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Improving germination indices of alfalfa cultivars under saline stress by inoculation with beneficial bacteria

M. ANSARI[1,2], F. SHEKARI[1]*, M.H. MOHAMMADI[1], B. BIRÓ[2] AND G. VÉGVÁRI[2,3]

[1] Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Zanjan, Zanjan, P.O. Box 313-45195, Iran (E-mail: shekari@znu.ac.ir)
[2] Department of Soil Science and Water Management, Szent István University, Budapest, Hungary (E-mail: biro.borbala@kertk.szie.hu; vegvari.gyorgy@kertk.szie.hu)
[3] Institute of Physiology, Biochemistry and Animal Health, Kaposvár University, Hungary (E-mail: vegvari.gyorgy@ke.hu; vegvari.gyorgy@kertk.szie.hu)

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Abstract

Salinity stress is one of the most significant factors affecting seed germination of alfalfa. In this study, we considered whether it is possible to reduce the deleterious effects of salinity by inoculating seeds with beneficial salt-tolerant bacteria. Seeds of nine alfalfa cultivars were inoculated with two beneficial bacteria, *Pseudomonas* sp. Proradix and *Hartmannibacter diazotrophicus.* Salinity stress reduced the germination indices in all nine alfalfa cultivars significantly. Inoculation with either bacteria could improve germination performance through increasing germination percentage, germination index, radicle length, plumule length, seed vigour and seedling fresh weight. Germination indices decreased in most cultivars at > 10 dS m^{-1} salt concentration (without inoculation), but were high at 20 dS m^{-1} (inoculation with either bacteria). Germination indices were improved by both the bacteria, however, germination percentage, germination index, plumule length and seed vigour were most improved by inoculation with *Pseudomonas* sp. Proradix; seedling fresh weight and radicle length were most improved by *Hartmannibacter diazotrophicus*. It is suggested that the bacteria could be used to inoculate alfalfa seeds and solve the problem of germination under saline conditions.

Introduction

Salinity stress is one of the most significant limiting environmental factors, affecting plant production in more than 34 million hectares of agricultural lands. Saline soil increases day by day because of unsuitable agricultural practice, using saline water from saline rivers for plant irrigation, low precipitation, high surface evaporation especially on dry lands, and weathering of native rocks such as basalt (Munns and Tester, 2008; Gupta and Huang, 2013). Salinity stress causes reduced water absorption of seeds by decreasing water osmotic potential and inducing osmotic stress (Munns, 2002). The toxic saline ions distort cell division, cell expansion and metabolic processes, and decreases germination percentage, seedling growth and, ultimately, all developmental stages (Zhu, 2001). If seeds can germinate under saline conditions, growth and survival could increase (Hubbard *et al.*, 2012).

* Author for correspondence

There are several strategies for coping with saline stress of which identifying and developing salt-tolerant crops is the most known. This strategy is much desired by researchers, but it has had little success to date (Ashraf *et al*., 2008; Athar and Ashraf, 2009). Another strategy is to identify and develop salt-tolerant microbes (Dodd and Pérez-Alfocea, 2012). *Hartmannibacter diazotrophicus*, which was identified from the rhizosphere of a natural salt-meadow plant in Germany (Suarez *et al*., 2014), *Bacillus amyloliquefaciens*, *Pseudomonas putida* and *P.* sp. Proradix, identified from non-saline soils, are plant-growth-promoting rhizobacteria (PGPR) (www.biofector.info). The bacteria help plants to tolerate biotic and abiotic stress by increasing nutrient uptake from soil and inducing systemic tolerance (Yang *et al*., 2009). PGPRs also help plants by improving biological nitrogen fixation, producing plant hormones, nutrient channelisation by solubilising in absorbable forms and inhibiting plant disease (Suarez *et al*., 2014).

Alfalfa is an important leguminous plant that is cultivated for forage across the world. This plant has high agronomic importance because of its high quality and N_2-fixing ability that improves plant yield and soil structure. Alfalfa is moderately salt-tolerant compared with other legumes but its production decreases in salinity above a threshold of 2 dS m^{-1} and shows 7.3% reduction for every 1 dS m^{-1} increase in salinity (Mass and Haffman, 1977). To be successful in alfalfa production under saline conditions, plants with high germination ability in saline soil are needed. It has been suggested that a combination of stress-tolerant cultivars and stress-tolerant microbes may result in synergistic advantages in the ability of legumes to grow and survive under saline conditions (Bertrand *et al*., 2015). This hypothesis has been supported by Hashem *et al*. (1998) and Nogales *et al*. (2002). Moreover, using beneficial microbes to cope with salinity stress is an economical solution compared with other methods. For this reason, this experiment was conducted to determine the effects of two beneficial saline-tolerant bacteria on germination indices of nine alfalfa cultivars. We sought to understand (i) how typical bacteria isolated from soil can tolerate increasing doses of salt under *in vitro* laboratory conditions; (ii) whether there are any differences in salt-tolerance between the two bacteria; (iii) whether it is possible to reduce the deleterious effect of salinity by inoculation with beneficial salt-tolerant bacteria; and (iv) which parameter is the most appropriate for testing the plant-bacterial interaction under saline conditions, to find further candidates for saline soils.

Materials and methods

Bacterial strains

Four representative soil bacteria (*Pseudomonas* sp. Proradix, *P. putida*, *Bacillus amyloliquefaciens* and *Hartmannibacter diazotrophicus*) were used to identify saline tolerant strains. Three bacteria, *Pseudomonas* sp. Proradix, *P. putida* and *Bacillus amyloliquefaciens*, were obtained from an industrial product used in BIOFECTOR project (www.biofector.info), and *Hartmannibacter diazotrophicus* was kindly provided from the collection of Prof. Sylvia Schnell, Justus Liebig University Gießen, Germany. To evaluate salinity tolerant of the bacterial strains, NaCl were added at 0 (Control), 15, 30, 45 and 60 dS m^{-1} to the nutrient media solution (0.5% meat peptone, 0.3%

extract of meat dry, 0.5% NaCl, 0.5% glucose and distilled water) and incubated 24 hours in the shaker at 23°C and 120 rpm. The growth of bacterial strains was measured by the spectrophotometric method at 530 nm.

Alfalfa cultivars
Nine Iranian cultivar of alfalfa (*Medicago sativa* L.), 'East Azerbaijan', 'Hamadan', 'Hashtrod', 'Heris', 'Ilkhchi', 'Shiraz', 'Kurdistan', 'Local253' and 'Urmia', were gathered from saline areas in northwest Iran in 2016.

Inoculum procedure and germination test
Alfalfa seeds were scarified for two minutes in 98% sulphuric acid and then surface-sterilised for three minutes in 5% sodium hypochloride. After washing, the seeds were placed in sterile Petri dishes and 10 ml microbial solution (1.6×10^{13} cells) added to each of them. The Petri dishes were placed at room temperature for one hour. After drying the seeds, 25 seeds from each cultivar were put in 90 mm-diameter Petri dishes. Germination tests were started by adding saline water to Petri dishes and put in an incubator at 23°C in dark. Germinated seeds were counted every two days and on the eighth day, radicle length, plumule length and seedling fresh weight were measured. The experimental design was factorial and comprised five levels of salt (0 (control), 5, 10, 15 and 20 dS m^{-1}) with nine alfalfa cultivars (randomised complete block design). Seeds was scored as germinated when at least 2 mm of the radicle was visible.

The following germination indices were calculated:

(i) Final germination percentage, excluding dead seeds
(ii) Seed vigour: germination percentage × plumule length
(iii) Germination index: $(8 \times n_2) + (6 \times n_3) + (4 \times n_4) + (2 \times n_5)$, where n_2, n_4, n_6, n_8, = number of germinated seeds, 2, 4, 6 and 8 days after sowing, respectively (Al-Mudaris, 1998)

Data were subjected to ANOVA and means compared using Duncan's multiple range test at $P < 0.05$. All calculations were performed using SAS software, version 9.4.

Results

Selecting salt tolerant bacteria
Analysis of variance showed significant effects of bacterial strain, NaCl concentration and the interaction of the two factors on bacterial growth. NaCl treatment decreased all bacterial strain growth compared with control and growth decreased with increasing NaCl concentration (figure 1). *Hartmannibacter diazotrophicus* had the highest growth at 0, 15 and 30 dS m^{-1} of NaCl compared with the other strains. *Pseudomonas* sp. Proradix had the highest growth at 45 and 60 dS m^{-1} of NaCl compared with the others strains. Based on these results, *Hartmannibacter diazotrophicus* and *Pseudomonas* sp. Proradix were selected as the most saline-tolerant bacteria for inoculating alfalfa seeds.

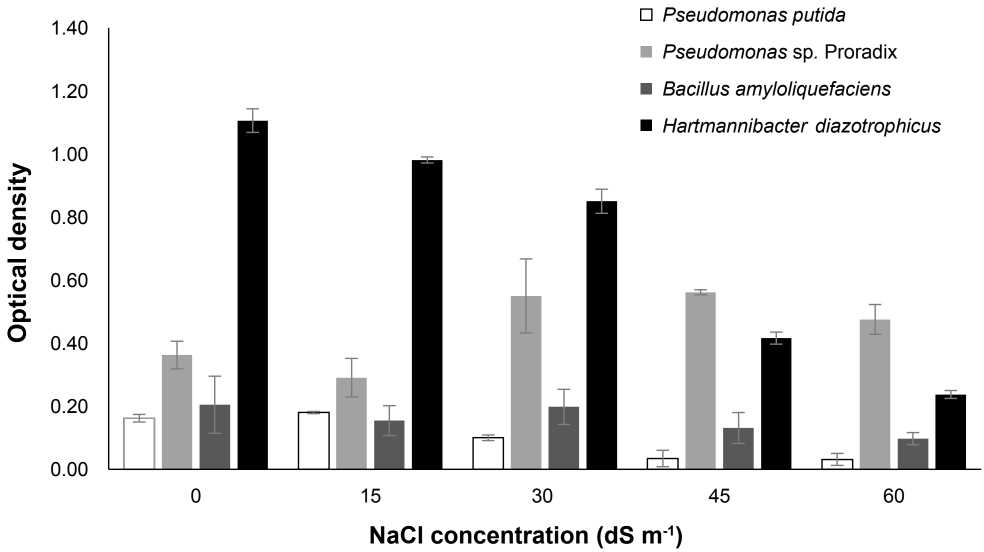

Figure 1. Growth of *Hartmannibacter diazotrophicus*, *Pseudomonas* sp. Proradix, *P. putida* and *Bacillus amyloliquefaciens* under salt stress. Error bars represent standard deviation of the three replicates.

Effect of inoculation on salinity tolerance
Analysis of variance showed significant effects of cultivar, NaCl concentration and bacterial strain on germination indices. There were also significant interaction effects for all germination indices. Salinity stress decreased seed vigour, germination percentage, germination index, radicle length, plumule length and seedling fresh weight compared with the control (figure 2). With increasing salinity concentration, the reduction in all the germination indices was more pronounced. All germination indices decreased at > 10 dS m^{-1} salt concentration and germination in some of the cultivars was near zero at 15 dS m^{-1} without inoculation. After inoculation with either bacteria, all germination indices except radical length were high even at 20 dS m^{-1}. Inoculation with both *Pseudomonas* sp. Proradix and *Hartmannibacter diazotrophicus* improved the germination percentage, germination index, radicle length, plumule length, seed vigour and seedling fresh weight at all salinity and control concentrations.

Differences among cultivars
'Hashtrod' and 'Heris' cultivars had the best germination indices when not inoculated and were identified as saline-tolerant (table 1). 'Local253' and 'Kurdistan' cultivars had the worst germination indices and were identified as saline-sensitive. After inoculation with either bacteria, all germination indices improved significantly. Moreover, differences among cultivars decreased. After inoculation with both bacteria, there were no significant differences among cultivars in the germination percentage and radicle length except for 'Local253'. Plumule length of seedlings from inoculated seeds of 'East Azerbaijan' and 'Hamadan' cultivars improved more than 95 and 108% compared with control. Inoculation with bacteria improved vigour in all cultivars, especially 'East Azerbaijan' and 'Hamadan'

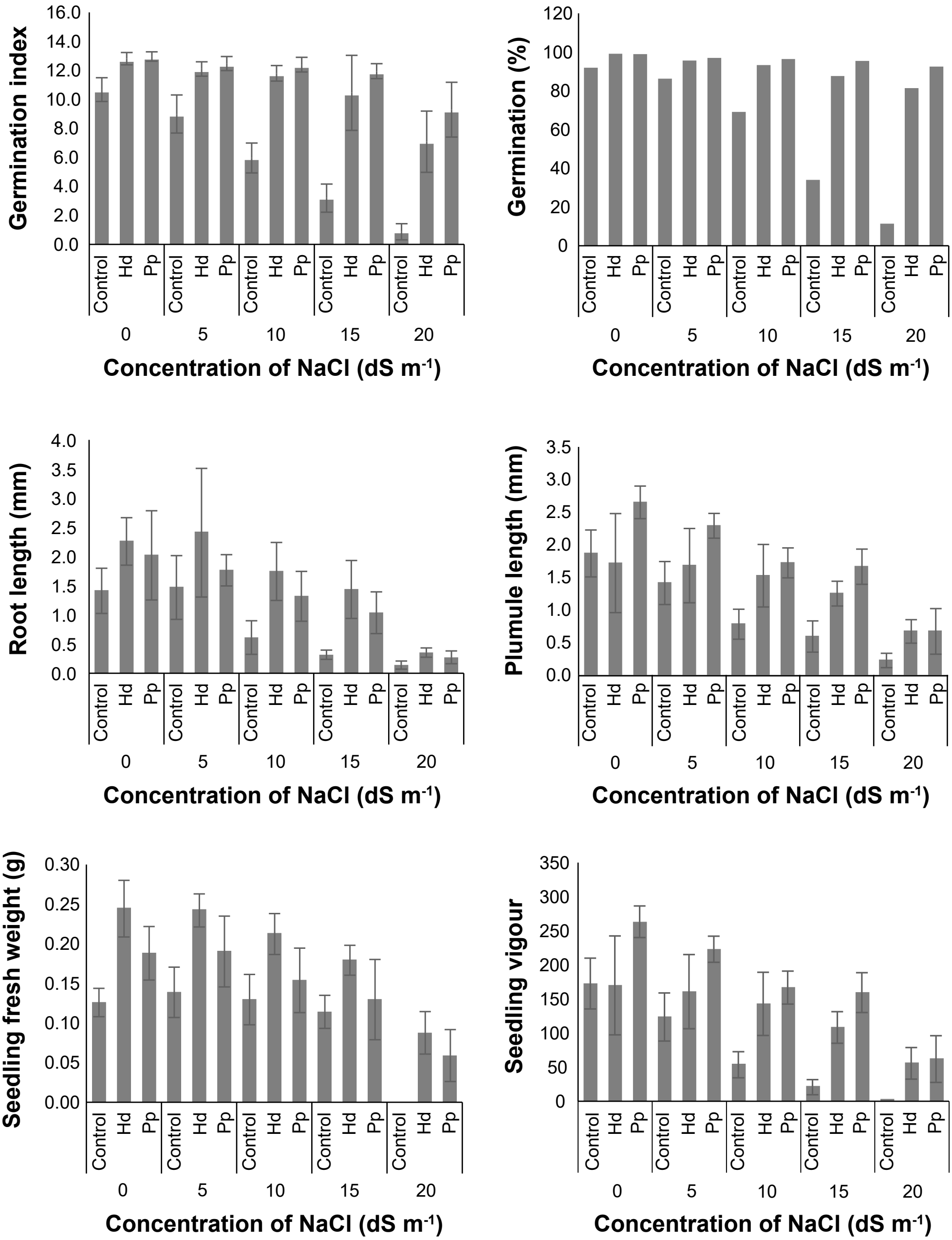

Figure 2. Mean germination index, germination percentage, root length, plumule length, seedling fresh weight and seed vigour across nine cultivars of alfalfa. Seeds were inoculated with *Pseudomonas* sp. Proradix (Pp) or *Hartmannibacter diazotrophicus* (Hd) with and non-inoculated seeds as control and sown at 0, 5, 10, 15 and 20 dS m^{-1} NaCl. Error bars represent standard deviation (three replicates).

Table 1. Mean values for germination indices across different NaCl concentrations for nine alfalfa cultivars, with and without inoculation with beneficial bacteria.

Cultivar	Germination (%)	Germination index	Root length (mm)	Plumule length	Seed vigour	Seedling fresh weight (mg)
			Control (without inoculation)			
East Azerbaijan	59	5.6	7.6	9.7	76.5	0.105
Hamadan	61.6	6.1	7.9	9.1	73	0.09
Hashtrod	65	6.4	9.9	11.5	93	0.113
Heris	64	6.5	7.9	11.0	84	0.095
Ilkhchi	59	6.2	8.5	1.06	84	0.108
Kurdistan	52.8	4.9	9.3	1.06	72	0.116
Urmia	61	6	7.3	10.0	81	0.09
Shiraz	55	5.1	8.6	9.8	75	0.103
Local253	48.5	5.2	4.2	5.7	39.8	0.095
			Inoculation with *Hartmannibacter diazotrophicus*			
East Azerbaijan	93.6	10.5	12.0	19.0	181.9	0.176
Hamadan	91.2	9.9	16.0	20.0	184.1	0.178
Hashtrod	95.2	11.4	16.0	10.0	92.7	0.182
Heris	95.5	11.5	20.0	12.0	115.7	0.208
Ilkhchi	93.3	11.3	19.0	11.0	103.3	0.216
Kurdistan	93.6	11.2	20.0	12.0	115.3	0.220
Urmia	93.9	11.0	16.0	10.0	98.7	0.196
Shiraz	94.1	10.8	19.0	12.0	114.3	0.202
Local253	72.3	8.2	11.0	17.0	148.4	0.168
			Inoculation with *Pseudomonas* sp. Proradix			
East Azerbaijan	97.6	11.4	12.0	19.0	184.5	0.174
Hamadan	96.8	12.2	14.0	18.0	177.4	0.174
Hashtrod	97.1	12.4	15.0	18.0	171.5	0.174
Heris	97.1	12.1	12.0	17.0	163.3	0.068
Ilkhchi	96.8	11.6	16.0	18.0	171.3	0.084
Kurdistan	95.7	11.3	12.0	18.0	176.9	0.154
Urmia	96.8	11.5	13.0	20.0	192.1	0.150
Shiraz	95.7	10.9	11.0	19.0	178.2	0.172
Local253	90.9	11.1	10.0	18.0	162.3	0.152
LSD ($\alpha = 0.05$)	–	0.6	2.7	1.2	11.8	0.25

(139 and 147% increase). Seedlings from inoculated seeds of 'Kurdistan' and 'Shiraz' had the highest fresh weight and increased more than 58 and 81%, respectively, compared with the non-inoculated control. 'Kurdistan' and 'Shiraz' had the highest seedling fresh weight among the alfalfa cultivars, but seedling fresh weight increased more following inoculation for 'Hamadan' and 'Urmia' (more than 95% compared with non-inoculated).

Pseudomonas sp. Proradix had more effect on germination percentage, germination index, plumule length and seed vigour, but *Hartmannibacter diazotrophicus* had more effect on radicle length and seedling fresh weight (figure 2). Inoculation with *Pseudomonas* sp. Proradix and *Hartmannibacter diazotrophicus* increased germination percentage from 11 to 92 and 81% at 20 dS^{-1} and improved germination index 100 and 84%, respectively, compared with non-inoculation. 'Kurdistan' and 'Local253' cultivars had the least germination percentages among the cultivars, but after inoculation with bacteria, germination percentage improved nearly two-fold. Moreover, there were not significant differences between germination percentage of seeds inoculated with *Pseudomonas* sp. Proradix or with *Hartmannibacter diazotrophicus*. 'Kurdistan', 'Shiraz' and 'Local253' cultivars had the lowest germination index among the cultivars before inoculation, but after inoculation, germination index improved 2-fold for 'Kurdistan' and 'Shiraz' (table 1).

Inoculation with either bacteria had a positive effect on radicle length and plumule length in all salinity concentrations, but inoculation did not affect radicle length at 20 dS m^{-1} (table 1). Inoculation with *Pseudomonas* sp. Proradix and *Hartmannibacter diazotrophicus* increased radicle length 65 and 110% and plumule length 84 and 43%, respectively, compared with non-inoculation (figure 2). In non-inoculated conditions, 'Hashtrod', 'Ilkhchi', 'Shiraz' and 'Kurdistan' cultivars had the highest radicle length and plumule length. After inoculation with either bacteria, 'Ilkhchi' had the highest radicle length and 'East Azerbaijan' and 'Hamadan' cultivars had the highest plumule length among the cultivars.

Inoculation with either bacteria improved seed vigour, however, inoculation with *Hartmannibacter diazotrophicus* improved seed vigour more effectively than *Pseudomonas* sp. Proradix in saline and control conditions, whereas *Pseudomonas* sp. Proradix was effective only in saline conditions (figure 2). Inoculation with *Pseudomonas* sp. Proradix and *Hartmannibacter diazotrophicus*, increased seed vigour 132 and 70%, respectively, compared with non-inoculation. 'Hashtrod', 'Heris', 'Ilkhchi' and 'Urmia' cultivars had the highest seed vigour when not inoculated. After inoculation with either bacteria, 'East Azerbaijan' and 'Hamadan' cultivars had the highest seed vigour among the cultivars. The greatest effect of inoculation with bacteria was in 'Local253', in which seed vigour increased nearly 4-fold. Inoculation with *Pseudomonas* sp. Proradix and *Hartmannibacter diazotrophicus*, increased seedling fresh weight 45 and 100%, respectively, compared with non-inoculation. 'Hashtrod' and 'Kurdistan' cultivars had the highest seedling fresh weight in non-inoculated conditions, but after inoculation, 'Kurdistan' had the highest seedling fresh weight among the cultivars.

Discussion

Salinity stress decreased germination percentage and germination index in alfalfa cultivars (figure 2). Similar results in alfalfa cultivars were reported by Wang *et al*. (2009), Li *et al*. (2010) and Cornacchione and Suarez (2015). Salinity stress in low concentration induces dormancy in seeds and decreases germination, but at high concentration, kills the seeds and decreases germination (Shannon and Grieve, 1999). Salt stress reduces and delays seed germination by various factors, such as shortage of water availability, reduces water absorption, changes in the mobilisation of carbohydrates, toxic effects of sodium and chloride ions on embryo viability and altering the structural organisation of proteins (Jahromi *et al*., 2008). Inoculation of alfalfa seeds with either bacteria, improved germination percentage and germination index significantly in all nine cultivars. Similar results were reported in other plants such as cotton (Yao *et al*., 2010), wheat (Ramadoss *et al*., 2013), tomato (Mastouri *et al*., 2010) and maize (Gholami *et al*., 2009).

Radicle length and plumule length decreased under saline stress because of the osmotic and toxic effects. Inoculation with beneficial bacteria mitigated the harmful effects of salinity stress on radicle length and plumule length. Inoculation with either bacterial strain had no significant effect on radicle length in high salinity suggesting that radicle growth is more sensitive to salinity stress than plumule growth. Since the radicle is the first organ to encounter salinity stress, it appears that plant growth reduction in saline stress is related to decrease in root growth under saline conditions. Focus on improving root growth under saline conditions is suggested as a priority for future studies

Decreasing seedling fresh weight under saline stress is related to reduction in radical and plumule growth. Salinity stress by decreasing mitotic division of radical and plumule meristems (West *et al.*, 2004) and inhibiting cell expansion result in decreased growth (Zörb *et al.*, 2015). Inoculation with bacteria via producing growth hormones, vitamins, and increasing higher uptake of nutrient cause increased growth of the radical and plumule and greater seedling fresh weight (Babalola, 2010). Due to improvement in both the germination percentage and plumule length after inoculation with either bacteria, seed vigour obviously improved. 'Hashtrod' and 'Local253' cultivars had the highest and lowest germination indices, respectively, among the cultivars without inoculation, but after inoculation, all germination indices improved. However, but in some cultivars, germination indices were improved more effectively with *Hartmannibacter diazotrophicus* and in other cultivars with *Pseudomonas* sp. Proradix (table 1). Using two saline tolerant bacteria together for inoculating alfalfa seeds may be more effective than using one bacteria alone. The results of this study showed that in alfalfa cultivars, seed germination problem under saline conditions could be solved completely by inoculation with beneficial bacteria. Investigating the positive effects of inoculation with these bacteria on other alfalfa growth stages is suggested for future studies.

Conclusion

Salinity stress significantly decreased germination indices for nine alfalfa cultivars significantly; with increasing salinity concentration, these effects were more pronounced. The most tolerable salt concentration was 10 dS m^{-1}, however, seedling fresh weight showed significant reduction only at 15 dS m^{-1} NaCl. Soil bacteria have variable salt-tolerance; two were identified as saline-tolerant for inoculation studies. Germination indexes in most cultivars decreased at > 10 dS m^{-1} salt concentration (without inoculation), but were high even at 20 dS m^{-1} (inoculation with either bacteria). Incubation with either bacteria improved all germination indices considerably. Germination percentage, germination index, plumule length and seed vigour improved following inoculation with *Pseudomonas* sp. Proradix and radicle length and seedling fresh weight improved with *Hartmannibacter diazotrophicus*.

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References

Al-Mudaris, M.A. (1998). Notes on various parameters recording the speed of seed germination. *Der Tropenlandwirt-Journal of Agriculture in the Tropics and Subtropics*, **99**, 147-154.

Ashraf, M. and Foolad, M.R. (2005). Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in Agronomy*, **88**, 223-271.

Ashraf, M., Athar, H.R., Harris, P.J.C. and Kwon, T.R. (2008). Some prospective strategies for improving crop salt tolerance. *Advances in Agronomy*, **97**, 45-110.

Athar, H.R. and Ashraf, M. (2009). *Salinity and Water Stress*, Springer, Netherlands.

Babalola, O.O. (2010). Beneficial bacteria of agricultural importance. *Biotechnology Letters*, **32**, 1559-1570.

Bertrand A., Dhont, C., Bipfubusa, M., Chalifour, F., Drouin, P. and Beauchamp, C.J. (2015). Improving salt stress responses of the symbiosis in alfalfa using salt-tolerant cultivar and rhizobial strain. *Applied Soil Ecology*, **87**, 108-117.

Cornacchione, M.V. and Suarez, D.L. (2015). Emergence, forage production, and ion relations of Alfalfa in response to saline waters. *Crop Science*, **55**, 444-457

Dodd, I.C. and Pérez-Alfocea, F. (2012). Microbial amelioration of crop salinity stress. *Journal of Experimental Botany*, **63**, 3415-3428.

Gholami, A., Shahsavani, S. and Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Academy of Science, Engineering and Technology*, **3**, 19-24.

Hashem, F.M., Swelim, D.M., Kuykendall, L.D., Mohamed, A.I., Abdel-Wahab, S.M. and Hegazi, N.I. (1998). Identification and characterization of salt- and thermotolerant *Leucaena*-nodulating *Rhizobium* strains. *Biology and Fertility of Soils*, **27**, 335-341.

Hubbard, M., Germida, J. and Vujanovic, V. (2012). Fungal endophytes improve wheat seed germination under heat and drought stress. *Botany*, **90**, 137-149.

Jahromi, F., Aroca, R., Porcel, R. and Ruiz-Lozano, J.M. (2008). Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. *Microbial Ecology*, **55**, 45-53.

Li, R., Shi, F., Fukuda, K. and Yang, Y. (2010). Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (*Medicago sativa* L.). *Soil Science and Plant Nutrition*, **56**, 725-733.

Maas, E.V. and Hoffman, G.J. (1977). Crop salt tolerance-current assessment. *Journal of the Irrigation and Drainage Division*, **103**, 115-134.

Mastouri, F., Björkman, T. and Harman, G.E. (2010). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology*, **100**, 1213-1221.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell and Environment*, **25**, 239-250.

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, **59**, 651-681.

Nogales, J., Campos, R., BenAbdelkhalek, H., Olivares, J., Lluch, C. and Sanjuan, J. (2002). *Rhizobium tropici* genes involved in free-living salt tolerance are required for the establishment of efficient nitrogen-fixing symbiosis with *Phaseolus vulgaris*. *Molecular Plant-Microbe Interactions*, **15**, 225-232.

Ramadoss, D., Lakkineni, V.K., Bose, P., Ali, S. and Annapurna, K. (2013). Mitigation of salt stress in wheat seedlings by halo tolerant bacteria isolated from saline habitats. *SpringerPlus*, **2**, 1-7.

Suarez, C., Ratering, S., Geissler-Plaum, R. and Schnell, S. (2014). *Hartmannibacter diazotrophicus* gen. nov., sp. nov., a phosphate-solubilizing and nitrogen-fixing alphaproteobacterium isolated from the rhizosphere of a natural salt-meadow plant. *International Journal of Systematic and Evolutionary Microbiology*, **64**, 3160-3167.

Yang, J., Kloepper, J.W. and Ryu, C.M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science*, **14**, 1-4.

Yao, L., Wu, Z., Zheng, Y., Kaleem, I. and Li, C. (2010). Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. *European Journal of Soil Biology*, **46**, 49-54.

Wang, W.B., Kim, Y.H., Lee, H.S., Kim, K.Y., Deng, X.P. and Kwak, S.S. (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant Physiology and Biochemistry*, **47**, 570-577.

West, G., Inzé, D., and Beemster, G.T. (2004). Cell cycle modulation in the response of the primary root of Arabidopsis to salt stress. *Plant Physiology*, **135**, 1050-1058.

Zhu, J.K. (2001). Plant salt tolerance. *Trends in Plant Science*, **6**, 66-71.

Zörb, C., Mühling, K.H., Kutschera, U. and Geilfus, C.M. (2015). Salinity stiffens the epidermal cell walls of salt-stressed maize leaves: is the epidermis growth-restricting? *PLoS ONE*, **10**, 1-15.