Journal of Plant Physiology **164** (2007) 1144—1151

Available online at www.sciencedirect.com

ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY

www.elsevier.de/jplph

Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi

Mozafar Sharifi[a,*], Mahlagha Ghorbanli[b], Hassan Ebrahimzadeh[c]

[a]*Department of Plant Biology, Faculty of Basic Sciences, Tarbiat Modarres University, P.O. Box 14115-175, Tehran, Iran*
[b]*Department of Biology, Azad University, Gorgan, Iran*
[c]*Department of Biology, Faculty of Basic Sciences, University of Tehran, Enghelab Ave., Tehran, Iran*

Received 5 April 2006; accepted 27 June 2006

KEYWORDS
Arbuscular mycorrhizae;
Glycine max;
Salt acclimation;
Salt stress;
Salt tolerance

Summary

Two sets of experiments to determine the effect of mycorrhiza on soybean (*Glycine max*) growth under saline conditions and to investigate the salt acclimation of mycorrhizal fungi were conducted. In the first experiment, the effect of an arbuscular mycorrhizal (AM) fungus *Glomus etunicatum* on mineral nutrient, proline and carbohydrate concentrations and growth of soybean. Under different NaCl concentrations (0, 50, 100, 150 and 200 mM) was evaluated. Salinity decreased AM colonization. In both the M and nonAM plants shoot and root proline and shoot Na and Zn concentrations were increased under salinity. Soybean plants inoculated with the AM fungus had significantly higher fresh and dry weight, root proline, P, K and Zn but lower shoot proline and Na concentrations compared to the non inoculated plants. In the second experiment, the AM fungus was pre-treated with NaCl (salt acclimation) then was used as inoculum for soybean plants subjected to 100 mM NaCl. Root colonization, fresh and dry weight, root proline, P, K and Zn concentrations were greater in soybean plants inoculated with the salt pre-treated fungus, compared to those inoculated with the nonsalt pre-treated fungus. However, for Na, the situation was the opposite. Based on these results, the AM inoculation helps the growth of soybean plants grown in saline conditions. When the AM fungus was pre-treated with NaCl with a gradual increase of concentration, and then exposed to a sudden salt stress, their efficiency was increased. This may be due to the acclimation of the AM fungus to salinity.

Abbreviations: AM, arbuscular mycorrhizal; CFU, colony forming unit; DW, dry weight; ECe, electrical conductivity; LSD, least significant difference; NonAM, nonarbuscular mycorrhizal

*Corresponding author. Tel.: +98 21 88011001; fax: +98 21 88009730.
E-mail address: msharifi@modares.ac.ir (M. Sharifi).

0176-1617/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2006.06.016

Introduction

One of the most widespread agricultural problems in arid and semiarid regions is soil salinity, which renders fields unproductive. In saline soils, NaCl is the most common salt. High salinity may induce imbalances in the soil:plant osmotic relationships (Wyn Jones and Gorham, 1983) and in plant metabolism (Singh and Jain, 1982). In general, salinity limits plant growth and productivity (Al-Karaki, 2000; Ashraf and Foolad, 2006; Ghazi and Al-Karaki, 2006). Incorporating factors that enable plants to tolerate salt stress could improve plant growth and production under saline conditions.

Many studies have demonstrated that inoculation with arbuscular mycorrhizal (AM) fungi improves growth of plants under salt stress (Jindal et al., 1993; Yano-Melo et al., 2003; Giri and Mukerji, 2004; Tian et al., 2004; Cho et al., 2006; Ghazi and Al-Karaki, 2006). The improved growth of AM plants has been attributed to enhanced acquisition of mineral nutrients such as P, Zn, Cu and Fe (Nelson and Safir, 1982; Al-Karaki et al., 2001; Ghazi and Al-Karaki, 2006). Increased plant growth under salinity following mycorrhizal colonization may be caused by enhanced P uptake by AM plants and subsequent increased growth (Poss et al., 1985; Duke et al., 1986). However, in some cases plant salt tolerance was not related to P concentration (Danneberg et al., 1992; Ruiz-Lozano et al., 1996).

Pearson and Schweiger (1993) showed a negative correlation between root carbohydrate concentration and percentage of root colonized by AM fungi. In contrast, Thomson et al. (1990) found a positive relationship between carbohydrate concentration in the roots and root colonization. Changes in the composition of carbohydrates of the host plant may play a role in increasing salt tolerance (Rosendahl and Rosendahl, 1991). Amino acid proline accumulation is one of the most frequently reported modifications induced by water and salt stresses in plants and is often considered to be involved in stress resistance mechanisms. Accumulation of this amino acid is thought to be involved in osmotic adjustment of stressed tissues (Delauney and Verma, 1993; Ashraf and Foolad, 2006). It has been suggested that salt stress induces proline accumulation in legumes (Ashraf, 1989; Sharma et al., 1990; Rabie and Almadini, 2005). There is evidence that proline concentration is greater in *Vigna radiat*a (Jindal et al., 1993) and *Vicia faba* (Rabie and Almadini, 2005) when were inoculated with AM fungi.

Salt tolerance in plants is increased by exposure to gradually increased salinity. This increase is known as acclimation and involves a range of alterations in cell solute concentrations (Rodriguez et al., 1997). A review of literature reveals that no investigations have been carried out in relation to salt acclimation of AM plants. However, it has been reported that exposure of AM fungi to low temperature prior to freezing resulted in increased freezing resistance (Addy et al., 1998).

The present work was carried out to investigate the effect of different concentrations of NaCl on mineral nutrient, proline and carbohydrate concentrations and growth of soybean inoculated or not with *Glomus etunicatum*. The effect of salt pre-treatment of the AM fungus on salt tolerance of inoculated soybean and on the mentioned parameters was also studied. Two experiments were conducted. In the first experiment, the effect of different concentrations of NaCl on the above-mentioned parameters was examined. The highest NaCl concentration at which the plant could grow without suppression of AM colonization was found and used as a salt stress treatment for soybean in the second experiment. In the second experiment, the effect of the salt pre-treatment of the AM fungus on growth and salt tolerance of the host plants under the salt stress treatment was studied. Our hypothesis was that AM fungi exposed to low salt concentration prior to salt stress have a higher capacity to alleviate the saline stress in plants.

Materials and methods

Experiment 1

The AM fungus (*G. etunicatum* Becker and Gerdemann, strain Sh21, Department of Applied Microbiology, Jahad-daneshgahi of Tehran University, Tehran, IRAN) was isolated from a soybean (*Glycine max* [L.] Merr cv. Pershing) field in northern Iran and cultured in pots containing a 4:1 mixture of sterilized sand: soil using maize (*Zea maize*) as the host plant. A mixture of soil, root fragments (46% colonization) and spores (15 spores g^{-1} soil) obtained from the pots was used as AM inoculum. For plant growth, a 4:1 mixture of autoclaved sand: soil was used in 20 cm $\times$ 14 cm $\times$ 15 cm plastic pots (1.5 kg per pot). The mixed soil was analyzed for nutrients. The soil had 3.62 mg available P (Olsen P), 112 mg available K, 100 mg total N per kg soil, pH 8.1 (soil:water 1:1), $EC_e = 1.82\,dS\,m^{-1}$, 0.12% organic carbon, 93% sand, 3% silt and 4% clay. Half of the pots received the AM fungus *G. etunicatum* by adding 100 g of the inoculum, while the other half received 100 g of the autoclaved inoculum, as nonarbuscular mycorrhizal (nonAM) controls.