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Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots

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Abstract The effect of colonization with the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe on the growth and physiology of NaCl-stressed maize plants (*Zea mays* L. cv. Yedan 13) was examined in the greenhouse. Maize plants were grown in sand with 0 or 100 mM NaCl and at two phosphorus (P) (0.05 and 0.1 mM) levels for 34 days, following 34 days of non-saline pre-treatment. Mycorrhizal plants maintained higher root and shoot dry weights. Concentrations of chlorophyll, P and soluble sugars were higher than in non-mycorrhizal plants under given NaCl and P levels. Sodium concentration in roots or shoots was similar in mycorrhizal and non-mycorrhizal plants. Mycorrhizal plants had higher electrolyte concentrations in roots and lower electrolyte leakage from roots than non-mycorrhizal plants under given NaCl and P levels. Although plants in the low P plus AM fungus treatment and those with high P minus AM fungus had similar P concentrations, the mycorrhizal plants still had higher dry weights, soluble sugars and electrolyte concentrations in roots. Similar relationships were observed regardless of the presence or absence of salt stress. Higher soluble sugars and electrolyte concentrations in mycorrhizal plants suggested a higher osmoregulating capacity of these plants. Alleviation of salt stress of a host plant by AM colonization appears not to be a specific effect. Furthermore, higher requirement for carbohydrates by AM fungi induces higher soluble sugar accumulation in host root tissues, which is independent of improvement in plant P status and enhances resistance to salt-induced osmotic stress in the mycorrhizal plant.

Keywords Arbuscular mycorrhiza · Maize · Osmotic adjustment · Soluble sugars · Salt stress

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Introduction

Salt-affected soils occupy more than 7% of the earth land surface and represent a major limiting factor in crop production (Jain et al. 1989). The direct effects of salt on plant growth may involve (1) a reduction in the osmotic potential of the soil solution that reduces plant-available water, and (2) toxicity of excessive Na^+ or Cl^- towards the plasma membrane. Osmotic effects are associated with inhibition of cell wall extension and cellular expansion, leading to reduced plant growth (Staple and Toenniessen 1984). Osmotic adjustment enables plants to maintain higher turgor under salt stress (Munns 1993). One of the most important responses of glycophytes to salt and drought stress is the accumulation of low-molecular-weight solutes (Hasegawa et al. 2000). The accumulation of these solutes in plants under saline conditions occurs not only because of reduction in plant size but also due to their increasing synthesis (Hu and Schmindhalter 1998).

Arbuscular mycorrhiza (AM) occur naturally in saline soils (Sengupta and Chaudhuri 1990). Although salinity might affect the formation and function of mycorrhizas (Juniper and Abbott 1993; McMillen et al. 1998), several studies have demonstrated that inoculation with AM fungi improves growth of plants under a variety of salinity stress conditions (Hirrel and Gerdemann 1980; Ojala et al. 1983; Al-Karaki and Hammad 2001). Therefore, AM fungi have been considered as bio-ameliorators of saline soils (Singh et al. 1997). Some investigations have suggested that improvement of plant phosphorus (P) status is the most important mechanism of salinity stress tolerance in AM plants (Hirrel and Gerdemann 1980; AlKaraki 2000). However, other studies have shown that

mycorrhizal plants grow better than non-mycorrhizal plants under salt stress even when mycorrhizal and non-mycorrhizal plants have a similar P status (Poss et al. 1985; Ruiz-Lozano et al. 1996; Feng et al. 2000), implying that the advantages of AM for plant growth and development under salt stress are not always related to P status improvement. Ruiz-Lozano et al. (1996) concluded that the mechanisms underlying AM plant growth improvement under saline conditions are based on physiological processes (increased carbon dioxide exchange rate, transpiration, stomatal conductance and water use efficiency) rather than on nutrient uptake (N or P).

The role of roots in sensing conditions in the soil environment has been evoked (e.g. Davies and Zhang 1991). Under salt stress, the production by roots of growth regulators that modulate plant metabolism is believed to be the main cause of decreased growth rate in plants exposed to a sublethal level of salinity (Amzallag 1997). Roots are also the site of carbohydrate and mineral nutrient exchange between AM fungi and host cells. However, the effects of AM fungal colonization on the physiological characteristics of roots under salt stress and their relation to tolerance to osmotic stress endowed by mycorrhizal plants are poorly understood. The purpose of this study was to investigate the effect of an established AM association on root P and Na concentrations, plasma membrane permeability and soluble sugar concentrations under salt stress condition, in order to improve understanding of the mechanisms underlying alleviation of salt toxicity in AM plants.

Materials and methods

Plant growth conditions

The experiment was conducted from late August to October in a greenhouse at a temperature of 20–30°C and 5 h supplementary light per day in the afternoon and evening. Maize seeds (*Zea mays* L. cv. Yedan13) were sown in plastic pots containing 2 kg of autoclaved sand. Eight seeds were sown in each pot and seedlings were thinned to four per pot after emergence. The plants were supplied with nutrient solution of the following composition: $MgSO_4$ 2.6 mM, $Ca(NO_3)_2$ 8 mM, K_2SO_4 3 mM, KCl 8 mM, H_3BO_3 10 µM, $MnSO_4$ 1.6 µM, $ZnSO_4$ 1.0 µM, $CuSO_4$ 0.5 µM, $(NH_4)_6Mo_2O_4$ 50 µM, Fe-EDTA 20 µM. The solution pH was adjusted to 6.5±0.3.

AM fungal inoculum

Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe was multiplied in a 1-l pot with fine sand as substrate. Red clover was used as host and was cultured for 4 months in the greenhouse. Inoculum consisted of sand, spores and mycelium of *G. mosseae* and infected-root fragments. Each pot was inoculated with 30 g inoculum for mycorrhizal treatment or 30 g sterilized inoculum plus 10 ml mycorrhizal fungal-free filtrate from the inoculum suspension as the non-mycorrhizal treatment.

Experimental design

The experiment consisted of a randomized block design with three factors: (1) mycorrhizal treatments (minus or plus *G. mosseae*), (2) two P levels of 0.05 mM and 0.1 mM in order to obtain similar P concentrations in mycorrhizal and non-mycorrhizal plants, and (3) two salinity levels of 0 and 100 mM NaCl. Each treatment had three replicates. During the first 34 days, maize plants were grown without addition of NaCl but with at the two P levels in order to obtain plants with functional mycorrhizas and avoid salt effects on AM establishment. To avoid osmotic shock, NaCl was introduced gradually by increasing the concentration by 25 mM per day starting at day 35 after sowing. Nutrient solution (250 ml) was supplied every 2 days. The pots were leached with 1 l deionized water every week to reduce salt accumulation. Fresh nutrient solution was added immediately after each leaching to keep a constant NaCl concentration in the substrate. The experiment was terminated at 68 days after sowing. Shoots and roots were harvested separately.

Measurements

Shoots were severed from the stem about 0.5 cm above the sand surface, then rinsed three times with deionized water. The second youngest leaf of each plant was collected and chlorophyll concentration was determined by measuring the absorbance (654 nm) of an ethanol extract solution as described by Winterman and De Mots (1965). The dry weights of the remaining plant shoots were recorded after drying in an oven at 70°C for more than 72 h.

Shoot samples were digested with concentrated H_2SO_4 and H_2O_2 at a ratio of 5:2 (v/v). P concentration was measured by the molybdovanadophosphate method (Kitson and Mellon 1944). Sodium was measured with a flame photometer as described by Haddad and Higginson (1990).

Mycorrhizal colonization was detected by the following procedures: roots from each plant were collected by gently washing out the sand under running tap water and rinsed three times in deionized water. A subsample of 0.5 g root segments was collected and cut into 1-cm-long pieces. The root segments were bleached with 10% KOH at 90°C for 30 min and then acidified in 1 M HCl and stained with acid fuchsin (Kormanik and McGraw 1982). Colonization was measured by the grid-line-intersect method previously described by Giovannetti and Mosse (1980).

Permeability of root plasma membrane was measured as follows. Fresh roots were cut into 2-cm segments and 2.5 g of root segments was placed in a glass vial with 25 ml deionized water. The electrolytic conductivity of the bathing solution was measured with a conductivity meter at 10, 20 and 30 min after soaking root segments in water. The root segments were then heated to boiling, the bathing solution cooled to room temperature, and electrolytic conductivity measured again. The relative permeability of root plasma membrane was calculated as described previously (Zwiazek and Blake 1991):

$$\text{Relative permeability} = \frac{\text{Electrolytic conductivity of solution at 30 min before heating}}{\text{Electrolytic conductivity of solution after heating}} \times 100$$

The remaining root segments were dried and ground. P and Na concentrations were measured as described above for shoots and soluble sugar concentrations were evaluated using the anthrone method described by Fales (1951).

All data were subjected to factorial analysis of variance using one-way ANOVA and means were compared by Duncan's multiple range test at the 5% level.

Results

Mycorrhizal colonization

None of the maize plants from the non-inoculated treatments were colonized by *G. mosseae*. Plants inoculated with *G. mosseae* had root length colonization of 70–80%, but the extent of mycorrhizal colonization was not significantly affected by salt or P treatments.

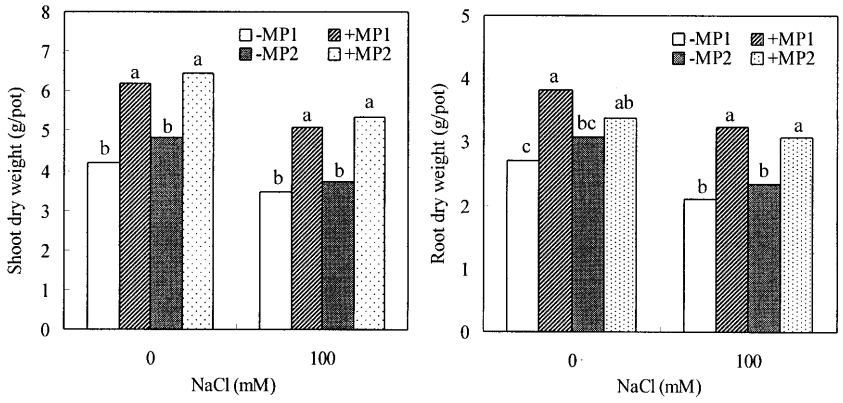

Fig. 1 Effect of *Glomus mosseae* on the growth of maize at 0 and 100 mM NaCl. *–M* and *+M* represent without and with inoculation of *G. mosseae*. *P1* and *P2* represent two phosphorus (P) levels (0.05 and 0.1 mM, respectively). Data labelled with different letters are significantly different (*P*=0.05) between treatments at a given NaCl level

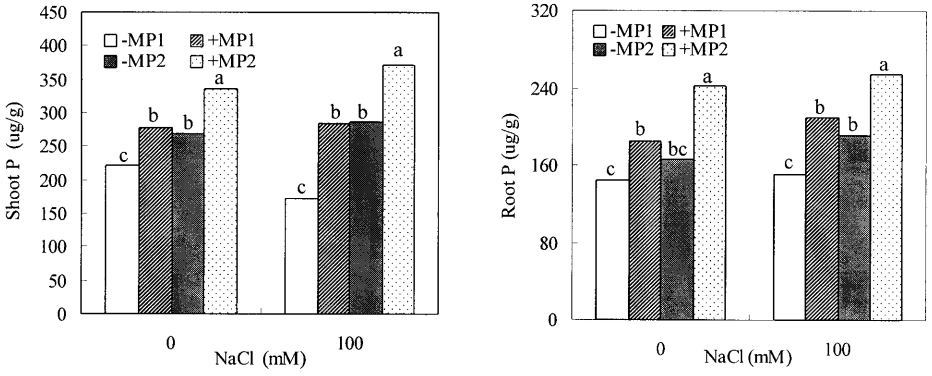

Fig. 2 Effect of *G. mosseae* on the P content of maize plants grown at 0 and 100 mM NaCl. Symbols as in Fig. 1

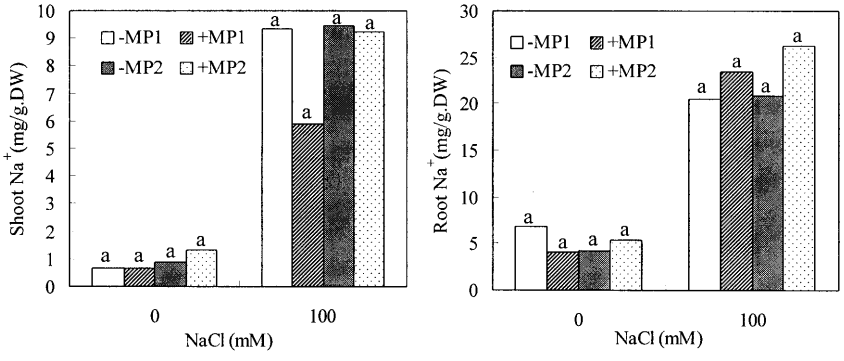

Fig. 3 Sodium concentration in shoots and roots of mycorrhizal and non-mycorrhizal maize plants grown in presence of 0 or 100 mM NaCl. Symbols as in Fig. 1

Plant growth

Root colonization by *G. mosseae* enhanced maize growth regardless of the P level with and without NaCl (Fig. 1). In the absence of added NaCl, the shoot dry weight of mycorrhizal plants increased by 47% in the 0.05 mM P treatment and by 34% in the 0.1 mM P treatment relative to the controls. In presence of NaCl at 100 mM, the shoot dry weight of mycorrhizal plants was 47% higher with 0.05 mM P and 43% higher with 0.05 mM P. Root dry weight was significantly higher in mycorrhizal than in non-mycorrhizal plants, except in the 0.1 mM P treatment in presence of 0 mM NaCl.

Phosphorus and sodium concentrations

At any given P level, root colonization by *G. mosseae* significantly increased tissue P concentration, especially under salt stress. The P concentration of shoots was increased by 12% and 27% at the 0.05 and 0.1 mM P levels, respectively, in AM plants under non-salt stress. Under salt stress, shoot P concentration in mycorrhizal plants was 82% higher in presence of 0.05 mM P and 28% higher with 0.1 mM P, relative to non-mycorrhizal control plants. The difference in P concentration in roots of mycorrhizal and non-mycorrhizal plants showed a trend similar to that in shoots (Fig. 2). P concentration in non-mycorrhizal plants grown at 0.1 mM P was similar to that in mycorrhizal plants grown at 0.05 mM P.

Sodium concentration increased in all plants as a consequence of salinity (Fig. 3). However, no significant differences in Na concentration in shoots and in roots were observed between mycorrhizal and non-mycorrhizal plants growing at any given salinity or P level.

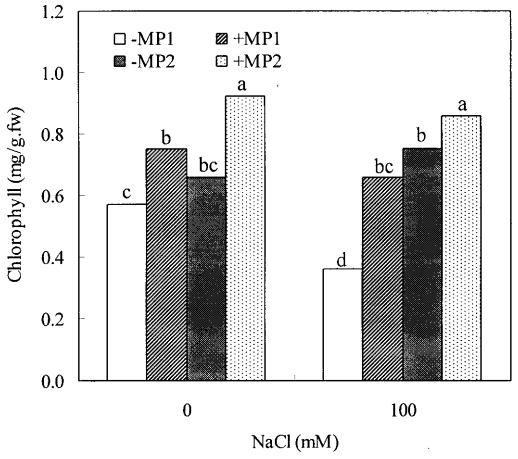

Fig. 4 Chlorophyll concentration in leaves of mycorrhizal and non-mycorrhizal maize plants grown in presence of 0 and 100 mM NaCl. Symbols as in Fig. 1

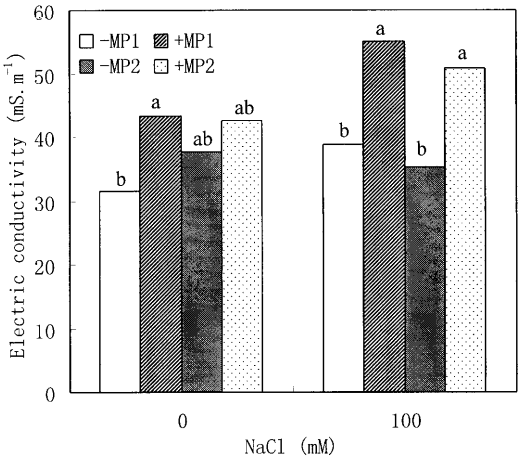

Fig. 6 Electrical conductivity in water extracts of root segments after boiling. Symbols as in Fig. 1

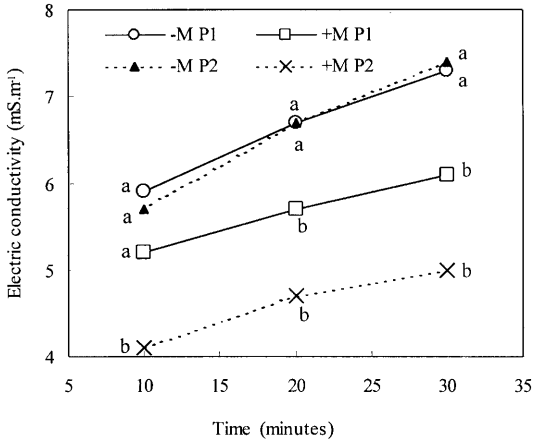

Fig. 5 Time course of electrolyte leakage from root segments of mycorrhizal and non-mycorrhizal plants grown at 0.05 and 0.1 mM P with addition of 100 mM NaCl. Symbols as in Fig. 1

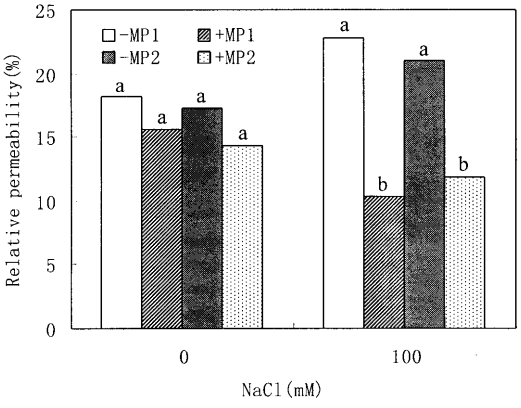

Fig. 7 Relative permeability of the root plasma membrane of mycorrhizal or non-mycorrhizal maize plants grown in presence of 0 and 100 mM NaCl. Symbols as in Fig. 1

Chlorophyll

At the same P and NaCl level, plants inoculated with *G. mosseae* had significantly higher chlorophyll concentrations than non-inoculated plants. In the absence of salinity stress conditions, chlorophyll concentration increased in AM plants by 32% at the lower P level and 40% at the higher P level. In the treatments with 100 mM NaCl, mycorrhizal plants had 81% higher chlorophyll concentrations at the lower P level and 15% at the higher P level than non-mycorrhizal plants (Fig. 4). As for P concentration, chlorophyll contents of non-mycorrhizal plants grown at 0.1 mM P were similar to those of mycorrhizal plants grown at 0.05 mM P.

Electrolyte leakage from roots

Electrolyte leakage from root segments increased with time for all treatments in which NaCl was added at 100 mM (Fig. 5). There was no difference in electrolyte leakage from non-mycorrhizal roots between the lower and higher P treatments under salinity stress, but electrolyte leakage from mycorrhizal roots was lower in the high P treatment than in the low P treatment (Fig. 5). Under salinity stress, electrolyte leakage from mycorrhizal was significantly lower than from non-mycorrhizal roots, irrespective of the P treatment (Fig. 5).

The electrical conductivity of the solution containing boiled roots indicated the total amount of electrolyte in roots. Roots of mycorrhizal plants had a higher electrical conductivity than those of non-mycorrhizal plants at the same P and NaCl levels and especially in presence of 100 mM NaCl. This implies that mycorrhizal roots had a higher total electrolyte content (Fig. 6).

There was no significant difference in electrolyte permeability of the root plasma membrane of mycorrhizal and non-mycorrhizal plants grown in the absence of NaCl. Mycorrhizal plants had a much lower root plasma membrane electrolyte permeability than non-mycorrhizal plants under 100 mM NaCl stress at both P levels (Fig. 7).

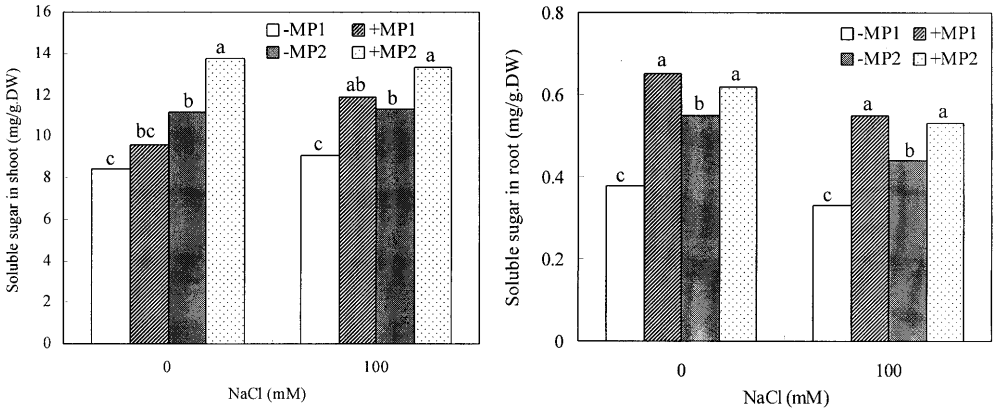

Fig. 8 Effect of *G. mosseae* colonization on soluble sugar concentration in roots of maize plants grown in presence of 0 and 100 mM NaCl levels. Symbols as in Fig. 1

Sugar concentration

Increasing P supply increased the concentration of soluble sugars in shoots and roots. In mycorrhizal plants, P supply increased sugar concentration only in the shoots of plants grown without NaCl (Fig. 8). Compared with non-mycorrhizal plants, the soluble sugar concentration in mycorrhizal plants was significantly higher in shoots at the same NaCl and P levels, except 0 NaCl and 0.05 mM P (Fig. 8). In roots, mycorrhiza formation significantly increased soluble sugar concentration regardless of NaCl and P level (Fig. 8).

Discussion

The higher dry weight, higher chlorophyll concentration in leaves and lower permeability of the root plasma membrane of mycorrhizal maize plants under NaCl stress conditions, compared with non-mycorrhizal plants, show that root colonization by *G. mosseae* can alleviate the deleterious effects of NaCl stress. These findings are consistent with previous reports for AM plants in soil (Hirrel and Gerdemann 1980; Ojala et al. 1983). It is essential to point out that the beneficial effects of AM on the maize plants occurred not only during NaCl stress but also in non-stress conditions, which implies that the mycorrhizal effect improving plant growth was not a specific process induced by salinity stress.

The primary effects of salt stress are through ion toxicity and osmotic stress (Levitt 1980). Sodium is mainly accumulated in older leaves because of its poor translocation (Lazof and Laüchli 1991; Jeschke et al. 1995). Accumulation of Na in plant cells decreases photosynthesis in old leaves and, thereby, transportation of carbohydrates from old leaves to young leaves and roots. On the other hand, a high concentration of Na in the apoplast generates osmotic stress, causing dehydration of cells (Munns 1993). Exclusion and rejection of Na at the cellular level are two strategies allowing plants to survive under salt stress (Levitt 1980). The lack of difference in Na concentration between mycorrhizal and non-mycorrhizal maize plants indicates that the mechanisms of enhanced tolerance to salt stress in mycorrhizal plants are not related to exclusion or rejection of Na by plants growing in the NaCl treatment.

It has been believed widely that alleviation of salt stress by AM is due to improvement of P nutrition (Hirrel and Gerdemann 1980). In the present study, mycorrhizal roots had higher electrolyte concentrations than non-mycorrhizal roots in presence of NaCl. This, together with the lower electrolytic conductivity in the bathing solution of mycorrhizal roots compared with non-mycorrhizal roots indicates a lower permeability of plasma membrane in the former than in the latter. This could be attributed to improved integrity and stability of the plasma membrane of mycorrhizal root cells due to increasing P uptake under salt stress. However, plants at low P plus *G. mosseae* had P concentrations similar to those at high P minus *G. mosseae*, although the dry weight of the mycorrhizal plants was significantly higher than that of the non-mycorrhizal plants. This suggests that AM can alleviate the deleterious effects of NaCl stress on plants by mechanisms which may not be related to improvement of P nutrition.

Glycophytes resist salinity-caused osmotic stress by regulating the osmotic potential of cells in two ways. One is to accumulate higher concentrations of low-molecular-mass organic solutes such as proline, betaine, soluble sugars or amino acids etc., which are generally at low concentration when a plant is not under salt stress. In addition, some plants are able to accumulate soluble solutes under non-salt-stress conditions and thus achieve higher resistance to salinity stress (Staple and Toenniessen 1984). Mycorrhizal maize plants appear to belong to the latter group according to the increased soluble sugar and electrolyte concentrations in roots colonized by *G. mosseae* in both NaCl-stress and non-stressed conditions. The soluble sugar concentration in roots of mycorrhizal maize plants grown at the lower P lever was significantly higher than that of non-mycorrhizal maize plants at the higher P level, in spite of similar P contents. This suggests that the higher soluble sugar concentration in roots of mycorrhizal plants was due to colonization by the mycorrhizal fungus, and not to improvement of the P status of the plants. Symbiotic interactions in AM associations are based on the exchange of carbohydrates and mineral nutrients between the plant and the fungus. Wright et al.

(1998a), using mycorrhizal and non-mycorrhizal clover plants of comparable plant size and growth rate and with similar N and P contents, demonstrated that AM fungal colonization stimulated the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus and to eliminate growth reduction of the autotroph. The consumption of carbon by AM fungi can be up to 20% of the host photosynthate (Harris et al. 1985; Jakobsen and Rosendahl 1990). Therefore, plant roots become a strong sink for carbohydrates when colonized by AM fungi (Amijee et al. 1993) and mycorrhizal sink strength influences the whole plant carbon balance (Wright et al. 1998b). In conclusion, the requirement for carbohydrates by AM fungi could cause an increased allocation to and accumulation of soluble sugars in the roots. This higher accumulation of soluble sugars in mycorrhizal plant tissue, especially in roots, could make mycorrhizal plants more resistant to osmotic stress induced by exposure to salt.

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