Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
Spanish Journal of Agricultural Research 2010, 8(S1), S144-S148
ISSN: 1695-971-X

Short communication. Effect of saline soil parameters on endomycorrhizal colonisation of dominant halophytes in four Hungarian sites

A. Füzy*, B. Biró and T. Tóth

Institute of Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences (RISSAC HAS). Herman O. út 15, H-1022 Budapest. Hungary

Abstract

Soil and root samples were collected from the rhizosphere of dominant halophytes (*Artemisia santonicum*, *Aster tripolium*, *Festuca pseudovina*, *Lepidium crassifolium, Plantago maritima* and *Puccinellia limosa*) at four locations with saline soils in Hungary. The correlations- between arbuscular mycorrhiza (AM) fungal colonisation parameters (% colonisation, % arbuscules) and soil physical, chemical and biological parameters were determined Endomycorrhiza colonisation was found to be negatively correlated with the electric conductivity of the soil paste, the salt-specific ion concentrations and the cation exchange capacity, showing the sensitivity of AM fungi at increasing salt concentrations, independently of the types of salt-specific anions. A positive correlation was detected between the mycorrhiza colonisation and the abundance of oligotroph bacteria known to be the less variable and more stable (k-strategist) group. This fact and the negative correlation found with the humus content underlines the importance of nutrient availability and the limitations of the symbiotic interactions in stressed saline or sodic soils.

Additional key words: correlations, halophytes, microbial abundance, salt stress, soil-parameters.

Resumen

Comunicación corta. Efecto de los parámetros de suelos salinos en cuatro sitios de Hungría sobre la colonización de endomicorrizas de halófitas dominantes

Se recolectaron muestras de suelo y raíces de la rizosfera de algunas halófitas dominantes (*Artemisia santonicum*, *Aster tripolium*, *Festuca pseudovina*, *Lepidium crassifolium, Plantago maritima* y *Puccinellia limosa*) en cuatro zonas de Hungría con problemas de salinidad. Se estudiaron las correlaciones/regresiones lineares entre los siguientes parámetros, determinados en los muestreos: colonización de la raíz por micorrizas arbusculares (HMA) (% micorrizas, % arbúsculos); recuentos de microorganismos cultivables (micromicetes y microorganismos del suelo heterótrofos y oligotrófos); y como parámetros físico-químicos se determinaron las concentraciones de iones salinos específicos, pH, contenido en agua y humus (H%) de los suelos. La colonización endomicorrícica presentó una correlación negativa con la conductividad eléctrica del suelo, las concentraciones de iones salinos específicos y la capacidad de intercambio catiónico, mostrando la sensibilidad de los HMA a una salinidad creciente, independientemente de los tipos de aniones salinos específicos. Se detectó una correlación positiva entre la colonización de la raíz por micorrizas y la abundancia en la rizosfera de bacterias oligotróficas, un grupo de bacterias estable y poco variable (estrategas k). Este hecho junto con una correlación negativa con el contenido de humus (H%) demuestra la importancia que puede tener la disponibilidad de nutrientes y las limitaciones de las interacciones simbióticas en suelos salinos o sódicos con problemas.

Palabras clave adicionales: abundancia bacteriana, correlaciones, estrés salino, halófitas, parámetros del suelo.

The physical, chemical and biological properties of saline soils are mainly determined by their water-soluble salts. Saline and sodic soils type and morphology change according to the quality and quantity of their salt-specific ions (Szabolcs, 1998). The macrobiota (halophytes) and microbiota (soil- and rhizosphere

* Corresponding author: fuzy@rissac.hu
Received: 23-09-09; Accepted:14-05-10.

Abbreviations used: A (arbusculum richness), AMF (arbuscular mycorrhizal fungi), CFU (colony-forming units), M (intensity of mycorrhizal colonisation).

microbes) are both influenced by the negative impacts of high salt concentrations (Hasegawa *et al.,* 1986), independently of the identity of salt-specific anions (Füzy *et al.,* 2008b). The interaction between higher plants (halophytes), the microsymbiont arbuscular mycorrhizal fungi (AMF) and beneficial plant-growth promoting rhizobacteria (PGPR) might affect the survival capacity of the plants in these stressed environments. There are several stress factors in these environments: high osmotic pressures, ion-toxicity, imbalanced ion concentrations, unfavourable soil structure and suboptimal soil pH, besides nutrient deficiency that also tends to occur in saline and sodic soils. The water regime of saline soils is imbalanced contributing to create an extreme environment for plant growth. When water is present in saline soils, these swell, become plastic, and the water present becomes unavailable to plants. When the soil dries out, it hardens, becoming less porous, hindering the roots development and subsequently, the nutrient uptake of the plants. Fluctuations of the water content, including flooding episodes are also a stress-factor that can result in oxygen deficiency and/or enhanced CO_2 content in the plant-rhizosphere.

A relatively high mycorrhizal colonisation rate and AM fungi spore numbers have been found in saline soils (Landwehr *et al.,* 2002) showing the importance of the symbiosis in these environments. In these saline soils, a specific mycorrhiza population has been found (Carvalho *et al.,* 2001; Landwehr *et al.,* 2002), with a low diversity, showing that salinity as a selection pressure acts not only on the plant-communities but also on the soil-microbial components. The aim of this work was to study the interrelations between the physical, chemical and biological properties of the saline or sodic soils and AMF root colonisation values in four sites in Hungary to assess the importance of the diverse environmental factors on the symbiosis.

Root and soil samples were collected from the rhizosphere of dominant halophytes *Artemisia santonicum* (L.), *Aster tripolium* (Jacq.), *Festuca pseudovina* (Hack.ex Wiesb.), *Lepidium crassifolium* (W. et K.), *Plantago maritima* (L.), *Puccinellia limosa* [(Schur.) Homberg.] at Spring (April 2001) from four sampling sites in Hortobágy- and Kiskunság National Parks, Hungary. The samples were collected at a depth of 5-15 cm, each sample was a mixture of 5 sub-samples from the rhizosphere soil of five individuals of each halophyte plant species studied. The soil physicochemical parameters measured were: soil-moisture, clay content, higroscopicity, electric conductivity, T-value, Na-content, pH, the salt-specific carbonate-content and the humus content (Buzás, 1988, 1993). Soil biological parameters studied were root colonization of AMF and abundance of some cultivable microbes (micromycetes, oligotrophs, heterotrophs). To assess mycorrhizal colonisation 1-2 g fresh fine roots were cleared by boiling in 15% KOH solution for 40 min, stained in aniline blue (30 min) and fixed in lactic acid (40%). Endomycorrhizal colonisation of the roots was determined by the method of Trouvelot *et al.* (1986). The abundance of hyphae, vesicles and arbuscules in the roots was evaluated; the intensity of mycorrhizal colonisation (M%) and the arbuscules richness (A%) were calculated for all the samples. Dilution series using 1g of the sampled soils were prepared and the appropriate soil suspensions were spread on three types of selective agar plates: nutrient agar for the heterotroph counts, 1/100 strenght nutrient agar for the k-strategist oligotroph counts (Horváth, 1980) and Martin agar for the micromycetes (Martin, 1950). Plates were incubated for 24-72 hours in 28°C and colony-forming units (CFU) were estimated.

Data sets were analysed by linear regression; and significant correlation ratios were determined. CFU data of countable microbes were log transformed; mycorrhizal colonisation values were arc-sin transformed before the appropriate variance analysis. Statistical evaluation was done for the whole range of root-samples, collected and also for the most common halophyte (*Plantago maritima* L.) in order to exclude host-plant effects.

Significant correlations between the mycorrhizal colonisation, both as the infection intensity (M %) and also as the arbuscules richness (A %) and some soil physicochemical properties are shown in Figure 1. The upper part of the figure shows the correlations considering all the six plant species sampled; the lower part shows the result of the statistical analysis limited to one plant species, the known salt-tolerant host, *Plantago maritima*. Statistically significant negative correlations were found between the mycorrhizal colonisation intensity (M %) and/or the arbuscules richness (A %) and some of the measured soil physicochemical parameters. Most of the significant physicochemical parameters relate to the soils salt content, such as the electric conductivity (Fig. 2a), cation exchange capacity and cation content of the soil samples, more particularly the Na^+ content of the soil (Fig. 2b). Some of these correlations are significant only for *P. maritima*. A

	Soil moisture	Clay content	Hygroscopicity	Electric conductivity	T-value	Na	Cation content	pH	Carbonat content	Humus content	Heterotroph CFU	Microscopic fungi	Oligotroph CFU
According to the data of several plant species													
Mycorrhizal colonisation	0	0	0	0	–	0	–	++	0	–	0	0	+
Arbusculum richness	0	0	0	0	0	–	–	+	0	0	0	0	0
According to the data of one plant species													
Mycorrhizal colonisation	0	0	–	– –	–	– –	– –	+	0	–	0	0	0
Arbusculum richness	0	0	0	– –	0	– –	– –	0	0	0	0	0	0

Figure 1. Correlation matrix between the main mycorrhizal colonisation values (infection intensity, arbusculum richness) and some other soil properties, including the physical-chemical and other biological parameters. Upper part of the figure is the cumulative results of all the six plant species tested (*Artemisia santonicum, Aster tripolium, Festuca pseudovina, Lepidium crassifolium, Plantago maritima* and *Puccinellia limosa*); lower part shows the data of *Plantago maritima* only. 0: no correlation, +/–: significant correlations at $p = 0.95$ level, ++/– –: significant correlations at $p = 0.99$ level.

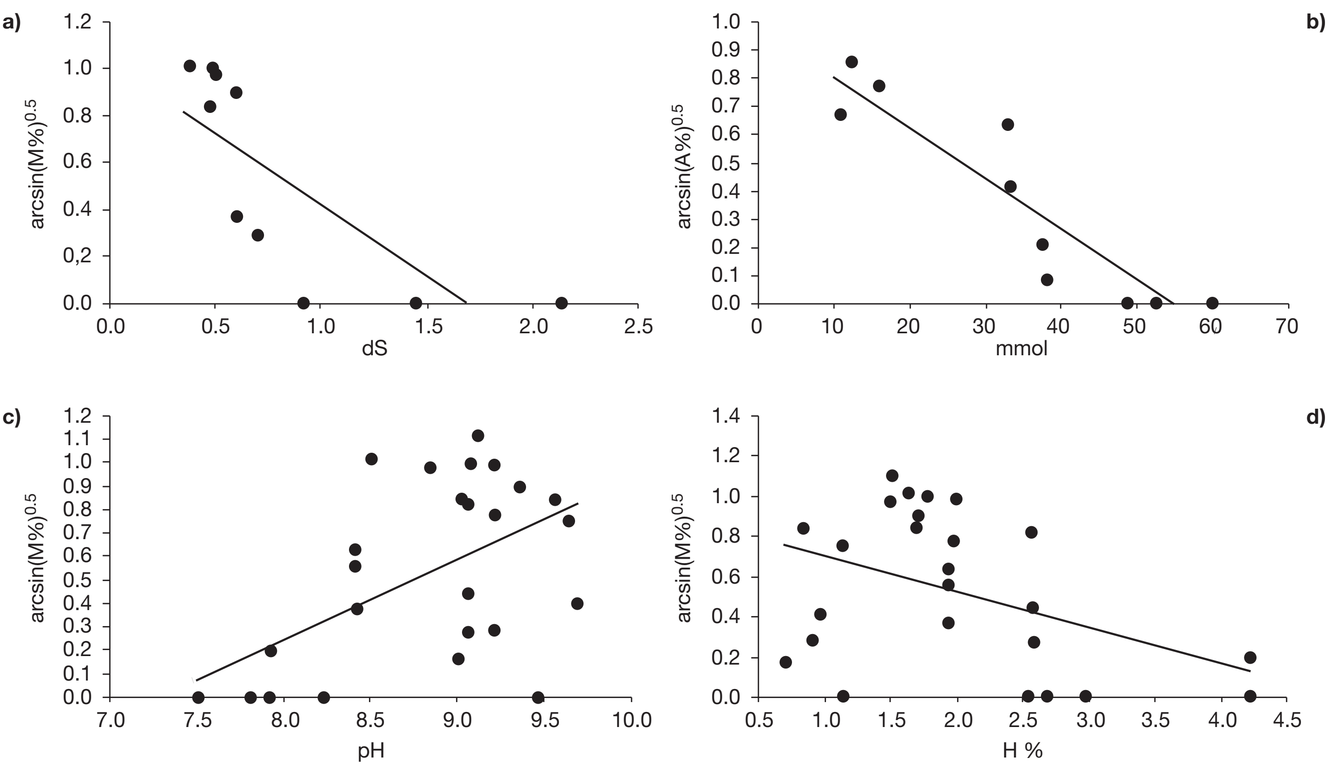

Figure 2. Results of linear correlation analysis between the mycorrhizal colonisation values and some of the soil physical-chemical-biological parameters studied. a) Infection intensity of mycorrhizal colonisation (M %) and electric conductivity (dS) of soil pasta. b) Arbuscules richness of roots (A %) and Na content of soil (mmol L^{-1}). c) Infection intensity of mycorrhizal colonisation (M %) and soil pH. d) Infection intensity of mycorrhizal colonisation (M %) and humus content (H %).

negative correlation was detected between mycorrhizal colonisation and the soil humus content (H %) (Fig. 2d), whereas soil pH values were correlated positively with the root colonisation rates (M%) (Fig. 2c), and the arbuscules content (A %).

Figure 1 shows the correlation data between the AM fungi and the three groups of cultivable microorganisms (micromycetes, heterotrophs and oligotrophs), assessed with selective plates from the rhizosphere of the studied halophytes. Among these cultivable microbes the oligotroph bacteria showed the most stable count; there were less than one order of magnitude variability among the sampling sites and sub samples. The variability in the counts of the heterotroph bacteria and the microscopic fungi from different host-plants rhizosphere was three orders of magnitude, meaning more than 1000-times difference in the counts. A positive correlation was found between the AMF colonisation values (M %, A %) and the abundance of oligotroph bacteria in the rhizosphere.

The soil physico-chemical parameters of saline and sodic soils have a defined effect on the studied endomycorrhizal colonisation. Soil physical parameters, have been shown to influence soil biological parameters (Al-Karaki, 2000; Hildebrandt *et al.,* 2000; Carvalho *et al.,* 2001). Seasonal samplings done at the same saline locations (Füzy *et al.,* 2006, 2008a) showed that soil water content have an effect on the soil biota and on the mycorrhizal colonisation. Parameters describing the quality and quantity of salts in the soil are strongly correlated with mycorrhizal colonisation. These results are in agreement with other studies, which showed a negative impact of high salt concentrations on the symbiosis formation (Juniper and Abbott, 1993; Aguilera *et al.,* 1998; Al-Karaki, 2000), the spore germination (Juniper and Abbott, 1993), and on hypha elongation (McMillen *et al.,* 1998). The negative effects of continuous salt stress might also select a more salt tolerant mycorrhizal population. Dominance (at about 80% abundance) of *Glomus geosporum* species was found by Landwehr *et al.* (2002) in salt-affected sites in Germany and in Hungary, irrespectively of the type of salt-specific anions in the sampled soil.

Mycorrhizal colonisation in saline and sodic areas has been found higher than average values found in other non stressed areas, showing an increase of mycorrhiza dependent plants in areas under this environmental stress (Hildebrandt *et al.,* 2000). Some AM fungi have shown to be able to tolerate a specific environmental stress (Regvar *et al.,* 2003; Ruiz-Lozano, 2003; Biró *et al.,* 2005) and might be capable to confer this tolerance to their non-adapted host-plants, as shown by Hildebrandt *et al.* (1999) on heavy metal contaminated sites. Besides salinity other factors linked to specific characteristics of the soil can influence the establishment and development of the symbiosis. In our study the alkalinity of the soil ranged from 7.5 to 10, the increase of pH was found to correlate positively with AMF colonisation. Sidhu and Behl (1996) showed that alkaline soils are favourable for mycorrhizal colonisation and for extraradical hyphal growth. The plant available P content of soils depends highly on the soil pH. As mycorrhizal symbiosis play an important role in phosphorus uptake, soil parameters involved in the P cycle can form a complex system with the biological parameters determining the ecophysiology of the host-plants. In saline conditions the need for a better nutrient supply and thus a stronger mycorrhizal colonization increases. The negative effect of the humus content (H %) of soil on the mycorrhizal colonisation also suggests the important role of the symbiosis in the nutrient uptake, specially in nutrient deficient soils (Marschner, 1998). When nutrient availability increases mycorrhiza dependency decreases, as found in this study.

There is a positive correlation between the mycorrhizal colonisation and the oligotrophic bacterial counts in the rhizosphere soil of halophytes. The abundance, ratio and dynamism of the so-called «k-strategist» oligotroph bacteria might be an important factor of soil quality and a biological indicator, as it was shown by Karlen *et al.* (1997) and Biró *et al.* (2002). The oligotrophs require a low-nutrient availability media, have a slow reproduction capacity, and are able to survive more efficiently in unfavourable (stressed) environmental (soil) conditions. This group of bacteria form a cluster of stable microbes, increasing the k-strategist ratio of the soil ecosystems. These k-strategist microbes, correlate positively with the mycorrhizal fungi, and might interact with the symbiosis acting either as mycorrhiza helper bacteria or have a plant growth promoting effect.

Adaptation to extreme environmental conditions is a complex phenomenon, specially when considering plant-microbe interactions. The plant's physiology and the rhizosphere microbiota are both capable of adaptative changes, interacting in multifactorial stressed environments (Schwarz and Gale, 1984). This interaction results in a complex, more plastic, system, which can tolerate saline habitats more efficiently. The tight relations between rhizobiological parameters and environmental stress factors can be the basis for the development good indicators for soil quality (O'Neill *et al*., 1986; Visser and Parkinson, 1992; Kling and Jakobsen, 1998).

Acknowledgements

Support of the COST Action 838. and the EU-kp 5 MYCOREM project is highly acknowledged.

References

AGUILERA L.E., GUTIÉRREZ J.R., MORENO R.J., 1998. Vesiculo arbuscular mycorrhizae associated with saltbushes *Atriplex spp*. (Chenopodiaceae) in the Chilean arid zone. Rev Chil Hist Nat 71, 291-302.

AL-KARAKI G.N., 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza 10, 51-54.

BIRÓ B., VILLÁNYI I., KÖVES-PÉCHY K., 2002. Abundance and adaptation level of some soil-microbes in salt-affected soils. Agrokémia, Talajtan, 50, 99-106.

BIRÓ B., POSTA K., FÜZY A., KÁDÁR I., NÉMETH T., 2005. Mycorrhizal functioning as part of the survival mechanisms of barley (*Hordeum vulgare* L.) at long-term heavy metal stress. Acta Biol Szegediensis 49, 65-68.

BUZÁS I., 1988. Talaj és agrokémiai vizsgálati módszerkönyv 2. Mezőgazdasági Kiadó. Budapest. 243 pp. [In Hungarian].

BUZÁS I., 1993. Talaj és agrokémiai vizsgálati módszerkönyv 1. INDA 423 Kiadó. Budapest. 357 pp. [In Hungarian].

CARVALHO L.M., CACADOR I., MARTINS-LOUCAO M.A., 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). Mycorrhiza 11, 303-309.

FÜZY A., TÓTH T., BIRÓ B., 2006. Seasonal dynamics of mycorrhizal colonisation in the rhizosphere of some dominant halophytes. Agrokémia, Talajtan, 55, 231-240.

FÜZY A., BIRÓ B., TÓTH T., HILDEBRANDT U., BOTHE H., 2008a. Drought, but not salinity determines the apparent effectiveness of halophytes colonized by arbuscular mycorrhizal fungi. J Plant Phsyol 165, 1181-1192.

FÜZY A., TÓTH T., BIRÓ B., 2008b. Soil-plant factors, others than the type of salt-specific anions are determining the mycorrhiza colonisations of halophytes. Community Ecol 9(Suppl), 1-8.

HASEGAWA P.M., BRESSAN R.A., HANADA A.K., 1986. Cellular mechanisms of salinity tolerance. Hort Sci 21, 1317-1324.

HILDEBRANDT U., KALDORF M., BOTHE H., 1999. The zinc violet and its colonization by arbuscular mycorrhizal fungi. J Plant Physiol 154, 709-714.

HILDEBRANT U., JANETTA K., OUZIAD F., RENNE B., NAWRATH K., BOTHE H., 2000. Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. Mycorrhiza 10, 175-183.

HORVÁTH S., 1980. Mikrobiológiai praktikum [Manual of Microbiology], Tankönyvkiadó, Budapest. 590 pp. [In Hungarian].

JUNIPER S., ABBOTT L., 1993. Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 4, 45-57.

KARLEN D.L., MAUSBACH M.J., DORAN J.W., CLINE R.G., HARRIS R.F., SCHUMAN G.E., 1997. Soil quality: a concept, definition, and framework for evaluation. Soil Sci Soc Am J 61, 4-10.

KLING M., JAKOBSEN I., 1998. Arbuscular mycorrhiza in soil quality assessment. AMBIO 27, 29-34.

LANDWEHR M., HILDEBRANDT U., WILDE P., NAWRATH K., TÓTH T., BIRÓ B., BOTHE H., 2002. The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. Mycorrhiza 12, 199-211.

MARSCHNER H., 1998. Role of root growth, arbuscular mycorrhizal, and root exudates for the efficiency in nutrient acquisition. Field Crops Res 56, 203-207.

MARTIN J.P., 1950. Use of acid rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci 69, 215-232.

McMILLEN B.G., JUNIPER S., ABBOTT L.K., 1998. Inhibition of hyphal growth of a vesicular-arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. Soil Biol Biochem 30, 1639-1646.

O'NEILL R.V., DEANGELES D.L., WAIDE J.B., ALLEN T.F.H., 1986. A hierarchical concept of ecosystems. Princeton Univ Press, NJ, USA. 263 pp.

REGVAR M., VOGEL K., IRGEL N., HILDEBRANDT U., WILDE P., BOTHE H., 2003. Colonization of pennycresses (*Thlaspi* spp.) of the Brassicaceae by arbuscular mycorrhizal fungi. J Plant Physiol 160, 615-626.

RUIZ-LOZANO J.M., 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13, 309-317.

SIDHU O.P., BEHL H.M., 1996. Response of three *Glomus* species on growth of *Prosopis juliflora* Swartz at high pH levels. Symbiosis 23, 23-34.

SCHWARZ M., GALE J., 1984. Growth response to salinity at high levels of carbon dioxide. J Exp Bot 35, 193-196.

SZABOLCS I., 1998. Concepts, assessments and control of soils affected by salinization. Adv Geo Ecol 31, 469-476.

TROUVELOT A., KOUGHT J.L., GIANINAZZI-PEARSON V., 1986. Mesure du taux de mycorhization VA d'un systéme radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. Proc 1st European Symposium on Mycorrhizae, INRA, Paris. pp. 217-221. [In French].

VISSER S., PARKINSON D., 1992. Soil biological criteria as indicators of soil quality: soil micro-organisms. Am J Alternat Agric 7, 33-37.