

Induction of Growth and Osmoregulation in Salt Stressed Barley by the Endophytic Fungus *Chaetomium coarctatum*

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ABSTRACT

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Endophytic fungi have been shown to increase the growth and improve plants tolerance to stressful conditions, especially salinity. The intent of this study was to determine the salt tolerance of barley inoculated by the fungus *Chaetomium coarctatum*, isolated from *Avena fatua* roots collected from saline soil (EC = 14 dS/m). A greenhouse experiment was conducted to test the effects of this selected fungus under increasing salinity levels (EC = 2.5, 8, and 14 dS/m) on seedling growth, leaf area and solute accumulation (proline and sugars) of barley seedlings. Results indicated a positive influence of *C. coarctatum* on barley salinity tolerance. Barley seedling emergence on heavily salted soil (14 dS/m) was improved by *C. coarctatum* (70%), compared to 60% recorded by control. Results showed that *C. coarctatum* increased soluble sugars (under moderate saline soil) and proline leaf contents (under unsalted soil, moderate and high saline soils). Inoculated barley has a higher leaf area (23 cm² under EC = 14 dS/m compared to 21.54 cm² recorded in the control) as well as sugar (29.1 mg/g FW under EC = 2.5 dS/m) and proline content (1.14, 1.99, and 2.21 mg/g FW under EC = 2.5, 8, and 14 dS/m, respectively). *C. coarctatum* fungus improves barley growth under salt stress conditions.

Keywords: *Avena fatua*, barley, *Chaetomium coarctatum*, endophytes, salinity

In nature, plants are confronted by a variety of environmental factors, resulting in adverse changes to growth and development. Among several environmental factors, salinity is one of the major abiotic stress limiting plant growth and productivity in many areas of the world (Alqarawi et al. 2014). Soil salinity affects plant growth by reducing

water uptake, causing toxic accumulation of sodium chloride and reducing nutrient availability. Salinity also induces water deficit by decreasing the osmotic potential of soil and therefore it is difficult for roots to extract water from their surrounding media (Jaleel et al. 2007). It is relatively difficult for producers to expand genetic yield potential of plants under salt stress conditions (Kalhor et al. 2016). High salt concentrations in soils or irrigation waters have devastating effects on plant metabolism, destruct cellular homeostasis and have various negative impacts on

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physiological and biochemical processes (Al-Razak and Al-Saady 2015).

Soil salinization can be a symptom of land degradation, as a result of inappropriate cultural practices and excessive agricultural use (Roy et al. 2014). Application of traditional desalination negatively affects soils. Recent studies have focused on identification of alternative methods to enhance plant productivity and protect the soil (Wei and Jousset 2017). In this context, the symbiotic association of rhizobia and roots of plants decreased severity of salinity (Mhadhbi et al. 2014).

Fungal endophytes may be of special significance because of their ability to colonize plants and help their partner to survive under extreme environmental conditions by secreting beneficial secondary metabolites such as auxin, gibberellin that helps in growth and development of the host plant (Dutta et al. 2014). Endophytic fungi favor plant growth against salt stress by improving the host plant nutrition, increasing Potassium/Sodium ions (K^+/Na^+) ratios and efficiently influencing osmoregulation by accumulation of compatible solutes such as proline, glycine betaine, and soluble sugars (Tuteja et al. 2012).

The current paper explored the effect of endophytic fungus, *Chaetomium coarctatum*, isolated from *Avena fatua* roots collected from saline soil located in Ghelizane; Algerian West (EC = 14 dS/m) on growth and osmoregulation of barley (*Hordeum vulgare*), under salt stress conditions, in order to give comprehensions on the mechanisms by which beneficial fungal endophytes are associated with host plant benefits, and establish the foundations for the design of ecologically friendly practices by using of microorganisms in agriculture.

MATERIALS AND METHODS

Barley inoculation and culture.

Strain 6 of *Chaetomium coarctatum* isolated from roots of *Avena fatua* collected in Ghelizane (Algeria) was used in this study (Kouadria et al. 2018).

Surface sterilized barley (*Hordeum vulgare*) var. Saida183 seeds were inoculated by immersion in *C. coarctatum* spore suspension at a concentration of 10^7 spores/ml for 24 h. Seeds immersed in distilled water were used as control.

Seeds were then placed into PVC pots (19 cm diameter and 50 cm height) filled with soils increasing salinity levels (EC = 2.5 dS/m (unsalted soil), 8 dS/m (moderate saline soil), and 14 dS/m (high saline soil) respectively). The experiment was conducted as a factorial design in randomized blocks with two factors and three-fold replications.

Growth parameters.

Barley seedling emergence rate was determined 15 days after treatment. Leaf area was calculated conventionally by the product of maximum leaf lengths and widths affected by the coefficient 0.75 (Li et al. 2004).

Proline leaf content estimation.

Free proline was estimated following the method of Troll and Lindsley (1955). One hundred mg of leaf was extracted in 2 ml of 40% methanol. One ml extract was mixed with equal volume of acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) and glacial acetic acid. The samples were then incubated at 100°C for 30 min and reaction was terminated by keeping the tubes in ice container. After cooling, proline was separated with 5 ml toluene and optical density was measured at 528

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

Soluble sugar leaf content estimation.

Soluble sugars were extracted in 80% ethanol from 100 mg of leaf fresh tissue and quantified using the anthrone method (Thimmaiah 2004). A standard curve was established using glucose and results are therefore expressed in mg/g of fresh weight (FW).

Statistical analysis.

Analysis of variance (ANOVA) was carried out, using Statbox v6.4 statistical software. Data were represented as mean \pm standard deviations (SD). $P < 0.05$ showed a significant effect. The purpose of these tests was to identify statistically significant effects and interactions among various test and control treatments.

RESULTS

Barley seedlings and leaf area.

Analysis of variance revealed significant effects of "salinity" and "endophytic fungi" factors on the emergence rate of barley seeds ($P < 0.05$). A total emergence rate (100%) was recorded by inoculated and non-inoculated barley grown under 2.5 and 8 dS/m of salinity. Emergence rate decreased under high saline soil (14 dS/m) in *C. Coarctatum*-colonized and non-*C. Coarctatum* colonized barley (Fig. 1A). However, *C. coarctatum* significantly increased the percentage of barley seedling emergence

under soil salinity 14 dS/m (70%), compared to the control (60%).

Salt stress and fungal inoculation significantly affected leaf area ($P < 0.05$). Moreover, saline stress reduced leaf area especially under moderate (17.36 ± 5 cm²) and high saline soil (21.54 ± 3 cm²) compared to the control (30.5 ± 5 cm²).

C. coarctatum inoculation enhanced leaf area of seedlings grown under soil salinity of 14 dS/m (23.74 ± 3 cm²) compared to non-*C. coarctatum* inoculated barley plants (21.54 ± 3 cm²) (Fig. 1B).

Proline and sugar leaf content.

Soil salinity significantly ($P < 0.05$) reduced soluble sugar levels in barley leaves (Fig. 2A).

With saline stress, the positive effect of *C. coarctatum* on barley leaf sugar content was significant under soil salinity of 8 dS/m (29.1 ± 1 mg/g FW) compared with control barley plants (15.57 ± 2.49 mg/g FW).

Results showed a significant increase in proline content as a consequence of both, saline stress and endophyte inoculation ($P < 0.05$). The significant interaction between those factors indicates that, under saline stress, endophyte inoculation induced a higher increase in barley leaf proline content (Fig. 2B).

The maximum proline content was noted in *C. Coarctatum*-inoculated barley under soil salinity of 14 dS/m (2.21 ± 0.38 mg/g FW).

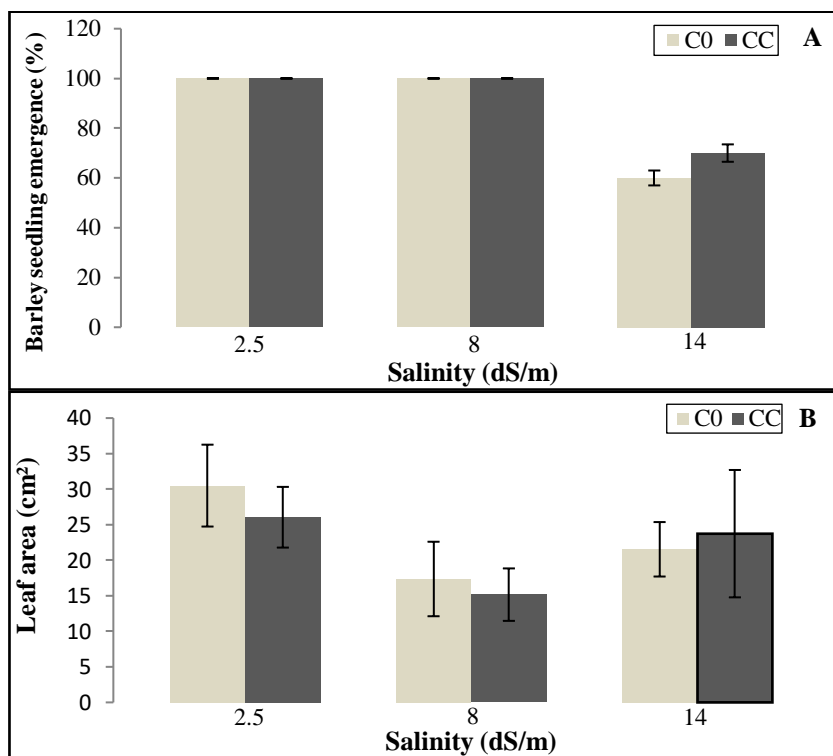


Fig. 1. Effect of salinity (C0) × *Chaetomium coarctatum* (CC) interaction on barley emergence rate (A) and leaf area (B). Segments are SD.

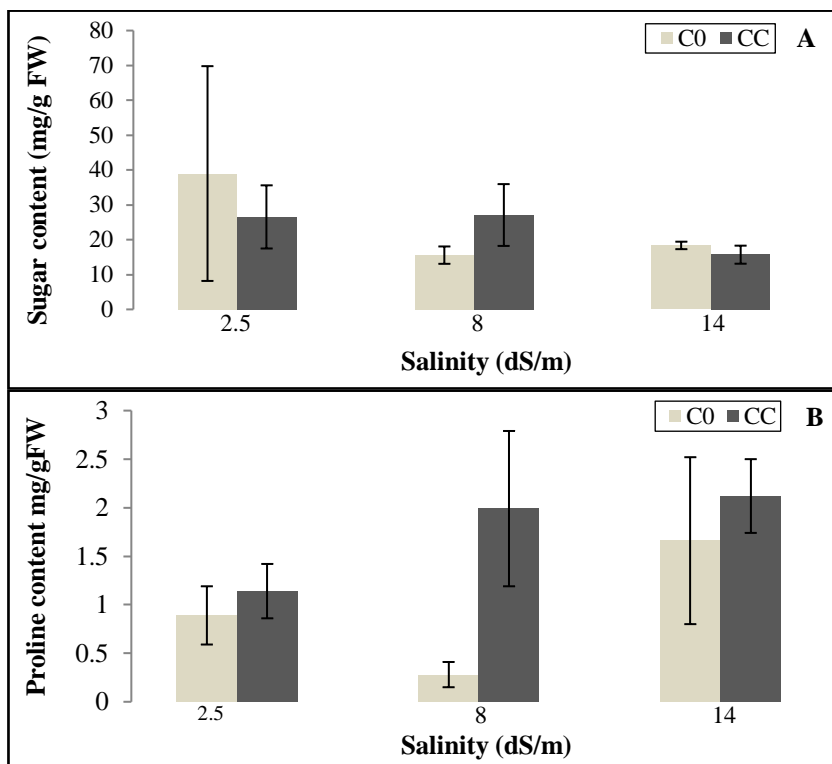


Fig. 2. Effect of salinity (C0) \times *Chaetomium coarctatum* (CC) interaction on barley leaf sugar content (A) and proline content (B). Segments are SD.

DISCUSSION

Climate change, growing food demand and inappropriate agricultural practices combination has stimulated a rapid saline soils expansion worldwide, principally among arid and semi-arid environments (Singh et al. 2015). Under stress conditions, the physiological and biochemical functions of both wild and cultivated plant species are reduced causing severe yield losses (Deinlein et al. 2014). Therefore, alternative and strategic tools to improve agricultural production are needed for the upcoming and challenging years (Roy et al. 2014). In this direction, the potential role of *C. coarctatum*, to expand the saline stress tolerance in barley was tested. Results showed that barley seeds presented sensitivity to salt stress, manifested by significant reduction in seedling growth and leaf area under high saline soil (14 dS/m). A similar decrease in growth has been reported in barley (Mallek-Maalej et al. 2004, El Goumi et al. 2014) and pistachio as salinity levels increased (Jamil et al. 2005). The reduction in leaf area because of salt stress has been considered an avoidance mechanism that permits decreasing water loss through transpiration (Blum 1996).

Inoculation with the endophyte *C. coarctatum* enhanced barley growth under high salt stress which supports the findings of Bae et al. (2009) for cacao (*Theobroma cacao*) colonized by *Trichoderma hamatum* and Rubio et al. (2014) for tomato inoculated by *Trichoderma parareesei*, under salt stress conditions. According to Fortin et al. (2008), endophytic fungi allow plants to have better access to nutrients and substrate water, which promotes their growth and improves plant tolerance to avoid stressful situations (Oses 2008). The host plant may be protected by secondary metabolites produced by

endophyte (Hamayun et al. 2009). Endophytic fungi can also enhance plant growth by phytohormone production, such as indole acetic acid and gibberellic acid (Egamberdieva and Kucharova 2009). Gibberellins are produced by *Penicillium* strains under salt stress to improve plant growth (Leitão and Enguita 2016).

Plants accumulate some organic solutes (proline, soluble sugars) and inorganic ions to maintain higher osmotic adjustment under salt stress conditions (Yang et al. 2009). In the current research, moderate and high salinity induced noticeable variations in proline and soluble sugar contents in barley leaves. In addition to acts as a compatible osmolyte, proline plays a protective role against salt stress in plants (Verbruggen and Hermans 2008). Soluble sugars are important indicators in response to abiotic stress (Azevedo-Neto et al. 2006). The increased accumulation of sugars in plants usually indicates a highly protective mechanism against oxidative damage caused by high salinity (Bartels and Sunkar 2005). Results showed that sugar content decreased with increased of salinity levels. Dubey and Singh (1999) also reported that sugar content increased more in susceptible cultivars than in tolerant cultivars.

Endophytic fungus, *C. coarctatum* has no significant effect on barley leaf sugar content. An opposite result was found following inoculation of perennial ryegrass with *Aspergillus aculeatus* under salt stress, where an increase in total sugar was observed (Li et al. 2017). However, colonization of barley plants by *C. Coarctatum* significantly increased proline levels compared to those of non-*C. Coarctatum* inoculated barley, suggesting that proline was accumulated to provide an energy source for plant growth and survival by

preventing ionic and osmotic imbalances in saline conditions (Manchanda and Garg 2011). Accumulation of proline under salt stress results from the enzyme upregulation involved in proline synthesis (Iqbal et al. 2015). Zhang et al. (2016) reported that *Trichoderma longibrachiatum* can significantly increase wheat seedling proline content under salt or non-saline stress. Similarly, significant accumulation of proline was observed in *Piriformospora indica* (Zarea et al. 2012) and *Paecilomyces formosus* (Khan et al. 2012) inoculated wheat compared to non-inoculated plants under salinity stress.

The current paper allowed exploring the possible mechanisms in which *C. Coarctatum* provides the ability of improving the suppression effect of salt stress. The mechanisms may include (i) *C. Coarctatum* increasing barley seedling growth to resist to salt stress, and (ii) enhancing the relative levels of proline in the stressed plants. However, there are some issues that need to be addressed in future studies, such as the efficacy of *C. Coarctatum* with other plant species and other abiotic stresses to determine the signaling role of *C. Coarctatum* in mitigating negative impacts of salinity stress.

RESUME

Kouadria R., Bouzouina M. et Lotmani, B. 2020. Induction de la croissance et de l'osmorégulation chez l'orge stressée par le sel avec le champignon endophyte *Chaetomium coarctatum*. Tunisian Journal of Plant Protection 15 (1): 19-27.

Les champignons endophytes augmentent la croissance et améliorent la tolérance des plantes aux conditions stressantes, en particulier la salinité. Le but de cette étude était de déterminer la tolérance au sel de l'orge inoculée par le champignon *Chaetomium coarctatum*, isolé des racines de *Avena fatua* prélevées dans un sol salin (CE = 14 dS/m). Une expérience en serre a été menée pour tester les effets de ce champignon sur l'orge sous des niveaux de salinité croissants (CE = 2,5; 8 et 14 dS/m) sur le taux de levée, la surface foliaire et l'accumulation des solutés (proline et sucres). Les résultats ont indiqué une influence positive de *C. coarctatum* sur la tolérance à la salinité de l'orge. Le taux de levée de l'orge sur le sol très salé (14 dS/m) a été amélioré par *C. coarctatum* (70%), contre 60% enregistré par le témoin. Les résultats ont montré que *C. coarctatum* a augmenté le contenu des feuilles en proline (sur les trois sols) et en sucres solubles (sur le sol moyennement salé). L'orge inoculée a une surface foliaire plus élevée (23 cm² sous EC = 14 dS/m comparé à 21,54 cm² enregistré avec le témoin) ainsi qu'une teneur élevée en sucre (29,1 mg/g FW sous EC = 2,5 dS/m) et en proline (1,14; 1,99 et 2,21 mg/g FW sous EC = 2,5; 8 et 14 dS/m, respectivement). Le champignon *C. coarctatum* améliore la croissance de l'orge sous des conditions de salinité.

Mots clés: *Avena fatua*, *Chaetomium coarctatum*, endophytes, orge, salinité

ملخص

قوادرية، ربيعة ومحمد بوزوينة وبرايم العثماني. 2020. حث الشعير على النمو والتنظيم الاسموزي تحت إجهاد الملوحة عن طريق الفطر *Chaetomium coarctatum*.

Tunisian Journal of Plant Protection 15 (1): 19-27.

تساهم الفطريات في زيادة نمو النباتات وتحسين تحملها للظروف الإجهادية، خاصة الملوحة. تهدف هذه الدراسة إلى تحديد درجة تحمل الشعير الملقح بالفطر *Chaetomium coarctatum* المعزول من جنور نبتة *Avena fatua* المأخوذة من تربة مالحة (EC = 14 dS/m). تم إجراء تجربة في بيت محمي من أجل اختبار آثار هذا الفطر على الشعير في ظل مستويات ملوحة متصاعدة (EC = 2,5 ; 8 ; 14 dS/m) على بروز نباتات الشعير ومساحات أوراقها وعلى تراكم البرولين والسكر فيها. بينت النتائج وجود تأثير إيجابي للفطر على تحمل الشعير للملوحة. سُجل تحسن نسبة بروز نباتات

الشعير على التربة عالية الملوحة ($EC = 14 \text{ dS/m}$) بوجود الفطر (70%) بالمقارنة مع الشاهد (60%). وبينت النتائج أن الفطر رفع في محتوى الأوراق من البرولين (مع أنواع التربة الثلاثة) والسكر الذائب (مع التربة متوسطة الملوحة). كان للشعير الملقح أعلى مساحة ورقية (23 سم² مع الملوحة $EC = 14 \text{ dS/m}$ مقارنة بـ 21,54 سم² لدى الشاهد) وكذلك محتوى عال من السكر (29,1 مغ/غ من الوزن الطازج مع $EC = 2,5 \text{ dS/m}$) ومن البرولين (1,14 و 1.99 و 2,21 مغ/غ من الوزن الطازج مع $EC = 2,5$; 8 ; 14 dS/m ، على التوالي). إن هذا الفطر *C. coarctatum* يحسن نمو الشعير تحت ظروف الملوحة.

كلمات مفتاحية: داخلي النبتة، شعير، ملوحة، *Avena fatua*، *Chaetomium coarctatum*

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