INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 6, p. 70-77, 2018

RESEARCH PAPER OPEN ACCESS

Salinity stress resistance of durum wheat (*Triticum durum*) enhanced by fungi

Rabiaa Kouadria*, Mohammed Bouzouina, Redouane Azzouz, Brahim Lotmani

Plant Protection Laboratory, Abdelhamid Ibn Badis University, Mostaganem, Algeria

Key words: Salinity, Endophytic fungi, Germination, Durum wheat.

http://dx.doi.org/10.12692/ijb/12.6.70-77 Article published on June 14, 2018

Abstract

Endophytic fungi are known for their biotic and abiotic resistance. We evaluated the ability of beneficial fungus to overcome salt stress. Fungi strains: *Alternaria chlamydospora*, *Embellisi aphragmospora*, *Phoma betae*, *Fusariume quseti*, *Fusarium graminearum* and *Chaetomium coarctatum* were tested for their ability to improve durum wheat germination under salinity stress conditions (0, 400, 600 and 800meq/l NaCl). Germination, radicle and coleoptile growths were inhibited in high salinity exposed durum wheat seeds, while fungal strains × salinity interaction significantly enhanced germination and growth of durum wheat. The highest germination percentage was showed by *Alternaria chlamydospora* under salt stress conditions (400, 600 and 800meq/L).

* **Corresponding Author:** Rabiaa Kouadria ✉ rabia-rk@hotmail.com

Introduction

Soil salinity is one of the most important factors that limit crop production in arid and semi-arid regions (Neumann, 1995). Benmahioul *et al.* (2009) showed that a large part of agricultural regions characterized by an arid and semi-arid climate in Algeria is affected by salinity process.

Salinity affects plant growth at all developmental stages; however, sensitivity varies from one growth stage to another (Jajarmi, 2009). Germination is an important developmental event in plants (Fowler, 1991) regulated by environmental factors such as salt (Baghbani *et al.*, 2013). Salt concentration completely inhibits germination at higher levels (Rafiq *et al.*, 2006). The plant growth is reduced by salt stress (Munns and Termaat, 1986). Endophytes are the plant-associated microbes that form symbiotic association with their host plants by colonizing the internal tissues (Dutta *et al.*, 2014), without causing any apparent disease symptoms (Petrini, 1991) which made them valuable for agriculture as a tool in improving crop performance (Dutta *et al.*, 2014). Fungal endophytes are ubiquitous; they play crucial roles as decomposers, mutualists, and parasites in ecological processes on earth (Liu *et al.*, 2015). Endophytic fungi have also been shown to impart plants with tolerance to salt, drought, heat and diseases (Khan *et al.*, 2011). Kavroulakis *et al.* (2007) indicate that endophytic fungi can show positive effects on the host plant such as improving growth, resistance and tolerance to biotic and abiotic stresses. They play a role in resistance to certain toxic elements by the production of certain organic acids (Rahman *et al.*, 2006). Many fungal endophytes produce secondary metabolites such as auxin, gibberellin that helps in growth and development of the host plant. Some of these compounds are antibiotics having antifungal, antibacterial and insecticidal properties, which strongly inhibit the growth of other microorganisms, including plant pathogens (Dutta *et al.*, 2014). Pestacin and isopestacin isolated from the culture broth of *Pestalotiopsis microspora*, an endophyte isolated from Terminalia spp. in New Guinea had antimicrobial as well as antioxidant properties (Harper *et al.*, 2003).

According to Hiruma *et al.* (2016), fungus *Colletotrichum tofieldiae* colonizes Arabidopsis roots and transfers the macronutrient phosphorus to its host to boost plant growth and increase fertility under phosphate-deficient conditions.

The goal of this study was to screen fungal strains which not only improve plant-growth but also extend greater salt stress tolerance to durum wheat (*Triticum durum* Desf.) at germination stage.

Materials and methods

Fungi strains

Endophytic fungi *Alternaria chlamydospora, Embellisi aphragmospora*, *Phoma betae*, *Fusariume quseti*, *Fusarium graminearum* and *Chaetomium coarctatum* were isolated from natural vegetation of salt soils located in Relizane; Algerian West (Lat. 35° 47' 46"N, Long. 0° 33' 11", Alt. 50m) and identified using ITS and 18S molecular methods (Unpublished results).

Study of durum wheat culture associated with fungal isolated from salt soil

Seeds of durum wheat variety: SIMETO (*Triticum durum* Desf.) were surface-sterilized for 10mn in 5% sodium hypochlorite, rinsed 3 times with distilled water and then germinated in a phytotron (BINDER) at 25° C in the dark for 4 days. Experimental design had four sets: control (seeds were given solely distilled water), fungal inoculums treated seeds (*Alternaria chlamydospora, Embellisi aphragmospora*, *Phoma betae*, *Fusariume quseti*, *Fusarium graminearum* and *Chaetomium coarctatum*), control of salt stress treatments (400, 600 and 800meq/L), and fungus inoculation with salt stress (Fungal strains were inoculated by spore suspension solution of 10^7 spores/mL).

Studied parameters

Final germination rate: was expressed by the ratio of germinated number seeds on the total seeds number.

Radicle and coleoptile length:were measured using a ruler to evaluate durum wheat growth.

Statistical analysis

Treatments were applied randomly and repeated five times, with 100 seeds in each Petri dish. Analysis of variance (ANOVA) was carried out, using Statbox v6.4 statistical software. Data were represented as mean±standard deviations. $P<0.05$ shows a significant effect. The purpose of these tests was to identify statistically significant effects and interactions among various test and control treatments.

Results

Germination rate

Results in fig.1A. showed a significant reduction in germination percentage under salt stress ($P<0.05$), reflecting that as salt stress increased durum wheat seeds showed a more pronounced decrease in germination percentage. Non-treatment durum wheat seeds showed germination rate of 100%. Germination percentage was estimated to be 15.6% under salt stress 400meq/L. Seeds suffered high mortality (100%) when they were treated with 600 and 800meq/L of NaCl. Endophytic fungi improved germination at all of NaCl concentration levels ($P<0.05$). *Embellisi aphragmospora and Chaetomium coarctatum* showed a germination percentage value of 100%. *Alternaria chlamydospora*, *Phoma betae*, *Fusariume quseti* and *Fusarium graminearum* presented a germination rate superior than 95% (Fig.2A.). Endophytic fungi exhibited significant enhancement of germination percentage under salt stress condition ($P<0.05$). Durum wheat inoculated seeds with fungi strains represented germination percentage with values between 30% and 49.8% under salt stress 800meq/l. The highest germination percentage was showed by *Alternariachlamydospora* under salt stress conditions (93.4%, 62.4% and 49.8%, respectively under salinity conditions 400, 600 and 800meq/L).

Radicle length

Analyses of variance (ANOVA) indicated that radicle growth was affected significantly by salt stress ($P<0.05$). Endophytic fungi presented a significant effect ($P<0.05$) on radicle length under nonsaline conditions. Durum wheat associated with endophytic fungi in saline conditions exposed a significant effect on radicle growth ($P<0.05$). Radicle was elongated deep to 6.36±1.13cm under control condition, while their growth was reduced to 0.26±0.08cm under salt stress 400meq/L (Fig.1B.); radicle growth was absent in seeds treated by 600 and 800meq/l of NaCl. The highest radicle growth was unregistered by seeds inoculated by *Embellisi aphragmospora* (5.52±0.08cm), while the lowest growth was estimated at 3.88±1.15cm by *Fusarium graminearum*.

Coleoptile length

Measures of the coleoptile length are shown in Fig. 1C. and fig. 2C. Increasing salinity significantly decreased coleoptile growth ($P<0.05$). Endophytic fungi revealed a significant effect on coleoptile growth under saline and nonsaline conditions ($P < 0.05$).

Coleoptile length was estimated at 0.13±0.11cm under moderate salt stress 400meq/l NaCl presenting a reduction rate of 27.70% compared to control. Coleoptile growth under high salt stress (600 and 800meq/L) was reduced by 100% compared to control condition (0meq/L). The highest and the lowest coleoptile growth were observed in seeds inoculated by *Phoma betae* (2.15±0.34cm) and *Alternari achlamydospora* (1.36 ±0.88cm), respectively.

Discussion

Germination rate, radicle and coleoptile length

Sensitivity of plants to salinity depends on plant species and their developmental stage (Prado *et al.*, 2000).

Application of increasing salt stress had substantial negative effects on final germination rate, radicle and coleoptile growths. Durum wheat seeds presented sensitivity to salt stress, manifested by significant reduction in germination percentage, radicle and coleoptile length under salt stress (400meq/L).

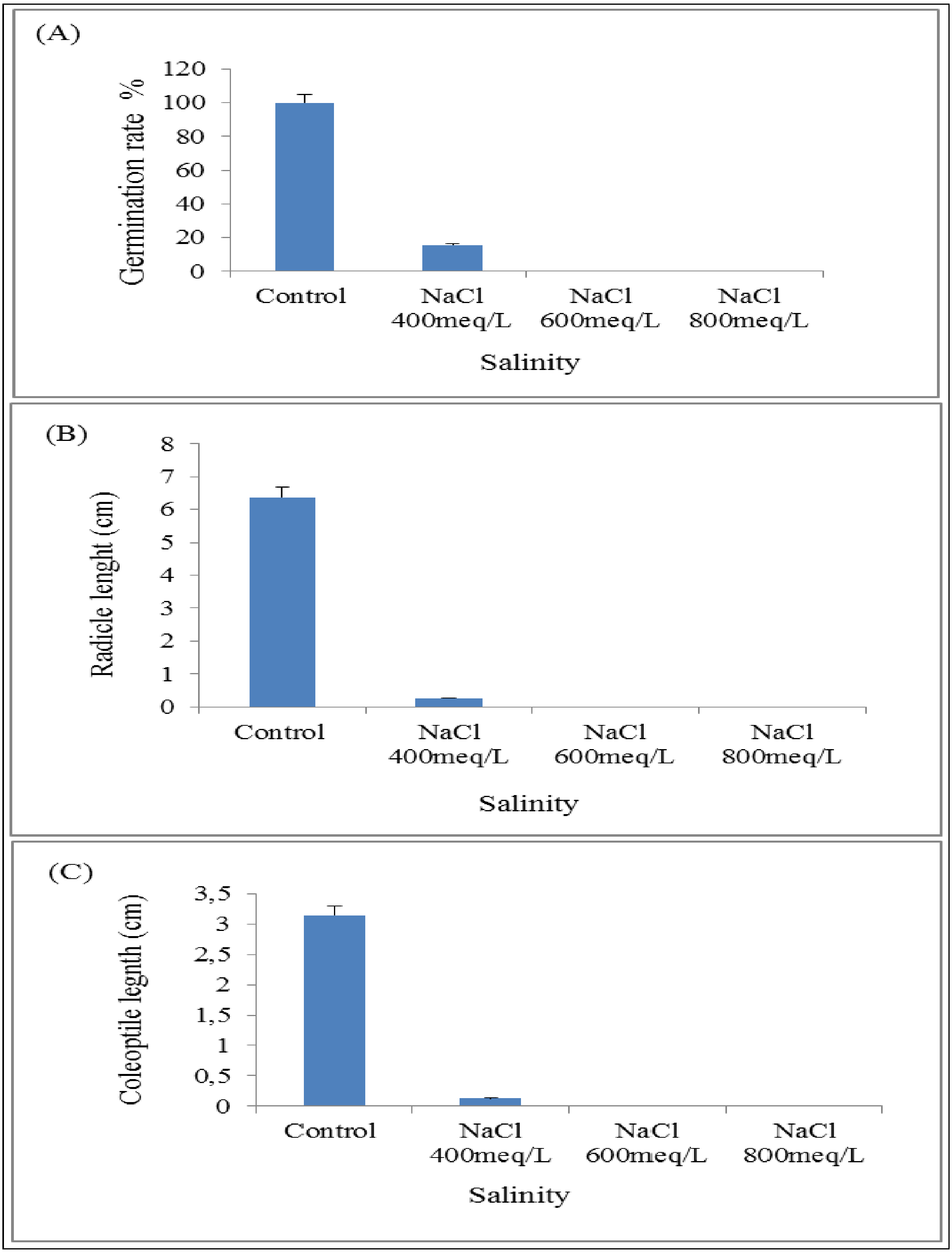

Fig.1. Effect of salinity on (A) germination rate, (B) radicle length and (C) coleoptile length.

A similar decrease in final germination percentage has been reported in all *Brassica* species as salinity levels increased (Jamil *et al.*, 2005). Decrease in final germination rate corresponds either to an increase in the external osmotic pressure, which affects the absorption of water by seeds and / or to an accumulation of Na^+ and Cl^- ions in the embryo (Groome *et al.*, 1991). Germination was totally inhibited under salt stress 600 and 800meq/L.

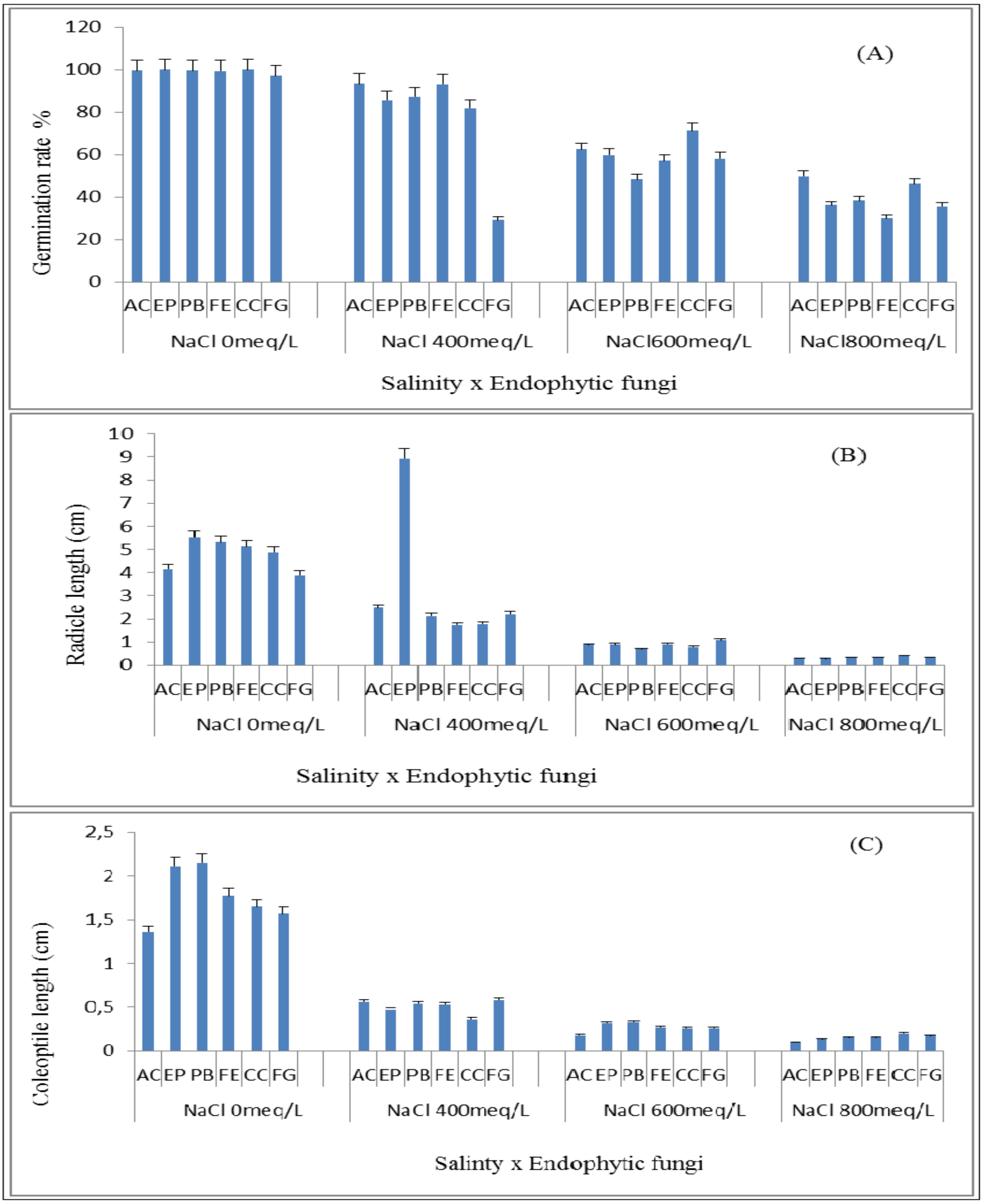

Fig. 2. Effect of salinity × endophytic fungi interaction on (A) germination rate, (B) radicle length and (C) coleoptile length.

Therefore, high salt concentration completely inhibits germination; it also reduces imbibition of water because of lowered osmotic potentials of the medium (Rafiq *et al.*, 2006). When plants were exposed to high salinity stress (100 and 200 mmol/L), Na^+ions were accumulated in plants, leading to osmotic stress and growth inhibition (Wu *et al.* 2017).

Endophytic fungi strains: *Alternari achlamydospora*, *Embellisi aphragmospora*, *Phoma betae*, *Fusariume quseti*, *Chaetomium coarctatum* and *Fusarium graminearum* enhanced germination and growth of durum wheat seeds treated with. According to Fortin *et al.* (2008), endophytic fungi allow plants to have better access to nutrients and substrate water, which promotes their growth. Fungi strains exhibited an enhancement of final germination percentage, radicle and coleoptile growth under saline conditions.

Pigmented endophytes: *Alternaria*, *Embellisia*, *Phoma and Fusarium*, play an important ecological role for plant survival and stress resistance (Sun *etal.*, 2012). Endophytic fungi play a role in tolerance to salinity and resistance to certain toxic elements by production of certain organic acids and other compounds (Rahman and Saiga, 2015).

Root-endophytic basidiomycete, *Piriformospora indica*, has been shown to improve plant resistance against root and leaf diseases and alleviate salt stress in barley (Waller *et al.*, 2005).

Malinowski and Belesky (2006) showed that fungi play a large part in the adaptation of plants, especially grasses, and survival even under severe biotic or abiotic stress.

Endophytic fungi can also enhance plant growth; this is due in part to the production of phytohormones by the endophyte, such as indole acetic acid and gibberellic acid, which induce more root growth, leading to increased nutrient uptake (Egamberdieva and Kucharova, 2009). Hamayun *et al.* (2010) have been conducted to investigate Gibberellic acids (Gas) production by endophytic fungi with plant interactions. Gibberellins are produced by *Penicillium* strains under salt stress to improve plant growth (Leitão and Enguita, 2016). Gibberellic acids (Gas) and indole acetic acid (IAA) secreting 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). Hasan (2002) indicated that gibberellin produced by *Fusarium* under high salt stress may reduce the effect of salinity to plant crops.

Conclusion

The present study indicated that all endophytic fungi were demonstrated their effects on durum wheat seeds to progress their germination and growth.

These findings indicate that endophytic fungi would be useful for agriculture to improve crop plant growth under salt stress conditions and more studies are needed to explore their potentiality in plant protection sector.

References

Baghbani A, Forghani AH, Kadkhodaie A.2013.Study of salinity stress on germination and seedling growth in greenhouse cucumber cultivars. Journal of Basic and Applied Scientific Research **3,**1137-1140.

Benmahioul B, Daguin F, Kaid-Harche M. 2009. Effect of salt stress on germination and in vitro growth of pistachio (Pistacia vera L.). Comptes rendus biologies**332**, 164-170.
http://dx.doi.org/10.1016/j.crvi.2009.03008

Dutta D, Puzari KC, Gogoi R, Dutta P. 2014. Endophytes: Exploitation as a Tool in Plant Protection. Brazilian archives of Biology and Technology. **57,**621-629.
http://dx.doi.org/10.1590/S151689132014020 43

Egamberdieva D, Kucharova Z. 2009. Selection for root colonizing bacteria stimulating wheat growth in saline soils.Biology and Fertility of Soils**45,** 563–571.

Fortin JA, Plenchette C, Piché Y. 2008. Les mycorhizes. La nouvelle révolution verte. MultiMonde Quae. (Eds.), Québec, 131 p.

Fowler JL.1991. Interaction of salinity and temperature on the germination of crambe. Agronomy Journal Abstract **83,** 169-172.

Groome MC, Axler S, Gfford DJ. 1991. Hydrolysis of lipid and protein reserves in lobolly pine seeds in relation to protein electrophoretic patterns following imbibition. Physiologia plantarum **83**, 99-106.

Hamayun M, Khan SA, Khan AL, Tang DS, Hussain J, Ahmad B, AnwarY, Lee IJ. 2010. Growth promotion of cucumber by pure cultures of gibberellin-producing Phomasp.GAH7. World Journal of Microbiology and Biotechnology **26**,889–894.

Harper JK, **Ford EJ, Strobel A, Grant DM, Porco J**, **Toner DP.**2003. Pestacin: a1, 3-dihydroisobenzofuran from Pestaliopsis microspora possessing antioxidant and antimycotic activity. Tetrahedron.**59,**2471-2476.

Hasan HAH. 2002. Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity-calcium interaction. ROSTLINNÁ VÝROBA. **48,** 101-106.

Hiruma K, Gerlach N, Sacristan S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramirez D, Bucher M, O'Connell RJ, Schulze-Lefert P. 2016. Root Endophyte Colletotrichum tofieldiae Confers Plant Fitness Benefits that Are Phosphate Status Dependent. Cell Press **2**,460-474. http://dx.doi.org/10.1016/j.cell.2016.02.028

Jajarmi V. 2009.Effect of water stress on germination indices in seven wheat cultivar, Proceedings of World Academy of Science, Engineering and Technology.**49,** 105–106.

Jamil M, Lee CC, Rehman SU, Lee DB, Ashraf M,Rha ES.2005. Salinity (NaCl) tolerance of Brassicaspecies atgermination and early seedling growth. Electronic Journal of Environmental, Agricultural and Food Chemistry.**4,**970-976.

Kavroulakis N, Ntougias S, Zervakis GI, Ehaliotis C, Haralampidis K, Papadopoulou KK. 2007. Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic Fusarium solani strain. Journal of Experimental Botany **58,** 3853-3864. http://dx.doi.org/10.1093/jxb/erm230

Khan AL, Hamayun M, Kim YH, Kang SM, Lu IJ. 2011. Ameliorative symbiosis of endophyte (Penicillium funiculosum LHL06) under salt stress elevated plant growth of Glycine max L. Plant Physiology and Biochemistry **49,** 852-861. http://dx.doi.org/10.1016/jplaphy.2011.03.005

Leitão AL, Enguita FJ. 2016. Gibberellins in Penicillium strains: Challenges for endophyte-planthost interactions under salinity stress. Microbiological research **183,**8-18. http://dx.doi.org/10.1016/j.micres.2015.11.004

Liu J, **Wang J**, **Gao G**, **Bartlam GM, Wang Y.** 2015. Distribution and diversity of fungi in freshwater sediments on a river catchment scale. Frontiers in Microbiology **6**, 329. http://dx.doi.org/10.3389/fmicb.2015.00329

Malinowski DP, **Belesky DP.** 2006. Ecological importance of Neotyphodium spp. grass endophytes in agroecosystems. Grassland Science.**52,** 1-14.

Munns R, **Termaat A.**1986. Whole-plant responses to salinity. Australien Journal of Plant Physiology **13,** l43–l60

Neumann PM.1995. Inhibition of root growth by salinity stress: Toxicity or an adaptive biophysical response. In: Baluska F, Ciamporova M, Gasparikova O, Barlow PW, Ed. Structure and Function of Roots. The Netherlands: Kluwer Academic Publishers. 299-304 p.

Petrini O. 1991. Fungal Endophytes of Tree Leaves. In: Andrews JH, and Hirano SS, Ed. Microbial Ecology of Leaves. New York: Springer-Verlag. 179-197 p.

Prado FE, Baero C, Gallardo M, Gonzalez JA.2000. Effect of NaCl on germination, growth, and soluble sugar content in Chenopodium quinoa Willd seeds. Botanical Bulletin Academia Sinica. **41,** 27-34.

Rafiq S, Iqbal T, Hameed A, Zulfiqar Ali R, and Rafiq N. 2006. Morpho biochemical analysis of salinity stress response of wheat, Pakistan Journal of Botany, **38,** 1759-1767.

Rahman MH, Saiga S, Sabreen S, Kodama Y, Tsuiki M. 2006. Neotyphodium endophyte infection affects the performance of tall fescue in temperate region Andisols. Grassland Science.**52,**23-28.

Rahman MH, Saiga S. 2005. Endophytic fungi (Neotyphodium coenophialum) affect the growth and mineral uptake, transport and efficiency ratios in tall fescue (Festuca arundinacea). Plant and Soil.**272,**163-171.

Sun Y, Wang Q, Lu XD, Okane I, Kakishima M. 2012. Endophytic fungi associated with plants collected from desert areas in China. Mycological Progress.**11,** 781–790. http://dx.doi.org/10.1007/s11557-011-0790-x

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M.2005. The endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proceedings of the National Academy of Sciences of the United States of America **102,**13386-13391. http://dx.doi.org/10.1073/pnas.0504423102

Wu GQ, Jia S, Liu HL, Wang CM, Li SJ. 2017. Effect of salt stress on growth, ion accumulation, and distribution in sainfoins (Onobrychis viciaefolia) seedlings. Prata-cultural Science.**34,** 1661–1668.