (2010) reported that salt stress enhances the generation of ROS and lipooxygenation of plasma membranes causing the degradation of several cellular components. In addition, Xu et al. (2016) concluded that, there was a marked decline of leaf water potential due to decline of water uptake under prolonged salinity stress. Whereas, Ahmad et al. (2013) suggested that salt stress might disorder phytohormone synthesis and induce ethylene synthesis

(senescence hormone) leading to reduction of elongation , and growth in addition to increase of defoliation of plants. In accordance with these views, the suppression in growth of NaCl-stressed barley plants, under this study, might be attributed to the destruction of plasma membranes integrity, which resulted in an inhibition of water and nutrients transportation as well as decreasing the biosynthesis of growth phytohormones and an increase of endogenous ethylene accumulation. It is seen that salinization of the growth medium with NaCl significantly increased ethylene accumulation in shoots and roots of barley plants (Fig. 1) and that was associated with a significant reduction of plant length and growth. Conversely, priming inoculation of barley grains with *B. subtilis* significantly increased the growth of both salinized and non-salinized plants compared to controls. These observations were accompanied with a significant decline of ethylene contents in the shoots and roots of these plants (Fig. 1). It has been attained that a wide range of PGPB can secrete ACC deaminase which suppress ethylene biosynthesis (Zahir et al., 2009; Kang et al., 2010). Timmusk et al. (2014) stated that *B.subtilis* strains are considered as IAA-producers to the rhizosphere. Therefore, the improvement of barley plant growth raised from *B.subtilis* inoculated seeds, in this study, might be attributed to absorption of IAA from rhizosphere reduction of ethylene biosynthesis. Results in Table 1 clearly demonstrated that *B. subtilis* can secrete IAA and ACC deaminase to the surrounding media. Salinization of the growth media with NaCl resulted in a significant increase of sucrose, trehalose, and proline accumulation in shoots and roots of barley plants, compared to controls. These findings might reflect the role of these soluble organic solutes as osmoprotectants (Szabados & Savouré, 2010; Hassan & Ali, 2014) and/or ROS scavengers (Yavuz, 2006; Nishizawa et al., 2008). It is seen that the accumulation of these solutes and growth biomarkers in salinized plants raised from *B. subtilis*-inoculated grains were significantly higher than those of the non-inoculated ones. Thus, these observations might indicate the involvement of *B. subtilis* in the alleviation of the inhibitory effect of NaCl stress on the growth of barley plants via improvement of plasma membranes integrity from lipoperoxidation and increase of water potential resulting in an increase of water status of cells. It is well known that salinity stress exerts several inhibitory effects on nitrogen metabolism through suppression of NO_3^- uptake and N-assimilating enzymes activities (Dai et al., 2015; Ashraf et al., 2018). In agreement with these views, application of NaCl significantly decreased NO_3^- content in shoots and roots of barley plants and increased NH_4^+ accumulation only in the roots. These findings were associated with a significant reduction of NR and GS activities. Ehlting et al. (2007) and Balliu et al. (2015) mentioned that the decrease of NO_3^- accumulation under saline conditions might be attributed to the reduction of water absorption and disruption of plasma membrane–associated NO_3^- transporters, and that might relate to the increase of the generation of ROS and oxidative stress (Oukarroum et al.,2015). Thus, the decline of NO_3^- content in NaCl-salinized barley plants, under this study, might be related to enhancement of ROS generation and lipooxygenation of plasma membrane which cause serious oxidative damages on NO_3^- carrier and disturbance of plasma membranes integrity; finally reduce water and NO_3^- uptake. Moreover, Meloni et al. (2004) and Debouba et al. (2007) reported that NR is an inducible enzyme, and the suppression of NR activity may be related to the degradation of the enzyme protein and restriction of NO_3^- uptake (Ashraf et al., 2018). Therefore, the suppression of NR activity in shoots of NaCl-stressed plants could be attributed to the decline of NO_3^- content, disorder of NR enzyme protein by generated ROS. Seong et al. (2007) stated that, there was a marked induction of plasma membrane-associated NADPH-oxidases under various stresses which use O_2 as H-acceptor. Thus, under this study, the competition between NR and NADPH oxidase on the source of H-donor (NADPH) might result in a decrease of NR activity in shoots of NaCl-stressed barley plants. There was a significant accumulation of NH_4^+ in roots of NaCl-treated barley plants that was accompanied with a significant decline of GS activity, revealing the inhibitory effect of salinity on NH_4^+-assimilating enzymes. Hossain et al. (2012) reported that salinity stress resulted in a marked suppression of GS/GOGAT activities, and this might be related to the reduction of the enzymes biosynthesis (Wei et al., 2008) and decline of enzyme protein (Plaza et al., 2009) as well as low glutamate availability (Kawakami et al., 2013). In addition, Khadri et al. (2001) stated that salt stress stimulates NAD-Glutamate dehydrogenase (NAD-GDH), which deaminates glutamate to α- ketoglutarate and NH_4^+. In this study, there was a marked decrease

of GS activity in NaCl-stressed barley roots, compared to control. Thus, the accumulation of NH_4^+ in roots might be considered as a toxic component resulting in the disturbance of barley growth. On the other hand, inoculation of barley grains with *B. subtilis* significantly increased NO_3^- accumulation in shoots and roots of NaCl-salinized plants, relative to those of the non-inoculated ones. These results were in accordance with various reports recorded by many authors (Baniaghil et al., 2013; Kang et al., 2014). The increase of NO_3^- content and its assimilating enzymes (NR, GS) in this study, in plants raised from *B. subtilis*-inoculated barley grains subjected to NaCl was associated with a significant increase of growth. Kumar & Verma (2017) reported that PGPB decline the generation of ROS and oxidative stress in plants exposed to various environmental stresses by enhancing the antioxidant enzyme activities, and expression of some gene coding proteins.

Conclusion

Priming inoculation of barley grains with *Bacillus subtilis* (as PGPB) might improve the growth of plants subjected to NaCl stress. The important roles of *Bacillus subtilis* could be related to improvement of plasma membranes integrity leading to enhancement of water and NO_3^- uptake by root cells, as well as protection of the cellular metabolic homeostasis *via* reduction of ROS generation and increasing of NR and GS activities. Moreover, the increase of barley growth and elongation might be resulted from the uptake of secreted IAA by bacteria and/or degradation of ethylene by bacterial ACC-deaminase enzyme.

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تأثير البكتيريا المحفزة للنموعلى بعض العمليات الفسيولوجية و البيوكيميائية لنبات الشعير (*Hordeum vulgare* L.) تحت تاثير الاجهاد الملحى

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تهدف هذه الدراسة إلى تأثير الاجهاد الملحى على بعض مؤشرات النمو، والتغيرات فى بعض العمليات الفسيولوجيه والبيوكيميائيه لنبات الشعير. أيضا تشير الدراسة إلى تأثير البكتيريا المحفزة للنمو PGPB- *Bacillus subtilis* على تقليل التأثير الضارللملوحة على نمو وتطور النبات. أدى نمو النباتات على بيئة سائلة تحتوى على 100 مللى مول من كلوريد الصوديوم إلى انخفاض معنوى فى نمو نبات الشعير مصحوبا بزيادة معنوية لمركبات حفظ التوازن الاسموزى مثل السكروز، التريهالوز والبرولين. أظهرت الدراسة زيادة معنوية فى تراكم هرمون الايثيلين في نبات الشعير النامي تحت الاجهاد الملحي. كما أدت الملوحة إلى نقصا معنويا فى محتوي النترات ونشاط إنزيمات تمثيل النترات فى وجود زيادة معنوية للأمونيا فى الجذور. نتج عن معالجة البذور ببكتيريا *B. subtilis* فى ظروف الاجهاد الملحى تحسين نمو نبات الشعيرمن خلال حماية الأغشية البلازمية وزيادة نشاط إنزيم النيترات ريدكتيز (NR) وإنزيم الجلوتامين سينسيتيز(GS) بالإضافة إلى أن وجود البكتيريا يدعم البيئات السائلة بهرمون إندول حامض الخليك الذى يساعد على الحد من تخليق هرمون الايثيلين من خلال افراز البكتيريا لأنزيم deaminase (ACC) وذلك تحت الاجهاد الملحى والذى قد يؤدى بدوره إلى زيادة امتصاص العناصرومن ثم زيادة النمو. ومن هنا قد تلعب بكتيريا *B . subtilis* دورا في تحفيز نمو نبات الشعيرالنامى تحت الاجهاد الملحى من خلال زيادة محتوى إندول حامض الخليك.