Endophytic Fungi from Salt Adapted Plants Confer Salt Tolerance in Barley

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ABSTRACT

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Endophytic fungi are known to improve plant tolerance under stressful conditions including salt stress. Considering this, the endophytic fungi, *Alternaria chlamydospora*, *Embellisia phragmospora*, *Phomabetae*, *Chaetomium coarctatum*, *Fusarium equiseti* and *Fusarium graminearum*, was isolated from roots of plants growing in salt environments and then, evaluated for their contribution in conferring salt stress tolerance in barley plants. The influence of inoculation with endophytic fungi, on germination, root and shoot lengths of barley seeds under different NaCl levels (0, 200, 300 and 400 mM) was investigated. Results showed that seed germination and root and shoot lengths were higher in seeds pretreated with endophytic fungi cultures than their controls under saline conditions. This study suggests that the tested endophytic fungi might be applied as a strategy for mitigating the stress-imposed salt in plants and, therefore, improving crop growth and productivity.

Keywords: Barley, endophytic fungi, salt stress, tolerance

Salinity is one of the most significant abiotic stresses that affect agricultural lands worldwide, limiting the crop productivity of the economically important crops (Elhindi et al. 2017). Salinity has affected more than 20% of agricultural land worldwide and the proportion will increase by 2050 (Otlewska et al. 2020).

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Salt stress decreases vegetative and reproductive development by inducing physiological and biochemical dysfunctions (Al-Razak and Al-Saady 2015), and this has profound implications on germination and growth (Jajarmi 2009). Germination is an important plant developmental stage (Fowler 1991) regulated by environmental factors such as salinity (Baghbani et al. 2013). Rafiq et al. (2006) revealed that germination is completely inhibited by high levels of salt.

Barley (*Hordeum vulgare*) is the fourth most important crop after wheat, maize and rice cultivated worldwide due to its nutritional and health benefits. It can be an excellent model crop for studies on the mechanisms of salinity tolerance and for developing approaches to promote crop growth in saline soil (Zhu et al. 2020). Increasing human populations request more crop yield for food security while barley yield damages in saline areas are significant; so salt stress tolerant barley is a widely sought quality in order to extend its cultivation to unfavorable regions (Baenziger et al. 2006).

Plant growth-promoting fungal association is an ideal strategy, suggested by Wei and Jousset (2017) to alleviate salt stress without compromising plant growth and yield. Endophytic fungi grow within plant organs without causing any disease symptoms; have been supposed beneficial to host-plants even during stress conditions (Rodriguez et al. 2009; Dutta et al. 2014).

These endophytes are well recognized for plant growth promotion under abiotic stresses conditions through different mechanisms; (1) phytohormone synthesis such as auxins, mainly indole-3-acetic acid (IAA) and gibberellins, which promotes plant growth; (2) the effect on the antioxidant enzymes activity (catalase, superoxide dismutase, and peroxidase), which prevents the harmful effects of reactive oxygen species (ROS); (3) the effect on the proline content, which structures the water content in plant cells and reduces the plants turgor pressure; and (4) increasing the availability of nutrients, which directly improves plant growth (Kumar et al. 2020; Brazhnikova et al. 2022).

In the last decade, it has been a known that these endophytic fungi, residing inside host confer abiotic stress tolerance (Rodriguez et al. 2012). However, the exact mechanism is still unexplored. In this study, six endophytic fungal strains were screened for their ability to enhance seed germination, root and shoot lengths of barley rown under different NaCl concentrations.

MATERIAL AND METHODS

Fungal material.

In this study, the endophytic fungi strains were previously isolated from halophytic plants (*Avena fatua*, *Beta macrocarpa*, *Anabasis prostrata*, *Salsola oppositifolia*, *Lolium rigidum*, *Suaeda fruticosa*), which were collected from wild populations growing at three saline sites in Relizane, Algeria, and then identified by ITS methods (Kouadria 2019). The endophytes *Alternaria chlamydospora*, *Embellisia phragmospora*, *Phoma betae*, *Chaetomium coarctatum*, *Fusarium equiseti* and *Fusarium graminearum* were deposited in a gene bank with KF993329, JQ796758, KM249076, NR_144822, KU377478 and KT211545 accession numbers respectively.

Screening of endophytic fungi for improving barley seed germination.

Barley seeds of cultivar Saida183 were used to screen fungal isolates that could improve seed germination under salt stress. Surface sterilized barley seeds were soaked in fungi suspension (10^7 spores/ml) or distilled water (control).

Germination test was carried out in Petri dishes at 25°C in phytotron. The Petri dishes contained two layers of filter paper, which were moistened with 0, 200, 300 and 400 mM of NaCl solution without and with endophytic fungi strains.

On the seventh day of the experiment, germination percentage, root and shoot were measured.

Data analysis.

The experiment was arranged in a randomized complete block design.

Experiments comprised 100 seeds per plate, each treatment comprised five replicates. Two-way ANOVA has been used for the analysis of the results with STATBOX v6.4 software, and means were compared with the LSD test ($P < 0.05$).

RESULTS

Effect of endophytic fungi on barley seed germination.

Results showed that salinity ($P < 0.05$, Fig.1) and endophytic fungi ($P < 0.05$, Fig.1) significantly affected the germination rate of barley seeds.

Under treatments without NaCl, all seeds inoculated or not recorded a 100% germination rate, with the exception *F. graminearum*-treated seeds, which recorded a percentage of 89.4%. For non-treated seeds (control), this rate decreases with the increasing of salinity level, showing a value of 33.2% at 200 mM NaCl; while a total inhibition of germination was noted with 300 and 400 mM of NaCl. Thus, we can consider the salinity level of 300 and 400 mM of NaCl as a threshold inhibiting the germination process of barley.

Application of endophytic fungi has significant results ($P < 0.05$, Fig.1). The association reduced the effect of salinity on barley germination. A significant increase in germination rate was revealed in *C. coarctatum*-associated barley (91%) under 200 mM of salinity. Moreover, the tested endophytic fungi have allowed the germination of barley under high salinity levels (300 and 400 mM). Indeed, inoculated barley seeds recorded a germination rate of 45.6% (*F. equiseti*) and 25% (*F. equiseti* and *P. betae*) respectively under 300 and 400 mM.

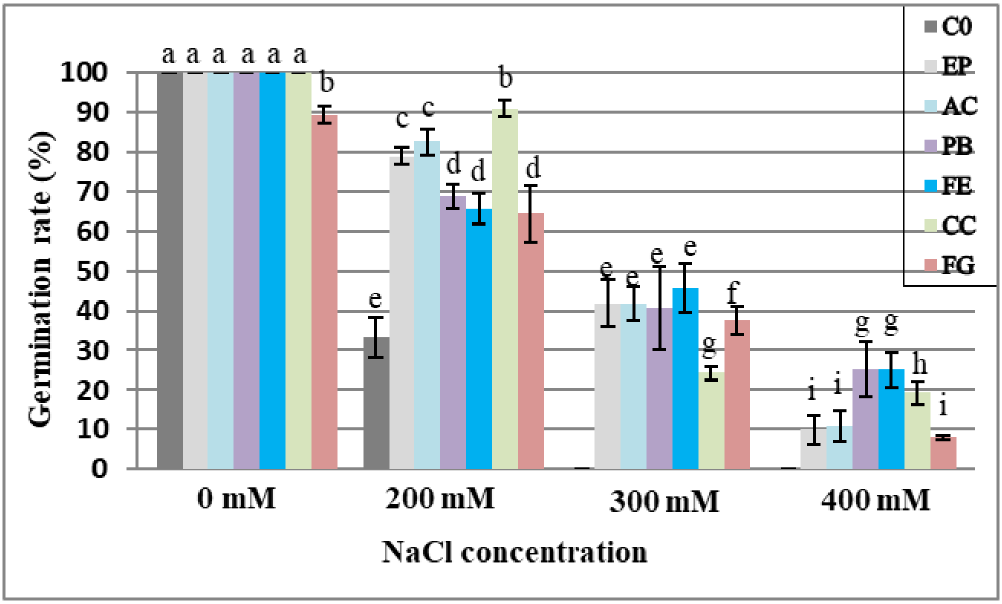

Fig. 1. Germination rate of barley seeds non-inoculated (C0) or inoculated with *Embellisia phragmospora* (EP), *Alternaria chlamydospora* (AC), *Phoma betae* (PB), *Fusarium equiseti* (FE), *Chaetomium coarctatum* (CC), and *Fusarium graminearum* (FG) under non-saline (0 mM) and saline conditions (200, 300 and 400 mM). Means followed by the same letter are not significantly different according LSD test at $P < 0.05$.

Effect of seed treatment with fungi on barley growth.

Salinity has significantly decreased root ($P < 0.05$, Fig. 2) and shoot ($P < 0.05$, Fig. 3) lengths. The tested fungal strains have significant effects on root and shoot lengths ($P < 0.05$).

The untreated control seeds (0 mM) recorded the highest root and shoot lengths by 7.07 and 8.26 cm, respectively.

Barley seeds treated with endophytic fungi (10^7 spores/ml) exhibited significant ($P < 0.05$) enhancement of growth (root and shoot lengths) when compared to the untreated control under saline conditions. *A. chlamydospora*-treated seeds had root length 5.56 cm and shoot length 3.99 cm, whereas in untreated control, root length was 1.08 cm and shoot length was 0.06 cm under 200 mM of NaCl. Barley growth was totally inhibited in untreated control under 300 and 400 mM salinity levels. However, some endophytic fungi allowed root and shoot growth under high levels of salinity but with reduced degrees.

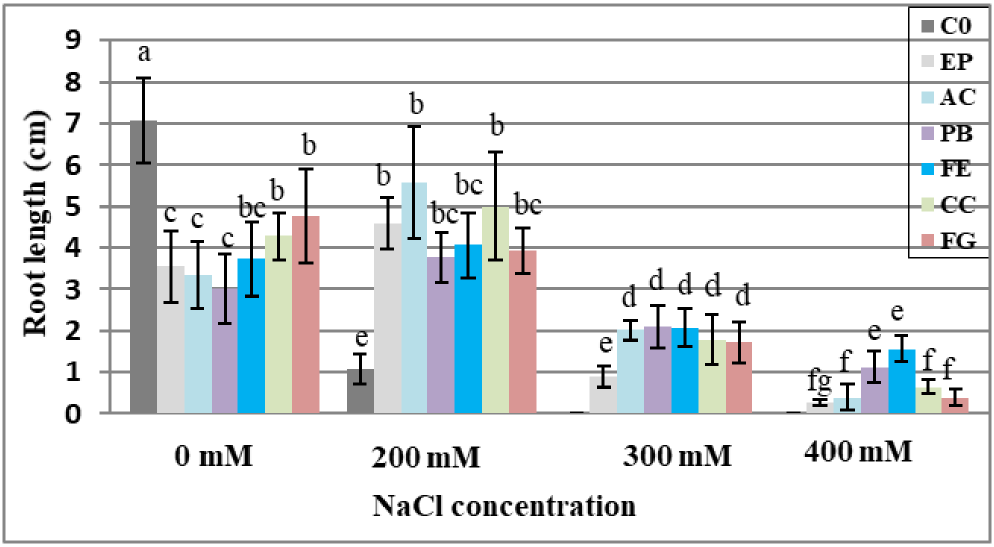

Fig. 2. Root length of barley seeds non-inoculated (C0) or inoculated with *Embellisia phragmospora* (EP), *Alternaria chlamydospora* (AC), *Phoma betae* (PB), *Fusarium equiseti* (FE), *Chaetomium coarctatum* (CC), and *Fusarium graminearum* (FG) under non-saline (0 mM) and saline conditions (200, 300 and 400 mM). Means followed by the same letter are not significantly different according LSD test at $P < 0.05$.

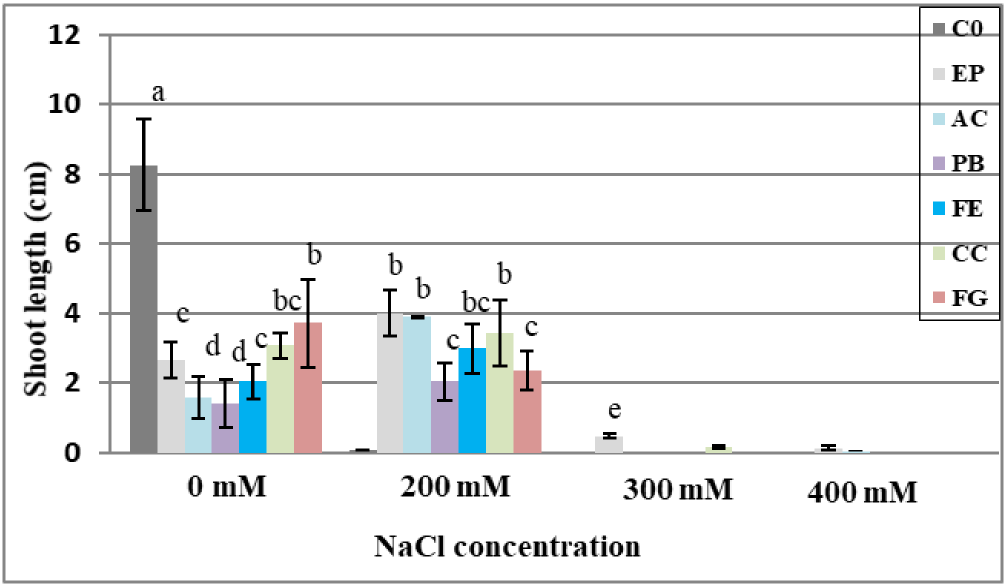

Fig. 3. Shoot length of barley seeds non-inoculated (C0) or inoculated with *Embellisia phragmospora* (EP), *Alternaria chlamydospora* (AC), *Phoma betae* (PB), *Fusarium equiseti* (FE), *Chaetomium coarctatum* (CC), and *Fusarium graminearum* (FG) under non-saline (0 mM) and saline conditions (200, 300 and 400 mM). Means followed by the same letter are not significantly different according LSD test at $P < 0.05$.

DISCUSSION

Endophytic fungi are non-harmful organims that colonize the internal tissues of healthy plants during their life cycle; they can often promote plant growth and alleviate biotic and abiotic stresses in plants (Pandey et al. 2019). Barley is one of the most salinity-tolerant crops. In this study, we attempted to screen the potential role of endophytic fungi in stimulating the salt-affected barley germination and groth. Fungal pretreatment significantly enhanced the biological response of barley under high levels of salinity. Similar result was obtained with fungal strains that colonize barley (Kouadria et al. 2020; 2023) and wheat (Bouzouina et al. 2021).

Furthermore, Ignatova et al. (2022) found that the endophytic halotolerant strain *Pseudomonas flavescens* D5 isolated from chicory had a complex of beneficial effects on plants under abiotic stress including IAA synthesis and enzymatic activity (amylolytic, cellulolytic, and proteolytic).

Seed treatment with endophytes enhanced germination root and shoot lengths compared to the untreated control under NaCl stress. Similar observations using *Epichloë bromicola* on barley plant (Wang et al. 2022), *Trichoderma viride* as seed treatment on cotton (Shanmugaiah et al. 2009), and *Penicillium chrysogenum* on pearl millet (Murali et al. 2013) were reported increasing seed germination and growth, and enhancing plant physiology status under salt stress. Endophytic fungi allow plants to have better access to nutrients and substrate water, which promotes their growth (Fortinet al. 2008).

Alternaria, *Embellisia*, *Phoma* and *Fusarium* are pigmented endophytes that play an important ecological role for plant survival and stress resistance (Sun et al. 2012). Rahman and Saiga (2005) showed that endophytic fungi play a role in plant salt stress tolerance by production of organic acids and other compounds. Endophytic fungi can also improve plant growth (root and shoot lengths); this is due to the production of phytohormones, such as IAA and gibberellic acid, which induce more root growth and lead to increased nutrient uptake (Egamberdieva and Kucharova 2009).

Hamayun et al. (2010) have been conducted to investigate gibberellic acid production by endophytic fungi. Gibberellins are produced by *Penicillium* strains under salt stress to improve plant growth (Leitão and Enguita 2016). Gibberellic acids and IAA produced by endophytic fungus *Penicillium funiculosum* had significantly enhanced soybean seed germination and mitigated negative effects of salinity stress by improving soybean growth and metabolism (Khan et al. 2011). Likewise, Hasan (2002) indicated that *Fusarium* produced gibberellin under high salinity levels that may decrease the negative effect of salinity in crop.

This study opens future directions for researchers to investigate the genetic mechanisms involved in the induction of salt tolerance by plant growth promoting endophytes (PGPE) in crops. A study of the stress-responsive gene expression in plants in response to PGPE inoculation might be helpful for understanding the molecular cross-talk between plants and endophytes (Afridi et al. 2019).

Endophyte-inoculation plants revealed low signs of the adverse effects of salinity. The findings of the current research reveal that endophytic fungal interactions can enhance the productivity of economically important crop species. Nevertheless, the favorable role of these fungal strains still needs to be examined under field conditions to confirm the obtained results.

RESUME

Kouadria R., Bouzouina M. et Lotmani, B. 2023. Les endophytes fongiques issus des plantes adaptées au sel confèrent à l'orge une tolérance au sel. Tunisian Journal of Plant Protection 18 (2): 63-70.

Les champignons endophytes sont connus pour améliorer la tolérance des plantes dans des conditions de stress, y compris le stress salin. Dans cette optique, les champignons endophytes *Alternaria chlamydospora*, *Embellisia phragmospora*, *Phoma betae*, *Chaetomium coarctatum*, *Fusarium equiseti* et *Fusarium graminearum* ont été isolés à partir des racines de plantes poussant en milieux salins, puis évalués pour leur contribution à la tolérance au stress salin chez l'orge. L'influence de l'inoculation avec ces champignons endophytes sur la germination et la croissance de la radicule et du coléoptile chez l'orge à différents niveaux de NaCl (0, 200, 300 et 400 mM) a été étudiée. Les résultats ont montré que la germination et la croissance des radicules et des coléoptiles étaient plus élevées chez les semences prétraitées avec des cultures de champignons endophytes par rapport aux semences témoins soumises au stress NaCl. Cette étude suggère que les champignons endophytes testés peuvent être appliqués comme stratégie pour atténuer les effets négatifs imposés par le stress chez les plantes et, par conséquent, pour améliorer la croissance et la productivité des cultures.

Mots clés: Champignons endophytes, orge, stress salin, tolérance

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ملخص

قوادرية، ربيعة ومحمد بوزوينة وبراهيم العثماني. 2023. الفطريات الداخلية المعزولة من النباتات المتكيفة مع الملح تعزز قدرة الشعير على تحمل الملوحة. Tunisian Journal of Plant Protection 18 (2): 63-70.

من المعروف أن الفطريات الداخلية تعمل على تحسين قدرة النبات على تحمل الظروف الإجهادية، بما في ذلك الإجهاد الملحي. مع أخذ هذا في الاعتبار، تم عزل الفطريات *Alternaria chlamydospore* و *Embellisia phragmospora* و *Phoma betae* و *Chaetomium coarctatum* و *Fusarium equiseti* و *Fusarium graminearum* من جذور النباتات التي تنمو في البيئات المالحة، ثم تم تقييم مساهمتها في تحمل إجهاد الملح لدى الشعير. تمت دراسة تأثير التلقيح بالفطريات الداخلية على إنبات ونمو الشعير عند مستويات كلوريد الصوديوم مختلفة (0 و400 و600 و800 ميلي مول). أظهرت النتائج أن الفطريات الداخلية تحسن إنبات الشعير ونموه. بذلك، كان إنبات ونمو الشعير أعلى مع البذور المعاملة بالفطريات الداخلية مقارنة بالبذور غير المعاملة والمعرضة لإجهاد كلوريد الصوديوم. تشير هذه الدراسة إلى أنه يمكن استخدام الفطريات الداخلية المختبرة كاستراتيجية للتخفيف من الآثار السلبية التي يفرضها الإجهاد الملحي على النباتات، وبالتالي تحسين نمو المحاصيل وإنتاجيتها.

كلمات مفتاحية: إجهاد ملحي، تحمل، شعير، فطريات داخلية

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