sciendo

Hellenic Plant Protection Journal **16:** 12-22, 2023
DOI 10.2478/hppj-2023-0002

Unraveling the role of endophytic fungi in barley salt-stress tolerance

R. Kouadria[1,2*], M. Bouzouina[2], B. Lotmani[2] and S. Soualem[3]

Summary Salinity is an agricultural and eco-environmental problem worldwide that decreases crop production. Endophytic fungi have been shown to improve plant tolerance to stressful conditions. The purpose of the paper is to examine the efficiency of *Embellisia phragmospora*, *Fusarium equiseti* and *Fusarium graminearum* to improve tolerance of pot-grown barley in greenhouse under different levels of soil salinity (2.5, 8 and 14dS/m) by estimating growth, relative water content, mineral nutrition, photosynthetic pigments biosynthesis, proline and sugar levels. Results showed that *E. phragmospora* increased barley emergence rate to 66.7% compared to 60% recorded by non-colonized barley under 14dS/m soil salinity. The tested endophytes increased barley root length, shoot and root dry weights under salt stress. Endophytic fungi reduced Na^+ accumulation and improved K^+ uptake in barely under salinity. *Fusarium equiseti* and *F. graminearum*-inoculated barley increased proline content under salinity. *Fusarium graminearum*-colonized barley showed the highest sugar content under salt stress. Our findings demonstrate the feasibility of endophytic fungi bio-inoculation in improvement of barley tolerance to salt stress, which qualify them to be a potent tool to provide substantial benefits to crops for sustainable agriculture.

Additional keywords: Barley, Bio-inoculation, Endophytic fungi, Salt stress, Tolerance

Introduction

Among the abiotic stresses, salinity is one of the major problems that affect crop growth and development (Ruiz-Lozano *et al*., 2012). Wang *et al*. (2003) suggested that increased salinity of agricultural land is expected to have damaging effects, resulting in 30% land loss within the next 25 years and up to 50% before the end of 21st century.

Salt-stressed plants suffer from K^+ deficiency and Na^+ toxicity which disrupts photosynthesis, enzymes activity, protein biosynthesis (Gupta and Huang, 2014) and accumulation of osmolytes, such as proline and sugars (Majumder *et al*., 2010).

Barley (*Hordeum vulgare* L.), the most important cereal crop in the world, is considered to be moderately salt sensitive (Munns *et al*., 2006). Barley yield damages in saline areas are significant; therefore salt stress tolerance is a widely sought quality in order to extend its cultivation to unfavorable regions (Baenziger *et al*., 2006).

Desalination and deployment of salt tolerant barley varieties can both be used to combat salinity stress. However, development of salt tolerant varieties through traditional breeding or advanced molecular techniques is time consuming and highly expensive. Quite recently, considerable attention has been paid to identification of alternative methods to enhance plant productivity under abiotic stresses (Wei and Jousset, 2017). Endophytic fungi have been gaining importance in recent years to explain their role in reducing negative effects of environmental stresses, such as salinity (Aghilia *et al*., 2014).

Munns and Tester (2008) showed that phytohormones are regulated by signaling genes involved in salt and osmotic stress alleviation under salt stress conditions. Leitão and Enguita (2016) have demonstrated that endophytic fungi can synthesize phytohormones such as gibberellins, auxins (IAA) and

[1] Higher School of Agronomy, Kharrouba-Mostaganem 27000.
[2] Plant Protection Laboratory, Abdelhamid IBN Badis - Mostaganem University, Kharrouba, 27000 Mostaganem, Algeria.
[3] Ibn Khaldoun University - Tiaret 14000.
* Corresponding author: rabia-rk@hotmail.com
ORCID: https://orcid.org/0000-0003-2632-0937

© Benaki Phytopathological Institute

abscisic acid (ABA) to improve plant tolerance under stressful conditions. As reported by Hasan (2002) the gibberellin produced by *Fusarium* spp. under salt stress conditions can reduce the negative effect of salinity and promote plant growth.

One of the examples of the feasibility of endophytic fungi to increase plant tolerance to salt stress was presented by Gill *et al*. (2016) reporting that the endophytic basidiomycete, *Piriformospora indica*, can colonize plants roots and improve plant tolerance under salinity. Furthermore, it has been shown that *Piriformospora indica* avoids Na^+ uptake and its translocation to leaves and increases K^+ uptake under salt stress (Yun *et al*., 2018). Endophytic fungi increased proline (Yang *et al*., 2004) and sugar content (Robert-Seilaniantz *et al*., 2007) as an osmoprotectant under salt stress conditions.

Positive impact of endophytes on wheat plants under salt stress conditions was evaluated, *in vitro*, in our previous study (Kouadria *et al*., 2018). The study showed that endophytic fungi improved wheat seedlings germination and growth under saline conditions (400, 600 and 800 meq/l NaCl) compared to non-inoculated plants where germination was inhibited under 600 and 800 meq/l NaCl. Moreover, the endophytic fungus *Chaetomium coarctatum* has been shown to improve salt stress tolerance of barley plants (Kouadria *et al*., 2020).

The paper aims to give comprehensive account of the ability of endophytic fungi to improve barley agricultural traits under salt stress. The ability of endophytic fungi to increase barley salt stress tolerance was assessed through measuring growth, relative water content, photosynthetic pigments biosynthesis, mineral nutrition and osmoregulation under both saline and non-saline conditions.

Materials and Methods

Spore suspension preparation

Fungi strains used in this research, *Embellisia phragmospora*, *Fusarium equiseti* and *Fusarium graminearum*, were isolated from *Beta macrocarpa* Guss., *Salsola oppositifolia* Desf., and *Lolium rigidum* Gaudin, respectively (Kouadria *et al*., 2019).

Isolates were allowed to grow and sporulate on PDA medium. Cultures were incubated at 28-30°C for 7 days. Spores were scraped off from the agar surface with distilled water and suspended in 0.05% Tween 80 solution. The spore suspension was then collected in a sterile test tube and shaken for 2 min. The suspension was filtered through a double layered mesh serially diluted and its concentration was determined microscopically with Hemocytometer (Akagi *et al*., 2015).

Endophytes-barley association – Experimental set up

Barley (*Hordeum vulgare* var. Saida 183) seeds were surface sterilized (5min) by sodium hypochlorite solution (NaClO, 5%) and distilled water. Then, seeds were inoculated by immersion in fungi spore suspensions (10^7 spores / mL) for 24 h. Non-inoculated seeds (control) were placed in distilled water. Seeds were pre-germinated in phytotron at 25°C for 24 h without substrate.

Soils used in the experiment are naturally salty soils, and their electrical conductivity was calculated by the National Institute of Soils, Irrigation and Drainage, El Matmar, Relizane, Algeria.

Pre-germinated seedlings were transferred to pots (19 cm in diameter and 50 cm in length), filled with autoclaved soil; unsalted soil with EC=2.5 dS/m, moderate and high saline soil with EC=8 dS/m and EC=14 dS/m, respectively. In each pot ten seedlings were planted with three replicates for each treatment (10 seedlings/pot and 3 pots/treatment). The treatments comprised controls and endophyte-inoculated wheat plants under non-saline conditions (2.5dS/m), controls for salt stress (8 and 14dS/m), and endophyte-inoculated plants under salt stress conditions (8 and 14dS/m). Measurements of EC were conducted at the end of the tests to confirm that soil salinity had not changed after the treatment period. The experimen-

© Benaki Phytopathological Institute

tal design was randomized complete block with four sets of treatments. The experiment was carried out under greenhouse conditions with 12 h light, relative humidity of 55–65% and 25/18 °C (day/night).

Measurements

Growth parameters

Barley seedling emergence rate was estimated 15 days after treatment. After 90 days of growth under salt treatments, barley seedlings were harvested at the heading stage. Root length and biomass production (root and shoot dry weights were measured after drying in oven 75°C for 72 h) were measured. Leaf area was calculated according to Li et al. (2004) using the following equation (5leaves/treatment):

$$LA\ (cm^2) = (MLL+MLW) \times 0.75$$

MLL: maximum leaf length. MLW: maximum leaf width.

Relative water content (RWC)

The RWC of each leaf was calculated according to Scippa *et al*. (2004) using the following equation:

$$RWC\ (\%) = [(FW-DW)/(TW-DW)]\times 100$$

where: FW is the fresh weight of the sampled leaf tissue. TW is the turgid weight of a leaf soaked in distilled water overnight at 4°C, and DW is the dry weight of that leaf after 48 h at 80°C.

Mineral nutrients

Potassium (K^+) and sodium (Na^+) contents were measured using diacid method (nitric acid and hydrochloric acid) according to Chapman and Pratt (1961). Leaves were dried in an oven at 80° C for 48 hours and then ground. After cooling, 50 mg of dry matter were placed in crucibles, and then put in a muffle oven at 600° C for 6 h. After cooling, the recovered ashes were subjected to an acid attack in 4 mL of 35% nitric acid, and the crucibles were placed on a sand bath until the organic matter was completely dissolved. After complete solvent evaporation, the mineral elements were suspended in 10 mL of 0.1 mol l^{-1} hydrochloric acid (HCl) and filtered with Wattman # 1 filter paper. Results were obtained after flame photometry analysis (model PFP7; Jenway, Stone, UK).

Chlorophyll and carotenoids content

Chlorophyll a (Chlo a), chlorophyll b (Chlo b), total chlorophyll (Chlo T) and carotenoids (CART), of fresh fully-expanded leaves were determined as described by Arnon (1949). Fresh leaf samples (0.1 g) were homogenized in a mortar and extracted with 5 mL acetone 80 % solution using Whatman No. 42 filter paper. The absorbance of the extract was recorded at 470 (A470), 663 (A663) and 645 (A645) nm wavelengths using a Jenway 67155 UV/Vis spectrophotometer.

Soluble sugar content

Soluble sugars were measured by the method of Schields and Burnett (1960). Fresh leaf tissue (100mg) was added to tubes containing 5.25 mL of ethanol 80% for 24 hours. The extract obtained was diluted 10 times with ethanol 80%. To 2 mL of the homogenate, 4 mL of anthrone reagent (2 g anthron + 1000 mL sulfuric acid (H_2SO_4)) was added. The anthrone reagent must be prepared 4 h before carrying out the tests. The homogenate was delicately mixed and kept in melting ice. Then the mixture was boiled in water bath at 92° C for 8 min, and then cooled for 30 minutes in the dark. Absorbance was measured at 585 nm. A standard curve was established using glucose and results are therefore expressed in mg/g of fresh weight (FW).

Proline content

The method of Troll and Lindsley (1955) was carried out to monitor the proline content. Fresh leaf tissues (100 mg) were homogenized in 2 mL methanol 40%. To 2 mL of homogenate, 2 mL of acid ninhydrin (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL of 6 mol l^{-1} phosphoric acid) and 2 mL of glacial acetic acid were added. The mixture was boiled in a water bath at 100°C for 60 min. The boiled mixture was then toluenized with 5 ml of toluene. Ab-

© Benaki Phytopathological Institute

sorbance was measured at 528 nm. Proline concentration was determined by following a calibration curve and expressed as mg/g of fresh weight (FW).

Data analysis

Experiments comprised of 10 plants per pot, while each treatment comprised of three replicates. Data were analyzed by means of 2-way ANOVA with 2 factors (fungal inoculation * salt concentration). For each trait an analysis of variance (ANOVA) was performed to compare endophyte-colonized plants in each treatment to their endophyte-free counterparts. Each ANOVA was followed by a post hoc Fischer's least significant difference (LSD) test. P-values less than alpha levels of 0.05 were considered significant. Statistical tests were run using Statbox v6.4 statistical software.

Results and discussion

Salinity is one of the critical abiotic factors that severely disturb plant growth and metabolism (Deinlein *et al*., 2014). Barley is an important cereal cultivated worldwide. However, its production is under threat due to ever increasing environmental stresses including salinity (Baenziger *et al*., 2006).

The results showed that salinity (*P <0.05,* Fig. 1A) and endophytic fungi (*P <0.05,* Fig. 1A) significantly affected the emergence rate of barley seedlings. Moreover, emergence rate of salt-stressed barley was increased by fungal endophytes (*P<0.05,* Fig. 1A). Barley plants had a high emergence rate (100%) in both colonized and uncolonized plants under non-saline (2.5dS/m) and moderate saline conditions (8dS/m). However, high salinity (14dS/m) reduced barley emergence rate for non-colonized barley (60%), *F. equiseti*, and *F. graminearum* colonized barley (36.7 and 53.3%, respectively), while *E. phragmospora* increased emergence rate to 66.7%.

Salinity significantly decreased root length and all tested endophytes had significant effects on root length (*P*<0.05, Fig. 1B). Root growth showed significant differences between non-inoculated barley plants and the inoculated ones (*P*<0.05, Fig. 1B). Salinity significantly decreased both root (*P*<0.05, Fig. 1C) and shoot (*P*<0.05, Fig. 1D) dry weights. The tested endophytic fungi had significant impact on root (*P>0.05*, Fig. 1C) and shoot dry weight (*P*<0.05, Fig. 1D), indicating that inoculations of barley by fungal endophytes increased root and shoot biomass produced by salt-stressed plants (*P*<0.05*,* Fig. 1C and D).

Overall, salinity reduced barley emergence, growth (root length and leaf area) and biomass production. The results are consistent with other studies which have shown that barley growth is negatively affected by increasing salinity levels (Mallek-Maalej *et al*., 2004; El Goumi *et al*., 2014). Moreover, Albacete *et al*. (2008) reported that salinity affects the cytokinins / auxins hormonal balance disturbing growth and biomass production. On the other hand, the current study supports the concept that beneficial endophytic association enhances plants growth and metabolism under salt stress. The tested endophytes promoted emergence rate, root length and biomass production (shoot and root dry weight) of barley and also helped the plants to tolerate elevated salt stress as compared to control plants.

Endophytic fungi have shown to control some physiological and biochemical processes under stressful conditions (Dardanelli *et al*., 2009). Hamayun *et al*. (2010) revealed that endophytic could reduce the negative effects of salinity and progress plant germination, emergence, growth, and biomass production, by synthesizing phytohormones (gibberellins: GA1, GA4, GA8 and GA9, indole-acetic acid: IAA and abscissic acid: ABA). Also, Yurieva *et al*. (2018) reported that *Penicillium funiculosum* stimulated soybean growth and improved the dry root biomass. Furthermore, Ahmad *et al*. (2015) showed that the increase in plant growth and biomass production after *Trichoderma spp*. inoculation may be due to its ability to produce gibberellins and cytokines, which cannot only promote growth but also pro-

© Benaki Phytopathological Institute

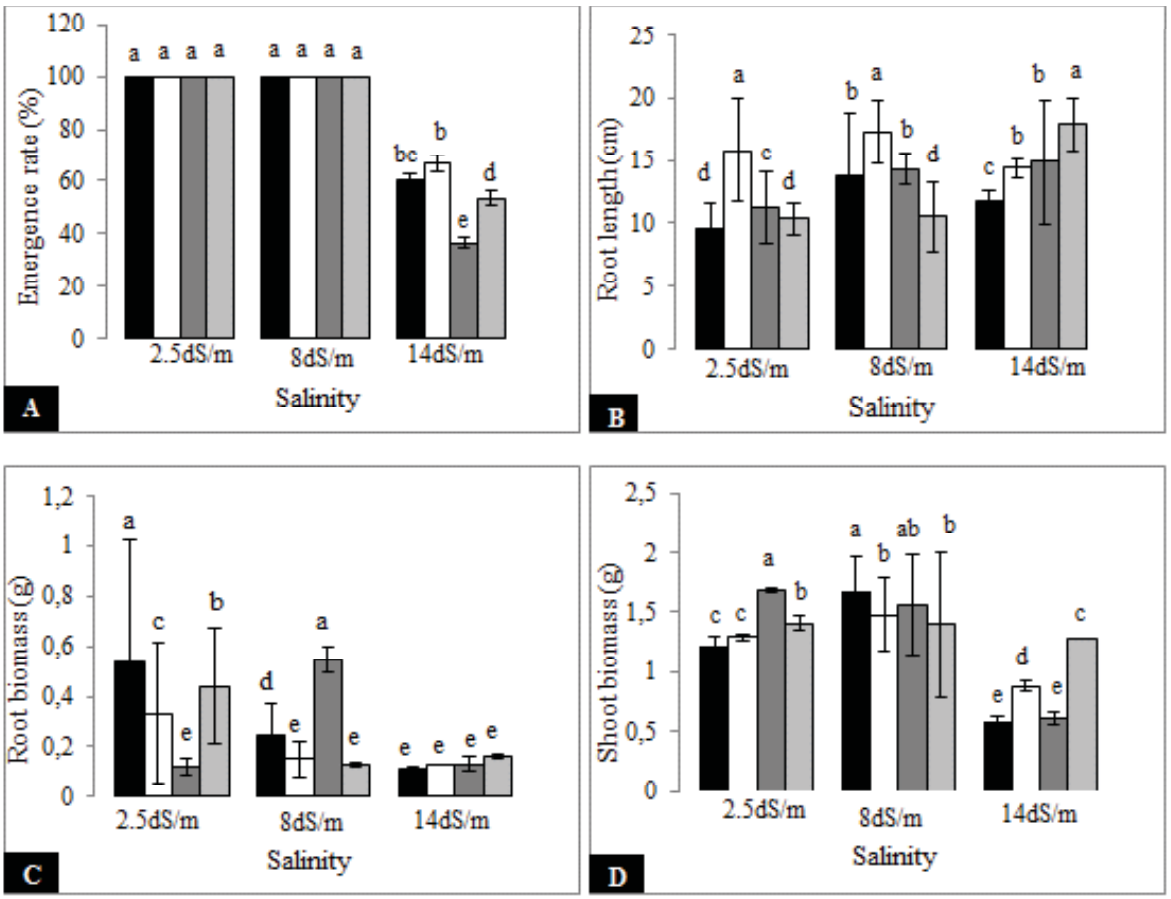


Figure 1. Emergence rate (A), Root length (B), Root biomass (C) and Shoot biomass (D) of barley non-treated (C0) or treated with *Embellisia phragmospora* (EP), *Fusarium equiseti* (FE), and *Fusarium graminearum* (FG) under non-saline and saline conditions. Means followed by the same letter are not significantly different ($P<0.05$).

C0 EP FE FG

vide plants salt stress tolerance.

The relative water content (RWC) was measured in order to indicate cellular volume and response level of plant to environmental stresses (Tátrai *et al*., 2016). Kara and Brinis (2012) indicated that keeping high RWC under saline conditions is a main strategy for maintaining optimal growth of plants under salinity. In the current study, the RWC decreased as salt concentration rose, while RWC values of salt-stressed plants were significantly increased by the tested endophytes under high saline conditions compared to the control plants ($P<0.05$, Fig. 2A). For instance, RWC of inoculated barley plants under non-saline stress increased in *F. equiseti* (81.3% RWC) associated barley as compared to *E. phragmospora* (68.3%) and *F. graminearum* (66.8% RWC) associated barley and control plants (68.12% RWC). While, under moderate saline conditions the RWC decreased with colonization of the tested endophytes and increased in control plants (82% RWC). However, *F. equiseti*-associated barley recorded the highest RWC under high salt stress (78.6% RWC) followed by *E. phragmospora*-inoculated barley (58.4% RWC). The present finding suggests that the effect of fungi on the RWC is greater, which is in close agreement with findings of Hashem *et al*. (2014) and Zhang *et al*. (2016). Siddiqui *et al*. (2014) showed that salinity significantly decreased RWC of rice crops; however, a significant increase was revealed due to *Trichoderma spp.* inoculation.

Mineral nutrition data indicated that the sodium (Na^+) leaf content increased in salt stress treatments as compared to the con-

© Benaki Phytopathological Institute

trol. Regarding the effect of endophytes on Na^+ concentration under salt-stress conditions, plant colonized with endophytes decreased Na^+ concentration compared with the endophyte-free control plants ($P<0.05$, Fig. 2B). Potassium (K^+) leaf content decreased in salt-stressed barley compared to control plants; however, endophytic fungi increased K^+ concentration compared to non-inoculated plants ($P<0.05$, Fig. 2C). Bouzid (2010) reported that sodium absorption by plants is caused due to increases in NaCl and Na_2SO_4 concentrations in the culture medium.. Haouala *et al*. (2007) showed that potassium contents of ryegrass leaves and roots were reduced at salinity exceeding of 50 mM NaCl.

The endophytes *E. phragmospora* and *F. graminearum* decreased Na^+ content under moderate and high salinity as compared to non-inoculated barley. Inoculation of *E. phragmospora* increased K^+ content compared to the non-inoculated barley under moderate salinity while in severe salinity *E. phragmospora*, *F. equiseti* and *F. graminearum*-colonized plants had higher K^+ content as compared to non-associated ones. Li *et al*. (2017) reported that endophytic fungi can protect plants against the toxic effects of ions by attenuating Na^+ absorption in saline conditions and conserving high K^+ assimilation. Moreover, endophytic fungi can be a source of minerals (Shankar *et al*., 2008). Ghorbani *et al*. (2019) indicated that *Piriformospora indica* improved K^+/Na^+ homoeostasis.

Regarding the sugar content results, salt treatment significantly decreased sugar leaves content in barley ($P<0.05$, Fig. 3B). When barley was grown under salt-stressed greenhouse conditions, none of fungal endosymbionts tested had a significant impact on soluble sugar levels ($P>0.05$; Fig. 3B); however, plants subjected to salt stress and colonized by endophytic fungi had greater proline content than uninoculated plants ($P<0.05$, Fig. 3A). Widodo *et al*. (2009) revealed that environmental changes affected plants metabolic homeostasis. Hu *et al*. (2014) exhibited that plants exposed to salt

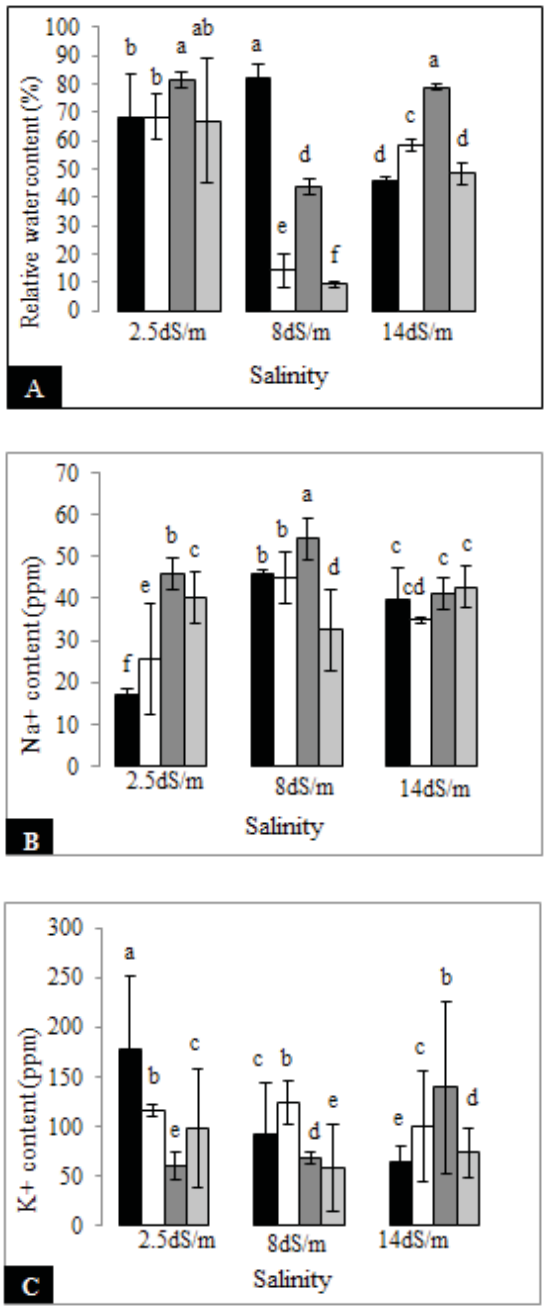


Figure 2. Relative water (A), Na+ (B) and K+ (C) contents of barley non-treated (C0) or treated with *Embellisia phragmospora* (EP), *Fusarium equiseti* (FE), and *Fusarium graminearum* (FG) under non-saline and saline conditions. Means followed by the same letter are not significantly different ($P<0.05$).

stress stimulate a range of metabolic responses, by producing several metabolites, such as proline and sugars.

The sugar content in barley decreased with increasing salinity. The current results

© Benaki Phytopathological Institute

are in agreement with those of Liu and Staden (2001), showing that salt-tolerant soybeans are characterized by a decrease in sucrose accumulation under saline conditions. Nevertheless, *F. graminearum* increased sugar content compared to non-inoculated barley and the other endophytes. The study of Sampangi-Ramaiah *et al*. (2020) demonstrates that a salt tolerant endophyte, *Fusarium sp*., from the salt-adapted Pokkali rice, can be successfully transferred to the cultivated salt-sensitive rice variety IR-64 to confer salt tolerance. Li *et al*. (2017) suggested that the high accumulation of soluble sugars in *Aspergillus aculeatus*-associated perennial ryegrass could contribute to plant protective mechanisms under salt stress by adjusting the osmotic balance.

Our data showed that proline levels increased with increasing salt concentration. Similar results have been shown in barley (Zerrad *et al*., 2008), durum wheat (Chorfi, 2009) and rice (Joseph *et al*., 2015). Proline is one of the osmolytes accumulated by plants under stressful conditions; its accumulation has a role in protecting the cell membrane and participating in osmotic adjustment (Verbruggen and Hermans 2008). Inoculated barley had higher proline content compared to non-inoculated plants. These results agree with those of Dardanelli *et al*. (2009), reporting an increase in proline content in plants colonized by endophytes under saline stress. High proline contents have been reported in *Aspergillus aculeatus*-colonized perennial ryegrass under salt stress and therefore the endophyte was hypothesized to increase the ability of the plants to tolerate salt stress (Li *et al*., 2017).

Results showed a significant increase in chlorophyll a ($P<0.05$, Fig. 4A), chlorophyll b ($P<0.05$, Fig. 4B), chlorophyll total ($P<0.05$, Fig. 4C) and carotenoids ($P<0.05$, Fig. 4D) biosynthesis with increase in salt concentration. However, the tested endophytic fungi had no significant impact on photosynthetic pigments biosynthesis since the pigments (chlorophyll a, b, total and carotenoids) in colonized and salt-stressed barley did not differ from that of control plants ($P>0.05$; Fig. 4).

Rahneshan *et al*. (2018) revealed that salt tolerant plants have increased or unchanged levels of chlorophyll under saline conditions, while chlorophyll levels decrease in salt sensitive plants. Results obtained by Zraibi *et al*. (2012) have shown that carotenoids are an effective antioxidant that protects and stabilizes photochemical processes of photosynthesis under stressful conditions. According to Ghorbani *et al*. (2018), inoculation of tomato plants with *Piriformospora indica* can promote plant growth via improving gas exchange, water potential, chloro-

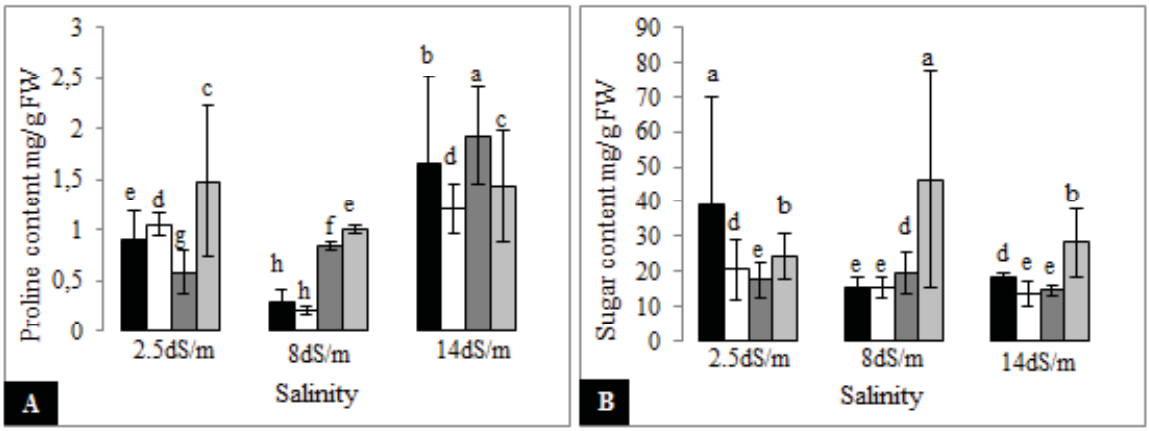


Figure 3. Proline (A) and Sugar (B) contents of barley non-treated (C0) or treated with *Embellisia phragmospora* (EP), *Fusarium equiseti* (FE), and *Fusarium graminearum* (FG) under non-saline and saline conditions. Means followed by the same letter are not significantly different ($P<0.05$).

C0 EP FE FG

© Benaki Phytopathological Institute

phyll content and chlorophyll fluorescence parameters.

Our results on inoculation of endophytic fungi on barley in saline environment are similar to those by Ban *et al*. (2017) showing that inoculation of the endophytic fungus *Gaeumannomyces Cylindrosporus* had no significant effect on photosynthetic pigments in maize plants under stressful conditions. However, Jogawat *et al*. (2013) indicated that *Piriformospora indica* enhanced barley and rice salt stress tolerance by increasing the activity of photosynthetic pigments in colonized plants. Xie *et al*. (2014) suggested that *Aspergillus aculeatus* can colonize plants and improve photosynthesis and chlorophyll content and facilitate plant growth under stress.

The present study has revealed that endophytic fungi appear to confer salt tolerance of barley as evident by changes in physiological and biochemical indexes, involving several mechanisms. As the mechanisms by which endophytic fungi interact with host plants and increase plant growth and yield under salt stress are incompletely understood, future *in vitro* and *in planta* research into the cellular, epigenetic and molecular mechanisms is merited. The capacity of endophytic fungi to increase barley tolerance under salinity stress and to improve growth could be applicable to achieve a sustainable agriculture.

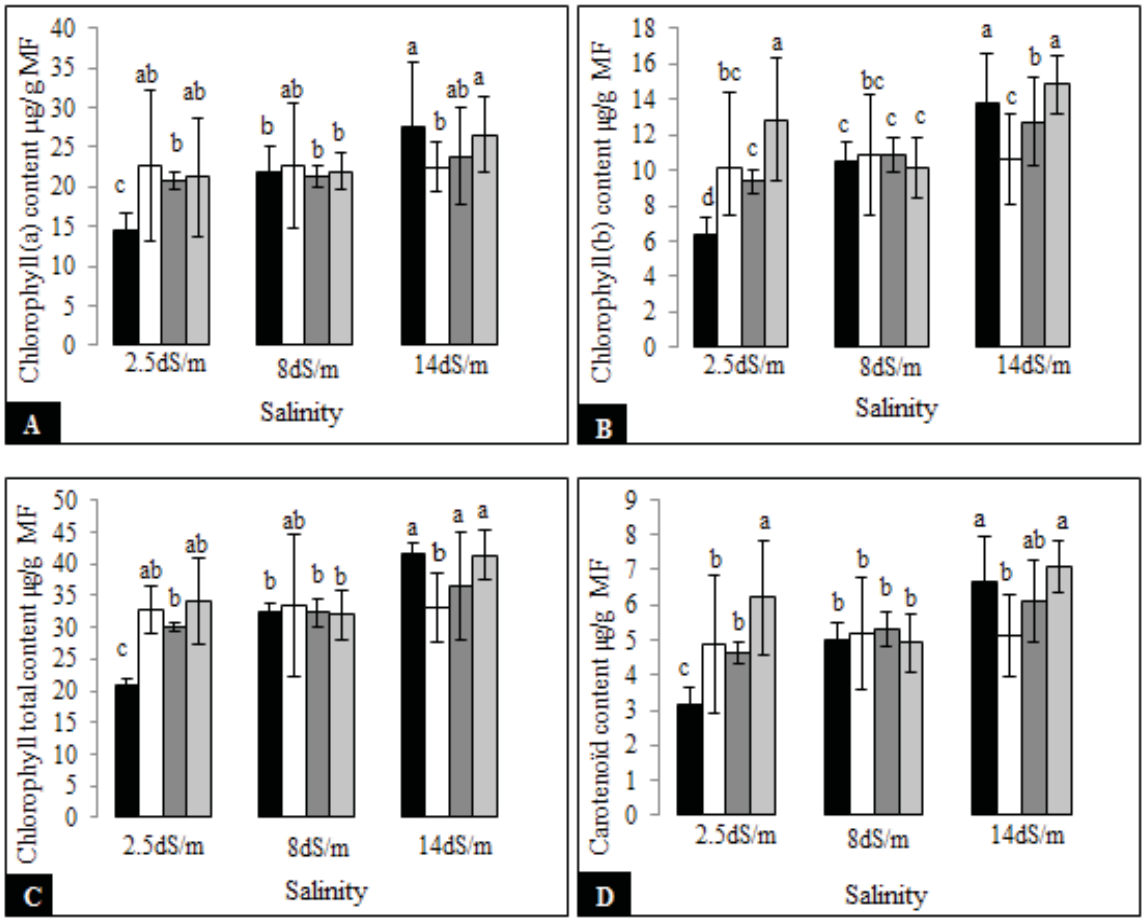


Figure 4. Chlorophyll a (A), Chlorophyll b (B), Chlorophyll total (C) and Carotenoid (D) contents of barley non-treated (C0) or treated with *Embellisia phragmospora* (EP), *Fusarium equiseti* (FE), and *Fusarium graminearu*m (FG) under non-saline and saline conditions. Means followed by the same letter are not significantly different ($P<0.05$).

C0 EP FE FG

© Benaki Phytopathological Institute

Literature cited

Aghilia, F., Jansab, J., Khoshgoftarmanesh, A.H., Afyunic, M., Schulind, R., Frossarda, E. and Gampera, H.A. 2014. Wheat plants invest more in mycorrhizae and receive more benefits from them under adverse than favorable soil conditions. *Applied Soil Ecology*, 84: 93-111.

Ahmad, P., Abeer, H., Elsayed, F.A.A., Alqarawi, A.A., Riffat, J., Dilfuza, E. and Salih, G. 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system. *Frontiers in Plant Science,* 6: 868.

Akagi, A., Jiang, C.J. and Takatsuji, H. 2015. *Magnaporthe oryzae* Inoculation of Rice Seedlings by Spraying with a Spore Suspension. *Bio-Protocol,* 11: 1-5.

Albacete, A., Ghanem, M.E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V., Lutts, S., Dodd, I.C. and Pérez-Alfocea F. 2008. Hormonal changes in relation to biomass partitioning and shoot growth. Impairment in salinized tomato (*Solanum lycopersicum* L.) plants. *Journal of Experimental Botany* 59 (15): 4119-4131.

Baenziger, M., Setimela, P.S., Hodson, D. and Vivek, B. 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. *Agricultural Water Management,* 80: 212-224.

Ban, Y., XU, Z., Yang, Y., Zhang, H., Chen, H., and Tang, M. 2017. Effect of dark septate endophytic fungus *Gaeumannomyces cylindrosporus* on plant growth, photosynthesis and Pb tolerance of maize (*Zea mays* L.). *Pedosphere,* 27: 283–292.

Bouzid, N. 2010. Étude de la résistance d'*Atriplex halimus* subsp. schweinfurthii aux sels solubles. *Acta Botanica Gallica*, 157 (4): 787-791.

Chapman, H.D. and Pratt, P.F. 1961. Methods of Analysis for Soils, Plants and Water. Univ. California, Public Division of Agricultural Sciences, Berkeley, CA, USA. 150-179.

Chorfi, A. 2009. Contribution à l'étude de la résistance à la salinité chez une variété de blé dur algérien (*Triticum durum* Desf.) Var. mohamed ben bachir. *Sciences and Technologie,* 29: 41-44.

Dardanelli, M.S., Manyani, H., Gonzalez-Barroso, S., Rodriguez-Carvajal, M.A., Gil-Serrano A.M., Espuny, M.R., López-Baena, F.J., Bellogín, R.A., Megías, M. and Ollero, F.J. 2009. Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinum* Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. *Plant Soil,* 328: 483-493.

El Goumi, Y., Fakiri, M., Lamsaouri, O. and Benchekroun, M. 2014. Salt stress effect on seed germination and some physiological traits in three Moroccan barley (*Hordeum vulgare* L.) cultivars. *Journal of Materials and Environmental Science*, 5: 625-632.

Ghorbani, A., Omran, V.O.G., Razavi, S.M., Pirdashti, H. and Ranjbar, M. 2018. *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* L.). *Plant Biology,* 20: 729-736.

Ghorbani, A., Omran, V.O.G., Razavi, S.M., Pirdashti, H. and Ranjbar, M. 2019. *Piriformospora indica* confers salinity tolerance on tomato (*Lycopersicon esculentum* Mill.) through amelioration of nutrient accumulation, K^+/Na^+ homeostasis and water status. *Plant Cell Reports*. Springer-Verlag GmbH Germany, part of Springer Nature 2019. doi.org/10.1007/s00299-019-02434-w.

Gill, S.S., Gill, R., Trivedi, D.K., Anjum, N.A., Sharma, K.K., Ansari, M.W., Ansari, A.A., Johri, A.K., Prasad, R., Pereira, E., Varma, A. and Tuteja, N. 2016. *Piriformospora indica*: potential and significance in plant stress tolerance. *Frontiers in Microbiology,* 7: 332.

Gupta, B. and Huang, B. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics*, 2014:701596. https ://doi.org/10.1155/2014/70159 6.

Hamayun, M., Khan, S.A., Khan, A.L., Tang, D.S., Hussain, J., Ahmad, B., Anwar, Y. and Lee, I.J. 2010. Growth promotion of cucumber by pure cultures of gibberellin-producing *Phoma sp*. GAH7. *World Journal of Microbiology and Biotechnology,* 26: 889-894.

Haouala, F., Ferjani, H. and El Hadj, S. 2007. Effet de la salinité sur la répartition des cations (Na+, K+ et Ca+2) et du chlore (Cl-) dans les parties aériennes et les racines de ray-grass anglais et du chiendent. *Biotechnology, Agronomy, Society and Environment*, 11 (3): 235-244.

Hasan, H.A.H. 2002. Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity-calcium interaction. *Rostlinná výroba*., 48: 101-106.

Hashem, A., Abd-allah, E.F., Alqarawi, A.A., Al-Huqail, A.A. and Egamberdieva, D. 2014. Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum* (Bonord.) Bainier. *Journal of Plant Interactions,* 9: 857-868.

Hu, L., Chen, L., Liu, L., Lou, Y., Amombo, E. and Fu, J. 2014. Metabolic acclimation of source and sink tissues to salinity stress in bermudagrass (*Cynodon dactylon*). *Plant Physiology,* 155: 166-179.

I.N.S.I.D. 2008. Etat d'avancement du projet (ALG/5/022) de coopération technique avec l'AIEA. Les techniques nucléaires pour l'utilisation durable des terres salines et des eaux saumâtres pour la production agricole. 48p.

Jogawat, A., Saha, S., Bakshi, M., Dayaman, V., Kumar, M., Dua, M., Varma, A., Oelmüller, R., Tuteja, N. and Johri, A.K. 2013. *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *Plant Signaling and Behaviour*, 8: e26891.

© Benaki Phytopathological Institute

Joseph, E.A., Radhakrishnan, V.V. and Mohanan, K.V. 2015. A Study on the Accumulation of Proline- An Osmoprotectant Amino Acid under Salt Stress in Some Native Rice Cultivars of North Kerala. *India Universal Journal of Agricultural Research,* 3 (1): 15-22.

Kara, K. and Brinis, L. 2012. Réponse Physiologique au Stress Hydrique de Variétés de Blé Tendre (*Triticum Aestivum* L.) Cultivées en Algérie. *European Journal of Scientific Research,* 81: 524-532.

Kouadria, R., Bouzouina, M., Azzouz, R. and Lotmani, B. 2018. Salinity stress resistance of durum wheat (*Triticum durum*) enhanced by fungi. *International Journal of Biosciences,* 12: 70-77.

Kouadria, R., Bouzouina, M. and Lotmani, B. 2019. Endophytic fungi contribution in adverse factors tolerance of cultivated species: isolation of endophytic fungi and study of their contribution to salinity or pollutants tolerance (in French). PhD dissertation. 159p.

Kouadria, R., Bouzouina, M. and Lotmani, B. 2020. Induction of growth and osmoregulation in salt stressed barley by the endophytic fungus *Chaetomium coarctatum*. *Tunisian Journal of Plant Protection,* 15: 19-27.

Leitão, A.L. and Enguita, F.J. 2016. Gibberellins in *Penicillium* strains: Challenges for endophyte-planthost interactions under salinity stress. *Microbiological Research,* 183: 8-18.

Li, X., Han, S., Wang, G., Liu, X., Amombo, E., Xie, Y. and Fu, J. 2017. The Fungus *Aspergillus aculeatus* Enhances Salt-Stress Tolerance, Metabolite Accumulation, and Improves Forage Quality in Perennial Rye grass. *Frontiers in Microbiol*ogy, 8: 1664.

Li, F.S., Kang, S.Z. and Zhang, J. 2004. Interactive effects of elevated CO2, nitrogen and drought on leaf area, stomatal conductance, and evapotranspiration of wheat. *Agricultural Water Management,* 67: 221-233.

Liu, T. and Staden, J.V. 2001. Partitioning of carbohydrates in salt sensitive and salt-tolerant soybean callus cultures under salinity stress and its subsequent relief. *Journal of Plant Growth Regulation,* 33: 13.

Majumder, A.L., Sengupta, S. and Goswani, L. 2010. Osmolyte regulation in abiotic stress. Chap dans Abiotic stress adaptation in plants: Physiological, molecular and genomic foundation. Sous la direction de A. Pareek, S.K. Sopory, H.J. Bohmert et Govindjee. p. 349-370.

Mallek-Maalej, E., Boulasnem, F. and Ben Salem, M. 2004. Effet de la salinité sur la germination de graines de céréales cultivées en Tunisie. *Cahiers Agriculture,* 12: 6-153.

Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology,* 59: 651-81.

Munns, R., James, R. A., and Läuchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany,* 57: 1025-1043.

Rahneshan, Z., Nasibi, F. and Ahmadi Moghadam, A. 2018. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. *Journal of Plant Interactions,* 13: 73-82.

Robert-Seilaniantz, A, Navarro, L, Bari, R. and Jones, JD. 2007. Pathological hormone imbalances. *Current Opinion in Plant Biology,* 10:372-9.0.

Rodriguez, R., White, J., Arnold, A. and Redman R. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist,* 182: 314-330.

Ruiz-Lozano, J.M., Porcel, R., Azcón, R. and Aroca, R. 2012. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *Journal of Experimental Botany,* 63: 4033-4044.

Sampangi-Ramaiah, M.H., Jagadheesh, Dey, P., Jambagi, S., Vasantha-Kumari, M.M., Oelmüller, R., Nataraja, K.N., Venkataramana-Ravishankar, K., Ravikanth, G. and Uma-Shaanker, R. 2020. An endophyte from salt-adapted Pokkali rice confers salt-tolerance to a salt-sensitive rice variety and targets a unique pattern of genes in its new host. *Science Reports,* 10: 32-37.

Schields R. and Burnett W. 1960. Determination of protein- bound carbohydrate in serum by a modified anthrone method. *Analytical Chemistry,* 32: 885- 886. https://doi.org/10.1021/ac60163a053.

Scippa, G., Di Michel, M., Onelli, E., Patrignani, G., Chiatante, D. and Bray, E. 2004. The histone-like protein H1-S and the response of tomato leaves to water deficit. *Journal of Experimental Botany,* 55: 99-109.

Shankar Naik, B., Shashikala, J. and Krishnamurthy, Y.L. 2008. Diversity of fungal endophytes in shrubby medicinal plants of Malnad region, Western Ghats, Southern India. *Fungal Ecology,* 1: 89-93.

Siddiqui, Z.S., Cho, J.I., Hanpark, S., Kwon, T.R., Ahn, B.O., Lee, G.S., Jeong, M.J., Whankim, K., Konlee, S. and Chulpark, S. 2014. Phenotyping of rice in salt stress environment using high-throughput infrared imaging. *Acta Botanica Croatica,* 73: 149-158.

Tátrai, Z.A., Sanoubar, R., Pluhár, Z., Mancarella, S., Orsini, F. and Gianquinto, G. 2016. Morphological and physiological plant responses to drought stress in *Thymus citriodoru*s. *International Journal of Agronomy,* 2: 1-8.

Troll, W., and Lindsley, J. 1955. A photometric method for determination of proline. *Journal of Biological Chemistry,* 215: 655-660.

Verbruggen, N. and Hermans, C. 2008. Proline accumulation in plants: a review. *Amino Acids* 35 (4): 753-759.

© Benaki Phytopathological Institute

Wang, W.X., Vinocur, B. and Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta,* 218: 1-14.

Wei, Z. and Jousset, A. 2017. Plant breeding goes microbial. Trends Plant Sci 22: 555-558.

Widodo, J.H., Newbigin, E., Tester, M., Bacic, A. and Roessner, U. 2009. Metabolic responses to salt stress of barley (Hordeum vulgare L.) cultivars, sahara and clipper, which differ in salinity tolerance. *Journal of Experimental Botany*, 60: 4089-4103.

Yang, Y.L., Guo, J.K., Zhang, F., Zhaob, L.Q. and Zhang, L.X. 2004. NaCl induced changes of the H^+-ATPase in root plasma membrane of two wheat cultivars. *Plant Science*, 166: 913-918.

Yun, P., Xu, L., Wang, S.S., Shabala, L., Shabala, S. and Zhang, W.Y. 2018. *Piriformospora indica* improves salinity stress tolerance in *Zea mays* L. plants by regulating Na^+ and K^+ loading in root and allocating K+ in shoot. *Journal of Plant Growth Regulation,* 86 (2): 323-331.

Yurieva, O.M., Syrchin, S.O., Nakonechna, L.T. and Kurchenko, I.M. 2018. Influence of endophytic and saprotrophic *Penicillium* funiculosum strains on resistance of *Glycine max* L. under salt stress. *Біологія, біотехнологія, екологія* (*Biology, Biotechnology, Ecology*) 2 (72): 579-262.

Zerrad, W., Hillali, S., Mataoui, B., El Antri, S. and Hmyene, A. 2008. Comparative study of the biochemical and molecular mechanisms of resistance to water stress of two varieties of durum wheat. *Lebanese Science Journal*, 9: 27-36.

Zhang, S., Gan, Y. and Xu, B. 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers in Plant Science,* 7: 1405.

Zraibi, L., Nabloussi, A., Merimi, J., El Amrani, A., Kajeiou, M., Khalid, A. and Serghini Caid, H. 2012. Effet du stress salin sur des paramètres physiologiques et agronomiques de différentes variétés de carthame (*Carthamus tinctorius* L.). AFRIMED AJ - *Al Awamia,* 125: 15-40.

Received: 15 April 2020; Accepted: 6 December 2022

Διερεύνηση του ρόλου των ενδοφυτικών μυκήτων στην ανοχή του κριθαριού στην αλατότητα

R. Kouadria, M. Bouzouina, B. Lotmani και S. Soualem

Περίληψη Η αλατότητα του εδάφους είναι ένα γεωργικό και οικο-περιβαλλοντικό πρόβλημα σε παγκόσμιο επίπεδο, που προκαλεί μείωση της παραγωγής στις καλλιέργειες. Έχει αποδειχθεί ότι οι ενδοφυτικοί μύκητες βελτιώνουν την ανοχή των φυτών σε συνθήκες καταπόνησης. Σκοπός της εργασίας είναι να διερευνήσει σε συνθήκες θερμοκηπίου την αποτελεσματικότητα των ενδοφυτικών μυκήτων *Embellisia phragmospora, Fusarium equiseti* και *F. graminearum* στη βελτίωση της ανοχής φυτών κριθαριού που αναπτύσσονται σε γλάστρες σε διαφορετικά επίπεδα αλατότητας του εδάφους (2,5, 8 και 14dS/m) με βάση την ανάπτυξη, τη σχετική περιεκτικότητα σε νερό και μεταλλικά στοιχεία, τη βιοσύνθεση φωτοσυνθετικών χρωστικών, και τα επίπεδα προλίνης και σακχάρων των φυτών. Τα αποτελέσματα έδειξαν ότι, σε αλατότητα εδάφους 14dS/m, ο μύκητας *E. phragmospora* αύξησε την έκπτυξη των φυταρίων κριθαριού στο 66,7% σε σχέση με το 60% έκπτυξη που καταγράφηκε στο κριθάρι χωρίς ενδόφυτα. Σε αλατούχο έδαφος, οι ενδοφυτικοί μύκητες αύξησαν το μήκος της ρίζας του κριθαριού και το ξηρό βάρος του στελέχους και της ρίζας, μείωσαν τη συσσώρευση Na+ και βελτίωσαν την πρόσληψη K+. Επίσης, σε συνθήκες υψηλής αλατότητας, φυτά με τα ενδόφυτα *F. equiseti* και *F. graminearum* είχαν αυξημένη περιεκτικότητα σε προλίνη ενώ φυτά με *F. graminearum* εμφάνισαν τη μεγαλύτερη περιεκτικότητα σε σάκχαρα. Τα αποτελέσματα δείχνουν τη σημασία των ενδοφυτικών μυκήτων στη βελτίωση της ανοχής του κριθαριού στην καταπόνηση λόγω αλατότητας του εδάφους ως ένα εν δυνάμει ισχυρό εργαλείο με σημαντικά οφέλη στις καλλιέργειες στο πλαίσιο της βιώσιμης γεωργίας.

© Benaki Phytopathological Institute