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MYCORRHIZAL SYMBIOSIS AND THE RESPONSE OF SORGHUM PLANTS
TO COMBINED DROUGHT AND SALINE STRESSES

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ABSTRACT

Arbuscular mycorrhizal (AM) symbiosis has been reported often to improve the abilities of host plants to tolerate drought stress. The physiological mechanism is uncertain, but one idea is that the effect might be linked to resistance to salt stress. Several studies have shown more growth in crop plants colonized with AM fungi than nonAM control plants under salt stress. Drought and salt stresses frequently occur together in nature and their initial symptoms in plants are similar. It may be interesting to scrutinize their physiological interaction in host plant as a function of AM fungi. Therefore, the objectives of my studies were to investigate if AM influence on plant response to drought is more evident in saline soils. I hypothesized that 1) AM and nonAM plants would have different values of water relation parameters with exposure to drought and 2) AM-induced drought tolerance would be greater when plants are subjected to salt stress during drought.

In two separate greenhouse experiments, sorghum was colonized with *Glomus intraradices*, *Gigaspora margarita*, or a mixture of AM species isolated from semiarid grasslands in Arizona (AZ). To induce drought stress to the host plants, watering was held after applications of soil solution. NaCl (40 mM and 80 mM) was applied to pot soil to initiate salt stress and macronutrient solution (-0.4 MPa and -0.8 MPa) was used for exposure of osmotic stress to host plants in experiment 2. To eliminate remaining salt ion in soil, a group of pots were leached heavily with distilled water in experiment 2. The pots receiving same amount of water as salt solution served as control plants. Several

parameters in relation to leaf and soil water status were monitored to determine the effect of AM symbiosis under drought and salt stress.

Significant decline of stomatal conductance was often observed when salt solutions (NaCl or macronutrient solution) were applied to the pot soil. All the sorghum plants reached stomatal closure in 9 to 12 days in experiment 1 and 9 to 16 days in experiment 2. Higher stomatal conductance often was observed in mycorrhizal plants before and after application of salt solution relative to nonAM plants; mycorrhizal promotion of stomatal conductance was generally 10 to 20% and even 100% near the stomatal closure point. The days to reach stomatal closure and the lethal point (point at which most foliage had died) varied with soil treatments and mycorrhizal association. It took the most days to reach the lethal point when plants were subjected to the macronutrient solution.

The shortage of available soil water and increasing solute level generated by osmotic stress resulted in decreasing leaf and soil water potential. The salt-treated soil maintained higher water content and higher soil water potential than pot soil treated with just water or leached with distilled water, but lower leaf water potential was observed at the stage of stomatal closure and lethal point in plants in salt-treated soil. There were a few significant different values in leaf and soil water potential between AM and nonAM plants during experimental period. AM plants showed lower lethal leaf water potential than nonAM plants when colonized with *Glomus intraradices* under drought alone. Symbiosis with *Glomus intraradices* did not have any marked effect on the parameters when drought and salt stresses were combined. There were no mycorrhizal effects on most parameters when plants were colonized with *Gigaspora margarita* or AZ.

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LIST OF ABBREVIATIONS

AM = arbuscular mycorrhizal

nonAM = non-arbuscular mycorrhizal

Gi = *Glomus intraradices*, mycorrhizal fungi

Gm = *Gigaspora margarita*, mycorrhizal fungi

AZ = species assemblage of AM fungi from a semi-arid grassland in Arizona

Osmotics = macronutrient solution with highly concentrated ions for osmotic stress

EC = electrical conductivity (dS/m)

g_s = stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)

Leaf Ψ = leaf water potential (MPa)

Leaf Ψ_π = ambient leaf osmotic potential (MPa)

Leaf Ψ_π^{100} = leaf osmotic potential at full turgor after rehydration (MPa)

Soil Ψ = soil water potential (MPa)

Soil θ = soil mass water content (g/g dry weight)

Leaf [P] = phosphorus concentration in leaves

CHAPTER I

INTRODUCTION

Under field conditions in many temperate regions as well as arid or semi-arid areas, crop plants suffer from various abiotic stresses (cold or hot temperature, salinity, drought, wind, low irradiance, etc.) separately or together. These abiotic stresses can negatively affect plant physiology and metabolism, reducing growth and yield, and increasing occurrences of diseases. Among these environmental stresses, drought is a serious issue in many agricultural regions throughout the world. Prolonged drought stress can induce many morphological, phenological, anatomical, and physiological disorders in crop plants and alter parameters associated with plant water relations and CO₂ assimilation (Ludlow, 1989). When plants are subjected to drought stress, an immediate response might be changes in stomatal behavior (Kaya et al, 2001). The degree of the stress can be understood by measurements of water relations inside plants and soil. The direct measurement of stomatal conductance (g_s) helps to gauge the way plants respond to changing water status and stress because it is correlated with many photosynthetic parameters under progressive drought condition (Jones, 1990; Liu and Stützel, 2002; Medrano et al., 2002).

An association with mycorrhizal fungi can be defined as a sustainable biotrophic interaction between a fungus and a root (Hodge, 2000). Arbuscular mycorrhizal (AM) fungi associated with host plants have often improved plant growth and biomass yield by more effective absorption of various nutrients such as P, Zn, Cu, Mn and Fe, and water from adjacent soil than non-mycorrhizal plants (Safir et al., 1971; Miranda, 1989; Juniper

and Abbott, 1993; Jeffries et al, 2003). Mycorrhizal plants also can maintain more normal stomatal conductance and leaf water potential than non-mycorrhizal plants during drought stress (Augé, 2001), and mycorrhizal symbiosis can protect host plants against detrimental effects caused by drought stress (Quilambo, 2003). Those investigations proposed that mycorrhizal plants should have more potential tolerance against drought stress in semiarid areas of the world (Al-Karaki and Al-Raddad, 1997). The mycorrhizal mechanism of influence remains unclear, but one idea is that host plants inoculated with AM fungi have extensive hyphal systems which can play an important role in absorbing and conducting water and soluble ions over long distances (Safir et al, 1971; Ingold and Hudson, 1993, Augé et al, 2003). Therefore, the host plants benefit under drought stress when the water supply to the non-mycorrhizal plants is restricted. Another idea is that the tolerance might be linked to salt tolerance of host plants.

The water deficit condition can be accompanied by the build-up of saline ions in soil solution. Increasing soil salinity in cultivated soils commonly occurs in regions where crops were continuously cultivated or soil leaching was not sufficient due to a shortage of rainfall for a long period (Levitt, 1980). More than 7% of the earth's land is occupied by saline soil (Munns, 2002; Ruiz-Lozano and Azcon, 2000; Tester and Davenport, 2003) and approximately 30% of irrigated areas in the United States have been affected by excessive salinity resulting in large losses in yield (Sohan et al, 1999; Yano-Melo et al, 2003). Moreover, the supply of fresh water resources is also not enough to meet the requirement for crop production in many regions of the world (Patel et al, 2000).

When irrigated with saline water, crop plants experience osmotic stress (Shani and Dudley, 2001) and plants suffer from dehydration due to decreasing osmotic potential resulting from accumulation of salt in soil (Ueda et al, 2003). Additionally, salt stress causes an ion stress that negatively affects ion homeostasis and metabolic activity of plant cells. Jensen (1981) proposed that stressed plants could reduce the severe effect of low water potential in soil through some hardening processes such as osmotic adjustment, altered leaf morphology or changed elastic modulus of leaf cells, which could make the plants less sensitive to renewed stress. Recently much research has been conducted to investigate the beneficial effects of mycorrhizal symbiosis to reduce the influence of salt stress on crop growth inhibition in tomato (Copeman et al, 1996; Al-Karaki, 2000), maize (Feng et al, 2002) and banana plants (Yeno-Melo et al, 2003). Augé et al. (1992) reported higher stomatal conductance of mycorrhizal plants than non-mycorrhizal plants when exposed to short-term osmotic stress in cowpea and soybean. Further, AM symbiosis has been shown to influence osmotic adjustment and leaf elasticity (Augé et al., 1986; Augé et al., 1987).

Ruiz-Lozano (2003) suggested that both drought and saline conditions should act as limiting factors to induce negative effects on plant development and the two stresses might have several similar responses. Initial symptoms due to saline or water deficit stress are similar, which suggests that the initial physiological pathway or metabolism of plants responding to both stresses might be common. However, unlike the water deficit condition from drought stress, salinized plants cannot uptake sufficient soil water even though the soil has ample water, because the water is in an unavailable form.

Even though water and salt stresses are related, most of the aforementioned research provided information about each stress separately. Only a few studies compared the two stresses physiologically and ecologically, and fewer still have compared plant response to salt stress alone, drought stress alone, and combined salt and drought stresses. Furthermore, almost all studies on the enhancing role of AM fungi against stress mainly dealt with plant responses to a single stress. However, in nature plants have more opportunities to cope with several stresses at the same time and there is little information available about responses of plants to combined stresses.

The objective of my studies was to determine if mycorrhizal influence on the plant response to drought is more evident in saline soils. To test whether the mycorrhizal effect on plant drought resistance is somehow linked to salt tolerance, we hypothesized that 1) mycorrhizal and non-mycorrhizal plants would have different values of water relation parameters with exposure to drought and 2) mycorrhizal-induced drought tolerance would be greater when plants are subjected to salt stress during drought.

In two separate experiments, I investigated the roles of the symbiosis of some AM fungi, *Glomus intraradices*, *Gigaspora margarita* or a mixture of AM species from a semiarid grassland in Arizona (AZ mix) (Augé et al, 2003), in sorghum plants subjected to drought, salt or osmotic stress. Measurement of stomatal conductance, leaf and soil water potential, soil water content and osmotic potential were made until stomatal closure or until foliage died (lethal point) to compare the mycorrhizal effect to each or to combined stresses. Sorghum was selected as a host plant because it is an important crop,

it grows quickly, it is easily colonized with AM fungi, and it exhibits a moderate degree of drought resistance (Shackel and Hall, 1983).

CHAPTER II

LITERATURE REVIEW

Effect of Mycorrhizal Symbiosis on the Drought-Stressed Plants

Drought is a serious main issue in many agricultural regions of the world. Originally ‘drought’ was defined as the lack of rain, but because it always causes ‘water deficit stress’, these terms have been used interchangeably (Levitt, 1980). Drought conditions in temperate regions as well as in arid or semi-arid regions due to insufficient precipitation can cause serious reductions in crop yield. The decreasing rainfall due to global climate changes has raised serious drought problems resulting in yield reduction or growth inhibition in temperate areas (Trenberth et al., 1988; Michaels, 2000). Lack of an adequate irrigation system is a dominant limiting factor for crop production in arid and semi-arid regions (Al-Karaki and Clark, 1998; Wang et al., 2003). Drought stress can also be accompanied by a build-up of salinity in soil solution.

In higher plants, many physiological, morphological, or anatomical responses may be caused by water stress (Ludlow, 1989). Adaptation to water stress involves the reduction of cell dehydration by either avoidance or tolerance of stress. Some examples of avoidance of water stress are rapid completion of ontogeny, leaf shedding, leaf rolling, and low stomatal conductance to water vapor. Tolerance of water stress usually involves the development of low osmotic potential, which characterizes many plant species found in more arid environments (Morgan, 1984). Many studies have been conducted to investigate the effect of water stress on plant growth inhibition (Alves and Setter, 2000;

Aronson et al. 1987; Lecoecur and Sinclair, 1996), reduced stomatal conductance (Chapman and Augé, 1994; Tardieu and Davies, 1993; Ray and Sinclair, 1997), hormonal regulation (Wang et al, 2003; Ludewig et al., 1988; Sharp et al., 1994; Sharp and LeNoble, 2002; Zhang et al, 1995; Sauter et al, 2002), osmotic adjustment (Augé et al., 1986; Wang et al., 1995; Ranney et al., 1991), and changes in enzyme activity (Egert and Tevini, 2002; Zhang and Kirkham, 1996). Among them, the term 'osmotic adjustment' is widely used to describe active osmoregulation in response to water stress in higher plants where the term is also used to describe changes in solute content after recovery from water stress.

Under prolonged drought, the uptake of soil water by roots becomes very limited due to lowered soil water potential. Plant roots cannot provide sufficient water to the stems and shoot resulting in a water deficit situation for the whole plant. Levitt (1980) proposed that two major symptoms of water-stressed plants should be growth inhibition and stomatal closure. When plants were exposed to drought, leaf area generally reduced and stomatal activities became inactive to prevent loss of water in leaf tissue due to excessive dehydration and soil drying which lead to reduction in dry weight of amaranth (Liu and Stützel, 2002). Egert and Tevini (2002) reported that withholding water for 9 days elicited a significant reduction in relative leaf water content (25 %) and a rise in the sap osmolarity (19 %) in chives plants.

Mycorrhizal symbiosis has been extensively investigated in relation to increased plant tolerance to drought stress through improving water relations, hormonal involvement or

osmotic adjustment (Augé, 2001). However, Simpson and Daft (1990) suggested that all mycorrhizal fungi should not have beneficial effects on the growth of water-stressed maize and sorghum plants. They compared the effects of six species of fungi, but only colonization with *Glomus clarum* increased total dry weight of maize and sorghum under water stress. In a comparative study of eight *Glomus* species, Ruiz-Lozano et al. (1995) found that *Glomus deserticola* was the most efficient fungus for improvement of water relations under drought. They showed that other species could increase CO₂ exchange and stomatal behavior to some degree.

Following recovery from moderate water deficit, mycorrhizal soybeans had lower resistance to water transport than nonmycorrhizal plants, which indicated that mycorrhizal colonization improved water utilization in the plant (Safir et al, 1971). Improving water relation of mycorrhizal plants can help to use the limited amount of soil water under stress very effectively. When Al-Karaki (1998) compared water use efficiency (WUE) of wheat plants, he found that the mycorrhizal plants used less water to produce one unit of shoot dry matter than nonmycorrhizal plants, but water-stressed and well-watered plants did not differ in WUE. Higher WUE in mycorrhizal plants may indicate an improved ability of roots to absorb soil moisture and maintain open stomata in leaves. Safir et al. (1971) explained that the enhanced ability to absorb water and nutrients of mycorrhizal plants might be due to increased surface area resulting from external hyphae. Mycorrhizal *Opuntia robusta*, a cactus in arid regions, can survive under severe drought due to improved water uptake (Pimienta-Barrios et al, 2002). According to Morte et al. (2000), the water potential, transpiration and net photosynthesis were

higher in mycorrhizal plant than nonmycorrhizal plant of *Helianthemum almeriense* under water stressed or well-watered conditions. Augé et al. (2001) reported that mycorrhizal soil had more water stable aggregates and higher extraradical hyphal densities than nonmycorrhizal soils.

Greater uptake of P, Zn, Cu, Mn and Fe also were reported in mycorrhizal wheat plants under water-stressed conditions, and it was proposed that mycorrhizal colonization had potential to protect wheat grown in semiarid regions of world from drought stress (Al-Karaki and Raddad, 1997). Some mycorrhizal fungi might have synergetic effects with nitrogen-fixing bacteria. In alfalfa, the most improved photosynthetic gas exchange under drought conditions was shown in mycorrhizal plants associated with nitrogen-fixing bacteria suggesting a beneficial effect of their interaction (Goicoechea et al., 1997). Al-Karaki and Clark (1998) also announced that mycorrhizal plants had tolerance against drought due to greater uptake of nutrients and higher WUE in wheat plants colonized by *Glomus monosporum*.

In general, water stress induces the production and the release of ethylene from papaya roots, but mycorrhizal colonization with *Gigaspora margarita* might act as an inhibitor of ethylene biosynthesis by blocking the 1-aminocyclopropane-1-carboxylic acid (ACC) conversion to ethylene (Cruze et al., 2000). Mycorrhizal plants lowered the amount of abscisic acid (ABA) moving in xylem (Duan et al., 1996); ABA is considered a signal for drought induction in plants.

Plant Behavior and the Role of Arbuscular Mycorrhizas under Saline Soil

Soil salinity is a measure of the total amount of soluble salt in soil (Kotuby-Amacher et al, 1997). Increasing soil salinity in cultivated areas has been a serious problem worldwide due to reduction in crop yield. Salt accumulation in soil commonly occurs in the regions where some crops were cultivated continuously or soil leaching was not sufficient due to a shortage of rainfall for a long period (Levitt, 1980). Generally regions where the average soil electrical conductivity (EC) exceeds 4 dS/m, is considered as a saline soils (Juniper and Abbott, 1993). More than 7 % of the earth is land occupied by saline soil (Ruiz-Lozano and Azcon, 2000; Munns, 2002; Tester and Davenport, 2003) and approximately 30% of irrigated areas in the United States are affected by excessive salinity and resultant yield losses (Sohan et al, 1999; Yano-Melo et al, 2003).

Excessive salt accumulation around the root-zone of crop plants lowers the osmotic potential of soil solution followed by a shortage of available water for plant metabolism (Feng et al, 2002). Munns (2002) proposed that there are two steps of soil salinity affecting plant growth inhibition. Firstly, initial salt accumulation around the crop root zone temporarily decreases the water uptake ability by plant roots, and root-generated hormonal signals lead to a reduction in shoot growth. These metabolic changes are similar to the plant response under water stress or drought conditions. Secondly, more excessive salt levels could be toxic to the plant cell causing premature senescence of the older leaves. Levitt (1980) described the first osmotic stress induced by salinity as 'secondary salt-induced stress' because it indirectly affects plant growth.

Plants suffer from dehydration because of decreased soil osmotic potential by salty ions in soil. According to the comparison of leaf water potential (leaf Ψ) and osmotic potential (leaf $\Psi\pi$) of sea aster leaves under control and NaCl stress, the salt-stressed leaves showed much lower leaf Ψ (-2.18 MPa) and leaf $\Psi\pi$ (-2.59 MPa) than control leaves (leaf Ψ , -1.01 MPa; leaf $\Psi\pi$ -1.43 MPa) after being subjected 300 mM NaCl (Ueda et al., 2003). Mickelbart and Marler (1996) also reported significantly lowered leaf $\Psi\pi$ (-3.8 MPa) of sapodilla leaves that received NaCl solution (20 dS/m around root zone). High salt levels in soil solution also decreased root hydraulic conductance in plants, subjecting the leaves to severe water deficit conditions. Treatment of sunflower plants with NaCl (100 or 150 mM) led to significantly lower stomatal conductance than those of control plants (Sohan et al, 1999). The salt stress may accelerate stomatal closure and therefore decrease transpiration rate from leaves due to turgor loss following dehydration in plants (Levitt, 1980). Increasing EC levels obtained by adding NaCl to the solution (from 1 dS/m to 20 dS/m) decreased remarkably net photosynthetic rate of sapodilla plant from $6.5 \pm 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $2.0 \pm 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ 9 weeks after treatment (Mickelbart and Marler, 1996).

At the same time, excessive salt has severe negative effects on maintenance of ion homeostasis and metabolic activity in plant cells. In tomato plants, high concentration of Na^+ and Cl^- resulted in nutrient imbalance of soil solutions and inhibited uptake of other essential nutrients (K, P, Mg, Ca) from soil solution (Kotuby-Amacher, 1997; Al-Karaki, 2000). High levels of sodium ions in plant cells decreased dry matter production and the leaf content of K, Mg, and Ca in spinach and lettuce (Matar et al., 1975).

In opposition to the secondary salt injury elicited from osmotic stress or nutrient imbalance uptake, primary injury is the negative effect of specific ion toxicity on plant growth (Levitt, 1980). In most major crop plants, high concentration of sodium ions in soil solution is the dominant reason for ion toxicity and imbalance (Demidchik and Tester, 2002; Tester and Davenport, 2003). Elevated sodium ions in the soil might displace the calcium ion attached on the cell surface leading to disruption of membrane integrity that makes the unwanted sodium ions transport into the root easily (Cramer et al., 1985). The chloride ion accumulation by plants occurs more rapidly than the sodium ion and, therefore Cl^- toxicity might be severe in crops under saline stress. Mickelbart and Marler (1996) reported that the accumulation of Na^+ and Cl^- decreased the net CO_2 assimilation.

An accepted method to eliminate the salt ions from saline soil is leaching by watering. However, it is not a good method due to the rising cost of water (Sohan et al, 1999) and the limiting sources of fresh water in many agricultural regions where the high rates of evaporation might lead to increases in salt content even after application of nitrogen fertilizer (Patel et al, 2000). Another method of managing salt stress is selection and development of salt tolerant cultivars which have special mechanisms to exclude Na^+ from the shoots (Tester and Davenport, 2003), are able to compartmentalize salt ions in the vacuoles of cells, or lower the transport rate of salt ions to the leaves and/or minimize the entry of salt ions into the plant or cytoplasm (Munns, 2002; Ueda, 2003). Because the mechanisms of sodium ion transport, toxicity and tolerance in plants are far from understood (Demidchik and Tester, 2002), those methods cannot solve the problems

completely. Effective alternative methods to alleviate salt stress would be highly beneficial to plant survival and to prevent growth inhibition and yield reduction in crop plants.

The basis for relationship between plants and vesicular-arbuscular mycorrhiza (VAM or AM) *per se* is known to be natural beneficial symbiosis, resulting in exchange of essential nutrients. Recently it was reported that VAM or AM enhances the abiotic- and biotic-stress resistance of crop plants (Junifer and Abbott, 1993; Jeffries et al., 2003). The development of mycorrhizal symbiosis in roots has been reported to alleviate the stresses and growth inhibitions under stress conditions by increasing phosphorus (Al-Karaki, 2000), Zn, Mn, Cu and Fe uptake (Al-Karaki and Al-Raddad, 1997), stimulating root development (Ruiz-Lozano and Azcon, 2000), changing root morphology (Berta et al, 1990), accumulating soluble sugar in roots (Feng et al, 2002), changing soil moisture retention properties (Augé et al, 2001), altering the osmotic balance (Gupta and Krishnamurthy, 1996), improving water use efficiency (Al-Karaki, 1998; Al-Karaki and Clark, 1998; Morte et al, 2000), lowering ABA levels in roots or increasing photosynthetic activity, leaf conductance and transpiration flux (Goicoechea et al, 1997).

A well-developed plant-mycorrhizal symbiosis can result in successful bioremediations against polluted environments (Jeffries et al., 2003). However Ruiz-Lozano and Azcon (2000) reported that soil salinity can negatively affect the infection of host plants by mycorrhizal fungi resulting in decreased hyphal growth and viability of *Glomus* spp. Increasing soil salt levels reduced the formation of AM in roots, but the growth of *Arachis hypogaea* was improved by symbiosis under salt conditions (Gupta

and Krishnamurthy, 1996). Mergulhao et al. (2002) reported that the root colonization rate and spore number of mycorrhizal fungi were not affected by increasing NaCl levels and *Glomus etunicatum* inoculation to the root zone of *Brachiaria humidicola* helped increase leaf and stem dry matter. Mycorrhizal maize plants had higher soluble sugar and lower electrolyte leakage than non-mycorrhizal plants under salt stress (Feng et al, 2002).

Total accumulation of P, Zn, Cu, and Fe was higher in AM tomato than nonmycorrhizal plants under normal and salt stress conditions (Al-Karaki, 2000). Mycorrhizal treatments decreased the inhibition by salt stress in banana (Yano-Melo et al, 2003). *Glomus clarum* colonization enhanced the dry weight of root (80%), shoot (83%) and the total leaf area (60%) of banana plants compared to non-mycorrhizal plants. According to Copeman et al. (1996), tomato plants colonized with combined mycorrhizal fungi (mostly *Glomus* sp.) had lower leaf Cl^- concentration than nonmycorrhizal control plants in saline soil (EC = 12.2 dS/m). They suggested that mycorrhizal colonization may aid in plant survival in saline conditions.

In opposition to that, mycorrhizal Carrizo citrange and sour orange accumulated more chloride in leaves than non-mycorrhizal plants (Hartmond et al., 1987). Further investigations will be needed with chloride uptake by mycorrhizal plants. In summation, mycorrhizal colonization of roots may help to promote plant growth or survival in crops subjected to salt stress. However the mechanism of salt tolerance is not understood entirely, except that mineral uptake by micorrhizal fungi is promoted.

Combined Effects of Drought / Salinity Stress on Plant Behavior

In field conditions, some environmental stresses (abiotic or biotic) commonly occur simultaneously and their effects are often interactive on organisms (Juniper and Abbott, 1993). A few studies were conducted to test the interactions of such combined stresses as NaCl and acid stress (Gupta and Krishnamurthy, 1996), or drought and high temperature (Machado and Paulsen, 2001). Combined effects of drought and salt stress on plant growth and yield in the field have been studied in beans (Jensen, 1981), cotton and pepper (Shalhevet and Hsiao, 1986), corn, melon, and alfalfa (Shani and Dudley, 2001), and sea aster (Ueda et al, 2003).

Many situations may induce drought and salt stress at the same time. First, as soil water contents decrease due to prolonged drought, the relative salt levels may increase and evaporation streams may pull up the salt ions to the soil surface (Hillel, 1998). Second, if drought stress is imposed on saline soil, the growth inhibition of crop plants would be more accelerated than those under drought or salt stress alone (Munns, 2002). Third, when crops were irrigated with saline water in region where fresh water sources are limited, considerable decreases in yield and biomass were reported (Shani and Dudley, 2001). Saline soil limits the ability of crop plants to absorb water from adjacent soil and the shortage of water in plants causes reductions in growth and alteration of metabolic processes, which are identical to those resulting from water stress (Munn, 2002). Both stresses decreased leaf water content, leaf water potential, stomatal conductance, and photosynthetic rate in a study with sea aster (Ueda et al., 2002).

In comparative experiments, using cotton and pepper plants, leaf water potential under water stress declined to lower levels than those under salt stress, and leaf osmotic potential was much less under salt stress than under water stress (Shalhevet and Hsiao, 1986). Leaf turgor potential was maintained under salt stress, but was reduced under water stress. Transpiration and net photosynthesis were higher in plants under salt stress than those under water stress (Shalhevet and Hsiao, 1986). Even though both stresses lowered soil water potential at the same rate (0.16 to -1.10 MPa), the water status in leaves was very different under drought and saline stress. An important observation was the lack of wilting under salt stress at soil water potentials that cause wilting under water stress. Since plants suffer when they take up water through salty soil solution, the amount of soil water in saline areas might be maintained much longer than that of normal soil under drought conditions.

Because salt stress can evoke ion toxicity in plants (Levitt, 1980), most researchers use polyethylene glycol (PEG) or concentrated macronutrients to induce water stress. Based on comparisons between NaCl and PEG at the same osmotic pressure, barley grew better in PEG than NaCl, but the results were very different in other crops (Munn, 2002). Lowered basal osmotic potential (leaf Ψ_{π} measured at full hydration) or osmoregulation can lead to the maintenance of turgor potential and hence continued plant growth after recovery from stress (Ludlow, 1989). Osmotic adjustment caused by uptake of inorganic solutes, before or during exposure to water and salt stress, can reduce the severe effects of low water potential in the root medium (Jensen, 1981). The loss of turgor was due not only to a smaller osmotic adjustment, but also a larger reduction in leaf water potential

under water stress and salt stress (Shalhevet and Hsiao, 1986). Combined stress of drought and salinity in soil occurs inevitably in many arid- and semi-arid regions, but clear-cut solutions to reduce the loss of crop yield has not been developed. Colonization of plants by AM fungi reportedly enhance the abilities of host plants to overcome the individual stress of drought and salt, but so far their protection effect against combined stresses of drought and salinity is unknown.

CHAPTER III

MATERIALS AND METHODS

Experiment 1: Symbiosis with Mycorrhizal Fungi, *Glomus intraradices*, and Response of Sorghum Plants to Combined Drought and Saline Stresses

Plant Materials and Culture

A species of AM fungus, *Glomus intraradices*, was grown on *Sorghum bicolor* 'Dekalb DK40Y' in an autoclaved soil mixture (50 sand: 50 loam soil, v/v) to maintain the cultures over nine months in the greenhouse. About 100 to 150g of the pot cultures colonized by *G. intraradices* was used as inoculum for each pot of sorghum plants in the mycorrhizal treatment. The AM inoculum was added to the middle of each mycorrhizal pot before planting and the remaining portion of the pots was filled with autoclaved silica sand (commercial medium grade, No.1962-51, Quikrete, Atlanta, GA). A similar amount of non-mycorrhizal pot culture, grown under the same conditions as AM pot culture, was added to the control pots, along with a water filtrate from AM inoculum, which was passed through a sieve having 44- μ m mesh openings (smaller than the spore and hyphae size of *G. intraradices*). That assured that some microbial activities were available to affect plant response in control pots, similar to mycorrhizal pots. In total, 105 pots were prepared for testing including 35 pots colonized with *G. intraradices* and 70 pots without colonization.

After the pots were prepared with AM or nonAM cultures, seeds of *Sorghum bicolor* 'Dekalb DK40Y' were planted on February 6, 2002. Plastic square 2.8-L pots (10cm width, 36cm depth, Stuewe & Sons Inc., Corvallis, OR) were used. Plants were fertilized

weekly with water soluble fertilizer (10.7 mM N; Peters, N:K=15:15, Scotts-Sierra Horticultural Product Company, Marysville, OH) and biweekly with a micronutrient solution (0.1 mM Fe) containing 5.43% Mg, 0.5% B, 0.05% Co, 1.5% Cu, 4.0% Fe, 4.0% Mn, 0.1% Mo and 1.5% Zn (Microplex[®], chelating agent-EDTA, Miller Chemical & Fertilizer Co., Hanover, PA). Phosphorus was supplied once per week as 0.8 mM KH₂PO₄ to mycorrhizal plants (AM) and one group of non-mycorrhizal plants (NML) and as 1.6 mM KH₂PO₄ to a second group of non-mycorrhizal plants (NMH). Plants were watered as needed prior to stress applications and grown in a glasshouse in Knoxville, TN. The temperature of the glasshouse was maintained at 25 to 29 °C / 18 to 23 °C (day/night) under natural light. The glasshouse was covered with 55% shade cloth to aid in temperature control during the period between May and October.

The experiment was conducted after AM and nonAM soil was established for 10 months. The sorghum plants were sheared back to the crown on May 1 and again on June 17, 2002, and allowed to regrow each time.

After confirming colonization of *G. intraradices* in roots of mycorrhizal plants, and the absence of AM colonization of nonAM plants, the plants were sheared and crowns removed on August 8, and five to six seeds were sown in each pot on August 16, 2002. Shoots were sheared on October 16. Because shoot re-growth after shearing on October 16 was poor, most likely due to frequent rainy and cloudy weather conditions, crowns were removed and pots were re-seeded on November 12. At this time, high-pressure sodium lamps (400 watt) were installed 80 cm above the pots and light was supplied (16 hours per day) for supplement low natural light during the winter months.

The shoots of extra five plants in each group (AM, NMH and NML) were sheared on January 14, 2003, collected in paper bags and dried in an 80 °C dry oven for 48 hours for measurement of dry weight. The AM and NML pots were selected for further study because they had similar shoot dry weights (4.54 ± 0.30 and 4.36 ± 0.29 g/pot respectively).

Osmotic, Saline and Drought Stress Application and Quantification

The experimental objective was to test whether AM effects on stressed plants would be more evident if soil solution was salinized prior to drying pots. Soil was exposed to three solutions before initiating the drought treatment: a macronutrient solution, a NaCl solution or distilled water. Macronutrient solution was used to lower soil $\Psi\pi$ and induce osmotic stress without NaCl. The macronutrient solution was composed of 40 mM MgSO_4 , 90 mM $\text{Ca}(\text{NO}_3)_2$, 1.6 mM KH_2PO_4 , 62 mM KNO_3 and 19 mM NH_4NO_3 (Augé et al., 1992). The solution was adjusted to -0.4 MPa for the first application (day -7 , the seventh day before beginning the drought treatment) on January 20, 2003 and -0.8 MPa for the second application (day 0) on January 27, 2003. Day 0 refers to the first day of the drought period (the last day that plants were watered). Water potential of solutions was measured with a chilled mirror dewpoint hygrometer (WP4, Decagon Devices, Inc., Pullman WA). The NaCl solution was prepared as 40 mM for the first application on day -7 and as 80 mM for the second application on day 0. Two hundred ml of distilled water, NaCl or macronutrient solution was applied to the pots for each application, day -7 and

day 0. By withholding water from the pots after the second application, all plants were subjected to continuous drought.

Soil electrical conductivity (EC) of each pot was measured with an EC meter (AR 20, Fisher Scientific Inc., Pittsburgh, PA) with the 1:5 soil/water suspension methods (Rayment and Higginson, 1992). The EC meter was calibrated with each use with a 0.01 N KCl solution. Air-dried soil (10 g) from each pot was put into a beaker filled with 50 ml distilled water. The flasks were shaken mechanically (100 rpm) at 25 °C for 1 hour to mix soil-water suspension and dissolve salt. After allowing the suspension to settle for 20 to 30 min, the EC probe was dipped into the supernatant and moved up and down slightly without disturbing the settled soil until a stable reading was obtained.

Determination of Soil Hyphae and Root Colonization

On day -10, the soil from five extra AM and NML pots was preserved in a refrigerator. Soil was mixed and subsampled for determination of soil hyphal density and for collection of roots for quantification of root colonization. Approximately 10 g of soil was suspended in a destaining solution (600 ml glycerol, 50 ml HCl and 350 ml distilled water) at medium speed in a blender. After the wet soil was sieved (40 µm) repeatedly to eliminate small particles, material remaining on the sieve was rinsed with distilled water into a beaker and brought to 200 ml. Approximately 10 ml of the suspension was placed on a membrane filter (GN6, 0.45 µm, 47-mm-diameter with a 3-mm grid line interval, Gelman Scientific, Ann Arbor, MI) in order to count the number of hyphae after staining with 0.05% trypan blue (Amber and Young, 1977).

Roots collected during sieving and rinsing were placed in a small vial in 10% KOH and autoclaved for 20 min at 121 °C. After cooling, the roots were stained with 0.05 % trypan blue for 1 hour and root pieces were placed on a glass slide parallel to one another; the number of vesicles, arbuscules, and hyphae were counted under a microscope (McGonigle et al, 1990).

Stomatal Conductance

Before and during the drought and salt stress period, the g_s of plants in each pot was measured on abaxial leaf surfaces until the average g_s of three leaves in each pot declined below $10 \text{ mmol m}^{-2}\text{s}^{-1}$ (defined as the stomatal closure point). Through preliminary experiments, the g_s below $10 \text{ mmol m}^{-2}\text{s}^{-1}$ was considered the point at which most stomata would be closed. The g_s was measured with an automatic-cycling porometer (AP4, Delta-T Devices, Cambridge, England). Once drought was initiated, g_s was measured each day, at the distal end of the largest, unshaded leaves. Three leaves per pot were measured, between approximately 1:00 pm and 3:00 pm. Stomatal conductance was measured also for plants of every treatment two to three times per week prior to application of treatment solutions.

Leaf Water Potential

For measurement of the initial (before application of drought and salt stress) leaf $\Psi\pi$, a leaf from each pot in each treatment (AM and nonAM) was excised on day -7. The leaves

were immediately cut into two halves. The base of the top half was submerged in distilled water in a covered beaker and rehydrated in a refrigerator (4 °C) overnight for measurement of leaf $\Psi\pi$ at full turgor ($\Psi\pi^{100}$). The bottom portion of the leaf was sealed in a 3- ml plastic syringe, frozen in liquid N₂, and placed in a freezer at –80 °C for later measurement of ambient leaf $\Psi\pi$ (osmotic potential of leaves at the time of excision). Following rehydration, the top portion of the leaf was removed from the beaker, quickly blotted to remove the surface water, sealed in a syringe and placed in a freezer until measured. The syringes were thawed until samples reached room temperature (about 30 minutes), and leaf $\Psi\pi$ of expressed leaf sap was measured with a vapor pressure osmometer (model 5500XR, Wescor Inc., Logan UT) calibrated with each use with standard NaCl solutions (Lang, 1967). Sampling of leaf $\Psi\pi$ and leaf $\Psi\pi^{100}$ was also conducted one day after application of each soil solution, and at the stomatal closure and lethal points in one leaf per pot. The lethal point of each plant was determined visibly when fewer than five live leaves remained having less than 25 % necrosis.

Leaf Ψ was measured with a thermocouple psychrometer (Tru Psi, Decagon Devices, Inc., Pullman, WA). Strips (about 1 cm x 8 cm) were cut from the middle of the lamina, parallel to the midvein, and immediately placed inside a thermocouple psychrometer chamber. Preliminary tests indicated that leaves reached equilibrium with the atmosphere in the psychrometer within 2 hours. The value of microvolt signal obtained from each measurement was converted into water potential (MPa) using a calibration curve generated on a graded series of NaCl solution (Comstock, 2000). The measurement of leaf Ψ was made on the same day as leaf $\Psi\pi$ and leaf $\Psi\pi^{100}$ measurements.

Soil Water Potential and Soil Water Content

Soil Ψ was measured on a sample (about 2.5 ml) from the middle of each pot with a dewpoint hygrometer (WP4, Decagon Devices, Inc., Pullman, WA) one day after solutions were applied to soils. At the stomatal closure point and lethal point, the soil was sampled on both mid- (14 to 17 cm from the top) and bottom-portion (27 to 30 cm from the top) of each pot. The soil sample was put in a plastic sample cup (4-cm-diameter and 1-cm-depth), sealed with a cap and placed on the WP4 to equilibrate thermally with it before measurement. The hygrometer was calibrated using NaCl solutions (Lang, 1967). After measurement of soil Ψ , the plastic sample cup was tightly sealed with a cap and immediately transferred to the laboratory for gravimetric measurement of soil water content (soil θ).

Leaf Phosphorus Concentration

Before treatments of salt solution and soil drying, the leaves of extra plants were harvested and dried in an oven at 80 °C. The dried leaves were ground and leaf phosphorus concentrations of nonAM and AM sorghum were determined spectrophotometrically using the vanadate-molybdate-yellow method on samples dry-ashed with magnesium nitrate at 700 °C for 2 hours and digested in nitric acid (Chapman and Pratt, 1961).

Size of Root Systems

Each soil from five extra nonAM and five extra AM pots was put into plastic bags and kept in a refrigerator before applications of salt solution and soil drying on day -10. For measurement of root length density, each soil was taken from the refrigerator and roots were carefully collected from 25 g of each pot. The collected roots were placed in a clear acryl tray (15 cm x 25 cm) and dispersed evenly by submerging in water. Total roots were scanned and counted using a scanning instrument (EPSON 1600; HP ScanJet 6100C) and imaging software (WinRhizo, Regent Instruments Inc., Quebec, Canada). Surface area, volume and average diameter of roots were also computed with the WinRhizo software.

Experimental Design and Statistical Analysis

Pots were arranged in a completely randomized block design having two mycorrhizal (AM and nonAM) treatments and three soil solution (water, NaCl and concentrated nutrient solution) treatments. By random selection of numbered sheets, each block was composed of 6 pots from each treatment. Six blocks were installed on a bench in the greenhouse. Thus the experiment was conducted as a 2 x 3 factorial design. Analysis of variance with linear contrasts was examined for each parameter using SAS program (PROC GLM procedure). Mean separation of each parameter was performed using ANOVA and Duncan's multiple range test ($P < 0.05$).

Experiment 2: Symbiosis with Mycorrhizal Fungi, *Gigaspora margarita* and Arizona Mix, and Response of Sorghum Plants to Combined Drought and Saline Stresses

Plant Materials and Culture

In the second experiment, host plants were colonized with other mycorrhizal fungi. Pot cultures of *Gigaspora margarita* (*Gm*) and a mixture of AM species from the semiarid grassland in Arizona (AZ mix) (Augé et al, 2003) were grown on *Sorghum bicolor* ‘Dekalb DK40Y’ in autoclaved soil mixture (50 sand: 50 loamy soil, v/v). Cultures were maintained for 12 months before being used as an inoculum for the experiment. About 100 to 150 g of pot culture of either *Gm* or AZ mix was added to the middle of each pot in the AM treatments before planting. The remainder of the pot was filled with autoclaved silica sand (commercial medium grade, No.1962-51, Quikrete, Atlanta, GA). A similar amount of nonAM pot culture was added to the control pots, along with a water filtrate from the AM inoculum, which was passed through a sieve with 44- μ m mesh openings. This assured that some microbial activities were available to affect the plant response in control pots. In total 108 pots were prepared for the experiment; 36 pots for nonAM, 36 for *Gm*, and 36 for AZ. Additionally five more pots for each treatment were prepared as extra plants.

Eight to ten seeds of *Sorghum bicolor* ‘Dekalb DK40Y’ were sown in each 2.8-L plastic square pot (10cm width, 36cm depth, Stuewe & Sons Inc., Corvallis, OR) on April 18, 2003. After emergence of seedlings, pots were thinned to six healthy seedlings per pot. Plants were watered weekly with water soluble fertilizer (10.7 mM N; Peters,

N:K=15:15, Scotts-Sierra Horticultural Product Company, Marysville, OH) and biweekly with a micronutrient solution containing 5.43% Mg, 0.5% B, 0.05% Co, 1.5% Cu, 4.0% Fe, 4.0% Mn, 0.1% Mo and 1.5% Zn (Microplex[®], chelating agent-EDTA, Miller Chemical & Fertilizer Co., Hanover, PA). Phosphorus was supplied once a week as 0.8 mM KH₂PO₄ to plants in each AM treatment and to nonAM plants. Plants were watered as needed prior to stress applications and grown in a glasshouse in Knoxville, TN. The temperature of the glasshouse was maintained at 25 to 29 °C / 18 to 23 °C (day/night) under natural light.

Application of Saline Solution

After confirmation of root colonization in mycorrhizal plants infected by either *Gigaspora margarita* or Arizona mix, the sorghum pots were carefully treated with three soil solutions prior to initiating a drought treatment. To establish saline conditions, NaCl solution was applied as 40 mM for the first application on day -7 (June 11, 2003) and as 80 mM for the second application on day 0 (June 18, 2003). Each saline-treated pot received 200 ml NaCl solution on the days of application. Pots treated with 200 ml distilled water served as control pots. A third group of pots was leached with distilled water (approximate 500 to 600 ml applied to each pot) in order to remove soil solutes (leaching). These treatments were applied to establish more solutes (NaCl solution) and fewer solutes (leaching) in the soil solution as plants entered a drought episode.

By withholding water from the pots after the second application of soil solutions, continuous drought was imposed on the sorghum plants.

Soil EC of each pot was measured with an EC meter (AR 20, Fisher Scientific Inc., Pittsburgh, PA) after calibration (0.01 N KCl solution) by the 1:5 soil/water suspension methods (Rayment and Higginson, 1992) before and after each application.

Soil Hyphae and Root Colonization by AM fungi

On day -8, each soil sample from five extra pots of each *Gi*, AZ and nonAM plant was put into a plastic bag and preserved in a refrigerator (5 °C) until measurement as described for Experiment 1.

Size of Root Systems

Soil samples from five extra *Gi* and AZ plants and five extra nonAM plants were placed in plastic bags and refrigerated (5 °C) prior to applications of salt solution and soil drying. The measurement method was same as described for Experiment 1.

Measurement of g_s , Leaf Ψ , Leaf Ψ_{π} , Leaf Ψ_{π}^{100} , Soil Ψ , Soil θ , and Leaf $[P]$

This experiment was conducted until stomatal closure (the time when the average measured stomatal conductance in each pot was $< 10 \text{ mmol m}^{-2} \text{ s}^{-1}$) using a porometer (same instrument and methods as used in experiment 1). Before applications, the measurements of stomatal conductance were done on several days for comparison with the data measured during the drying episode. Water or osmotic potential of leaves were

measured on April 8 (before application), 12 (1st application), 19 (second application), and the day of stomatal closure for each pot. The methods of each measurement were the same as explained in experiment 1.

Experimental Design and Statistical Analysis

The pots were arranged in a completely randomized block design having three mycorrhizal (nonAM, *G. margarita* and AZmix) inoculations and three soil solution treatments (NaCl, control and heavy leach). The experiment was conducted as a 3 x 3 factorial design and each treatment had six replicate pots and. Analysis of variance with specific linear contrasts was conducted for each data comparison and mean separation of each parameter was performed using Duncan's multiple range test ($P < 0.05$) SAS GLM procedure.

CHAPTER IV

RESULTS OF EXPERIMENT 1

Shoot Dry Weight, Root Colonization, Root Characteristics and Soil EC

This study was intended as a comparison of the responses of AM and nonAM sorghum plants when they were subjected to drought and/or salt stresses. Therefore before application of salt solution and soil drying, shoot dry weight, leaf phosphorus content, root colonization rates, and root characteristics for host plants of each AM treatment were measured from extra pots in order to select similar sizes of nonAM sorghum plant and AM plants. As a result of measuring dry weight of the shoot harvested from each host plant, nonAM (4.54 g/pot) plants receiving low phosphate (0.8 mM) during the preparation period and AM sorghum plants associated with *Gi* (4.36 g/pot) had similar shoot dry weights. These two groups were selected for further study (Table 1). Leaf [P] (1.52 mg/g DW) was similar in *Gi* plants and nonAM plants (1.45 mg/g DW). As expected, high phosphorus fertilization increased the leaf [P] of sorghum plants (data not shown) more than AM plants and nonAM plants receiving low phosphorus. The *Gi* plants showed higher soil hyphal density (1.16 m/g dry soil) and root colonization (53%) than nonAM plants (0.17 m/g dry soil and 0.4%). A very small portion of mycorrhizal fungi contamination was detected in nonAM plants. The AM plants associated with *Gi* were colonized by 51% hyphae, 6% arbuscule and 34% vesicle before stress applications (Table 2).

Table 1. Exp.1. Shoot dry weight, leaf phosphorus concentration (leaf [P]) and soil hyphal density of mycorrhizal and nonmycorrhizal sorghum plants before applications of salt solution and soil drying. Values represent means of 5 replicates of extra plants for each parameter.

Mycorrhizal treatment	Shoot dry weight (g/pot)	Leaf [P] (mg/g DW)	Soil hyphal density (m/g dry soil)
Non-mycorrhizal (nonAM)	4.54	1.45	0.17
<i>Glomus intraradices</i> (Gi)	4.36	1.52	1.16
P value ^z	0.67	0.58	<.0001

^zSignificant at 5% level (T-test).

Table 2. Exp.1. Mycorrhizal colonization of roots in mycorrhizal and nonmycorrhizal sorghum plants before applications of salt solution and soil drying. Values represent means of 5 replicates of extra plants for each parameter.

Mycorrhizal treatment	Hyphae (%)	Arbuscules (%)	Vesicles (%)	Root colonization ^z (%)
NonAM	0	0	0	0
<i>Gi</i>	51	6	34	53
P value ^z	<.0001	<.0001	<.0001	<.0001

^zRoot colonized with hyphae, arbuscules or vesicles

^ySignificant at 5% level (T-test).

According to the root scanning analysis, there were no significant differences in root characteristics between *Gi* and nonAM sorghum plants. Yet, *Gi* plants had larger root surface area and longer root length in general, and had 50% higher root volume density (total root volume per g dried soil) than nonAM plants (Table 3).

There were no changes in electrical conductivity (EC) (0.2 – 0.3 dS/m) of pot soil receiving distilled water (control), but the EC rapidly increased when the pots were treated with either NaCl or macronutrient solution to simulate osmotic stress. Application of macronutrient solution resulted in the highest EC (6.3 and 7.5 dS/m in nonAM and mycorrhizal pot) among the three treatments after the second application (full strengthen concentration of solution). These EC levels are too high for sorghum plants to survive under natural conditions. When the pot soil was treated with NaCl, soil EC was approximately 1.2 to 1.3 dS/m in each treatment. Based on linear contrasts, the EC of mycorrhizal soil was slightly but significantly lower than that of nonAM soil after the 1st application, but not different after the 2nd application (Table 4).

Table 3. Exp.1. Root characteristics of mycorrhizal and nonmycorrhizal sorghum plants before applications of salt solution and soil drying. Values represent means of 5 replicates of extra plants for each parameter.

Mycorrhizal treatment	Root surface area density (cm ² /g dry soil)	Root volume density (mm ³ /g dry soil)	Specific root length (cm/mg DW)	Root mass density (mg DW/g dry soil)	Root length density (cm/g dry soil)
NonAM	2.0	24.3	10.4	1.31	13.8
<i>Gi</i>	2.6	36.2	11.0	1.41	15.6
P value ^z	0.4	0.3	0.7	0.6	0.6

^zSignificant at 5% level (T-test).

Table 4. Exp.1. Electrical conductivity (EC) changes of mycorrhizal and nonmycorrhizal pot soil before and after the applications of salt solution and drought (dS/m).

Mycorrhizal treatment	Application	Before salt application (Day -9)	1 st application ^z (Day -7)	2 nd application (Day 0)
NonAM	Drought	0.2 a ^y	0.26 c	0.31 c
	Drought+NaCl	0.2 a	0.74 c	1.29 b
	Drought+osmotic stress ^x	0.2 a	2.16 a	6.31 a
<i>Gi</i>	Drought	0.2 a	0.29 c	0.25 c
	Drought+NaCl	0.2 a	0.65 c	1.19 b
	Drought+osmotic stress	0.2 a	1.68 b	7.52 a
Linear contrast ^w				
<i>Gi</i> vs. nonAM		NS	***	NS
Drought vs. Drought+saline solutions ^v		NS	***	**
NaCl vs. osmotic stress		NS	***	***
<i>Gi</i> drought vs. nonAM drought		NS	NS	NS
<i>Gi</i> drought+saline vs. nonAM drought+saline		NS	***	NS

^z 1st application; half strength NaCl (40 mM) and macronutrient solution (-0.4 MPa), 2nd application; full strength NaCl (80 mM) and macronutrient solution (-0.8 MPa).

^y Mean separation by Duncan's multiple range test at 5% level. Same letters indicate no significant differences between treatments

^x Osmotic stress was imposed with macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 9 mM NH₄NO₃ (Augé et al., 1992)

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5%(*), 1%(**), or 0.1%(***) levels.

^v NaCl and osmotic stress together were considered to be saline treatment for the linear contrast analysis.

Stomatal Behavior

Based on several measurements of stomatal behavior during drying episode, there were declines in g_s for most measurement days when the sorghum plants were exposed to NaCl or osmotic stress, irrespective of mycorrhizal colonization (Figs.1 and 2). Especially marked different values among three treatments were recorded in AM plants on day 0, 4, 5, 6, and 7 (Fig. 1), and in nonAM plants on day -5, -3, 0, 4, 5, and 6 (Fig. 2). Lower g_s values were shown in the plants with application of the macronutrient solution (osmotic stress) than in the plants receiving NaCl solution. The results of mean separation by Duncan's range test clearly confirmed these findings (Figs. 1 and 2). Water uptake by roots might be inhibited due to lowered soil water potential by build-up of salt ions in soil solution. As the drought stress developed, the g_s values generally decreased, but sometimes varied due to daily changes in irradiance. However, the value of g_s dropped sharply from day 8 in spite of normal irradiance. No different values of g_s among treatments were observed after day 8 in AM and nonAM plants, and most of sorghum plants reached stomatal closure in 9 to 11 days after withholding water. The leaves curled and changed in color at stomatal closure.

Through several preliminary measurements of g_s for *Gi* and nonAM sorghum plants before applications of salt solution and soil drying episode, mycorrhizal promotions of g_s were clearly confirmed from 23% to 77% (data not shown). During experimental period, I could confirmed the mycorrhizal promotion of g_s for several measurement days, but there were no significant differences among applications in both *Gi* and nonAM plants on each day from day 8.

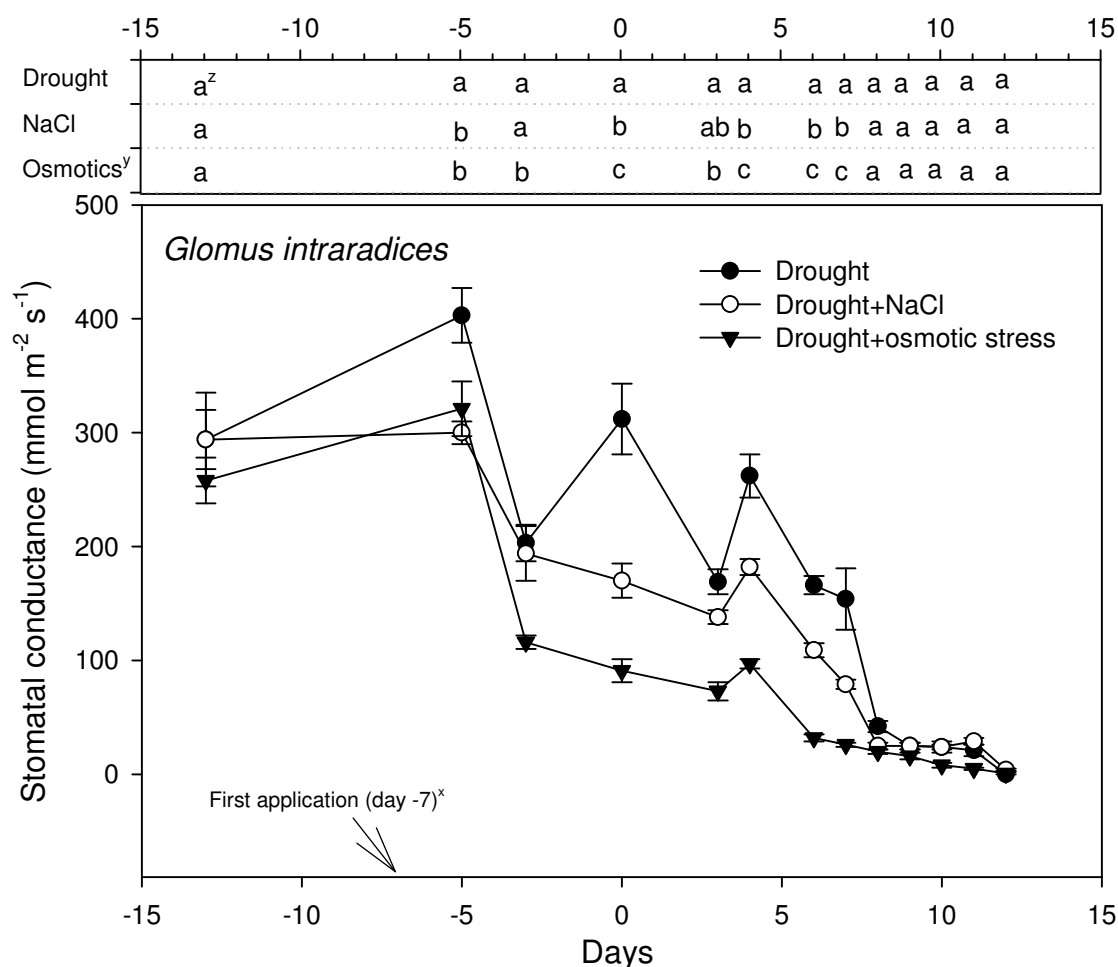


Fig.1. Exp.1. Effect of salt solution on the stomatal behavior of mycorrhizal sorghum plants subjected to soil drying. Vertical bars indicate standard error (SE) for 18 replications of each measurement.

^z Mean separation by Duncan's multiple range test ($P < 0.05$). The values of same letters mean no significant differences among treatments.

^y Osmotic stress was imposed by macronutrient solution composed of 40mM MgSO₄, 90mM Ca(NO₃)₂, 1.6mM KH₂PO₄, 62mM KNO₃, and 9mM NH₄NO₃ (Augé et al, 1992).

^x First application; half strength NaCl (40 mM) or macronutrient solution (-0.4 MPa) on day -7, second application; full strength NaCl (80 mM) or macronutrient solution (-0.8 MPa) on day 0.

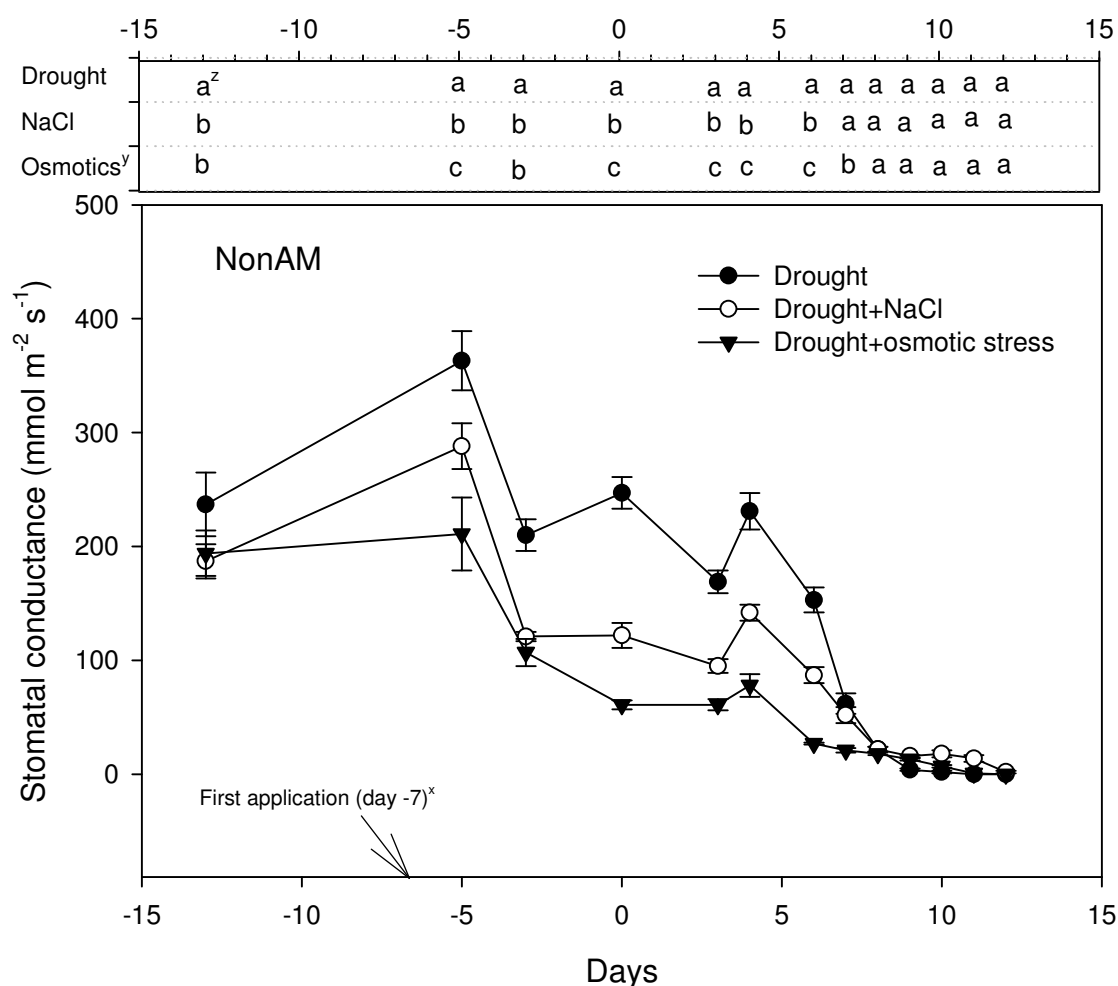


Fig.2. Exp.1. Effect of salt solution on the stomatal behavior of nonmycorrhizal sorghum plants subjected to soil drying. Vertical bars indicate standard error (SE) for 18 replications of each measurement.

^z Mean separation by Duncan's multiple range test ($P < 0.05$). The values of same letters mean no significant differences among treatments.

^y Osmotic stress was imposed by macronutrient solution composed of 40 mM MgSO_4 , 90 mM $\text{Ca}(\text{NO}_3)_2$, 1.6 mM KH_2PO_4 , 62 mM KNO_3 , and 9 mM NH_4NO_3 (Augé et al, 1992).

^ᵃ First application; half strength NaCl (40 mM) or macronutrient solution (-0.4 MPa) on day -7, second application; full strength NaCl (80 mM) or macronutrient solution (-0.8 MPa) on day 0.

Mycorrhizal association with *Gi* increased g_s of sorghum plants during much of measurement period. Stomatal behavior of *Gi* plants was more active than that of nonAM plants during the drying episode (Figs.3, 4, and 5). Stomatal conductance was significantly higher in *Gi* than in nonAM plants for 8 of the 12 measurement days when the plants were subjected to drought alone and the promotional rate was from 8% to 26% during day -7 through day 6. The promotional rate was increased markedly from day 7 (Fig. 3). When the sorghum plants were subjected to NaCl or osmotic stress under drought, the mycorrhizal promotion of g_s was recorded for more days (11 of 12 measurement days in either application) and their rates were higher than those of plants subjected to drought alone (Figs. 4 and 5). All g_s values were lower in the sorghum plants receiving NaCl or macronutrient solution and the extent of lowering was much more in nonAM than *Gi* plants. However, based on linear contrasts, these were no significant differences in g_s between *Gi* and nonAM plants subjected to osmotic stress under drought in spite of mycorrhizal promotion of g_s (Fig. 5).

It took more days for *Gi* sorghum plants to reach stomatal closure and lethal point than for nonAM plants except when subjected to drought and osmotic stress together (Table 5). More days to reach those points suggest more tolerance of plants to the stresses. The sorghum plants subjected to drought alone reached stomatal closure and lethal points first, whereas the sorghum plants receiving saline solutions required many more days. The g_s of sorghum plants treated with macronutrient solution remained above $10 \text{ mmol m}^{-2} \text{ s}^{-1}$ for 23 days in either nonAM or AM plants. The numbers of days required for sorghum plants to dry to the lethal point were significantly affected by NaCl and macronutrient solution.

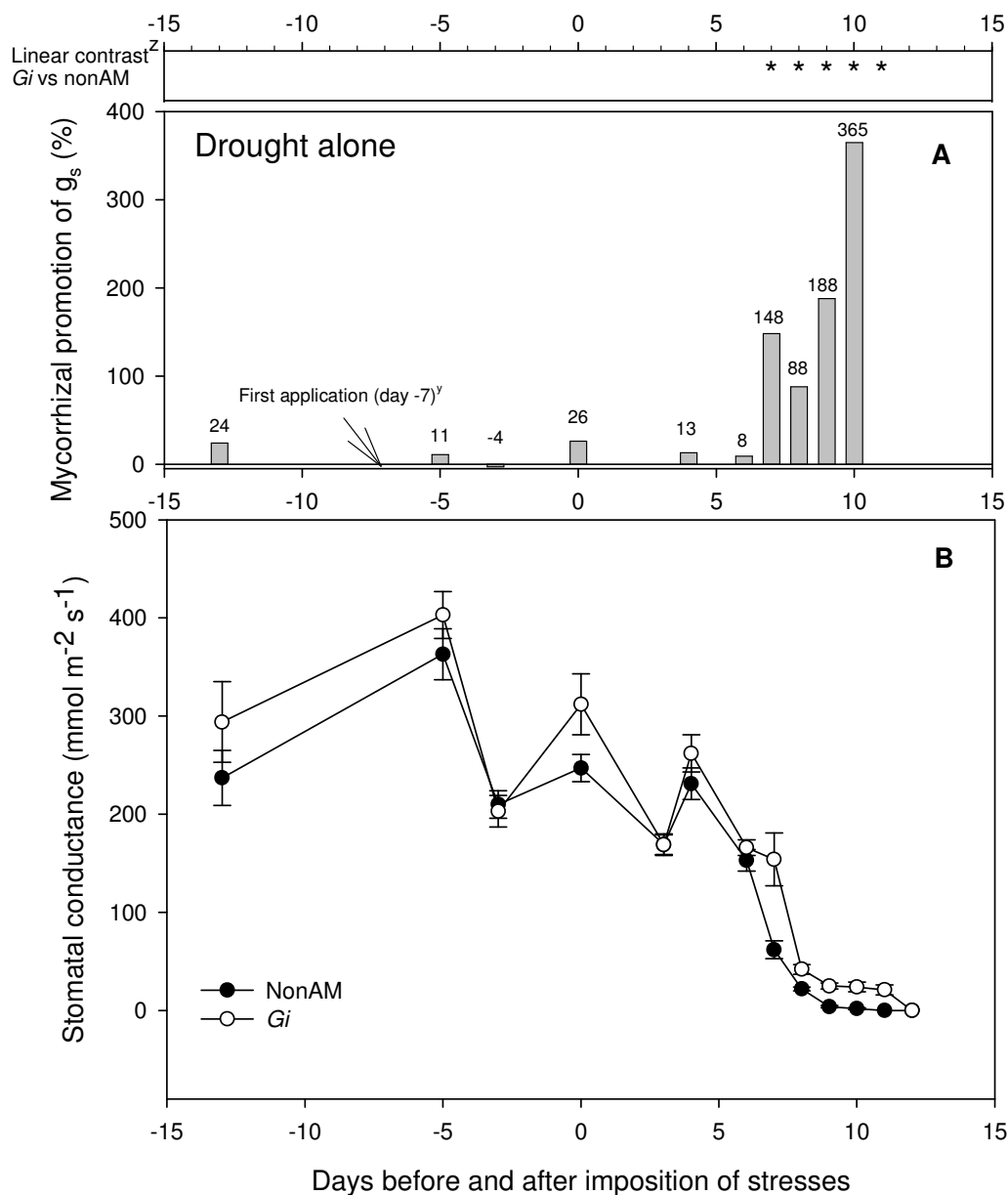


Fig.3. Exp.1. Mycorrhizal promotion of stomatal conductance (g_s) by *Glomus intraradices* (Gi) relative to nonAM plants (A), and changing g_s of Gi and nonAM sorghum plants exposed to drought (B). Vertical bars in (B) mean standard error (SE) calculated from 18 replications for each measurement.

^zThe symbol (*) indicates a significant difference between treatments based on linear contrast ($P < 0.05$).

^yFirst application; half strength NaCl (40 mM) or macronutrient solution (-0.4 MPa) on day -7, second application; full strength NaCl (80 mM) or macronutrient solution (-0.8 MPa) on day 0.

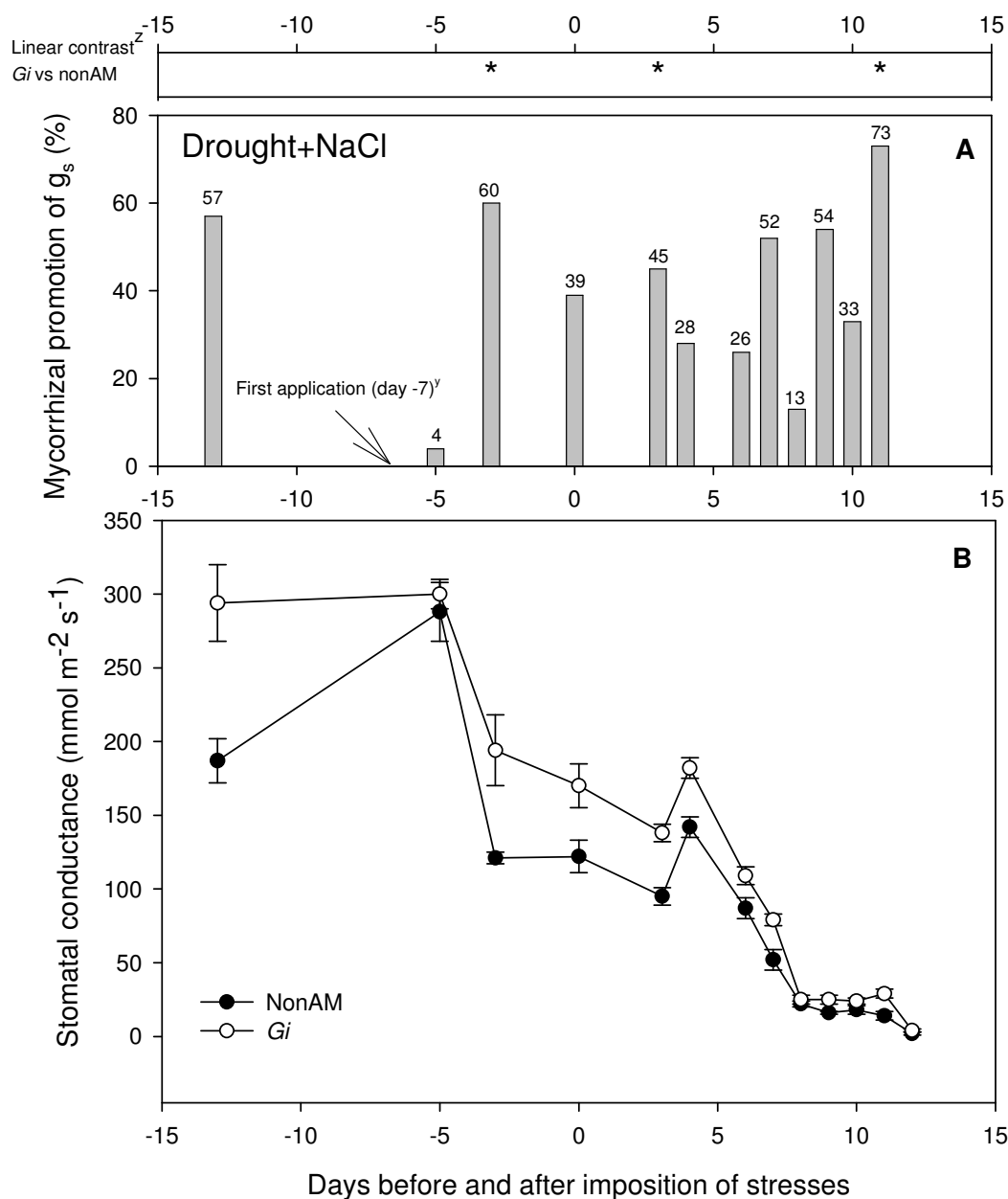


Fig.4. Exp.1. Mycorrhizal promotion of stomatal conductance (g_s) by *Glomus intraradices* (Gi) relative to nonAM plants (A), and changing g_s of Gi and nonAM sorghum plants exposed to drought and NaCl solution (B). Vertical bars in (B) mean standard error (SE) calculated from 18 replications for each measurement.

^zThe symbol (*) indicates a significant difference between treatments based on linear contrast ($P < 0.05$).

^yFirst application; half strength NaCl (40 mM) or macronutrient solution (-0.4 MPa) on day -7, second application; full strength NaCl (80 mM) or macronutrient solution (-0.8 MPa) on day 0.

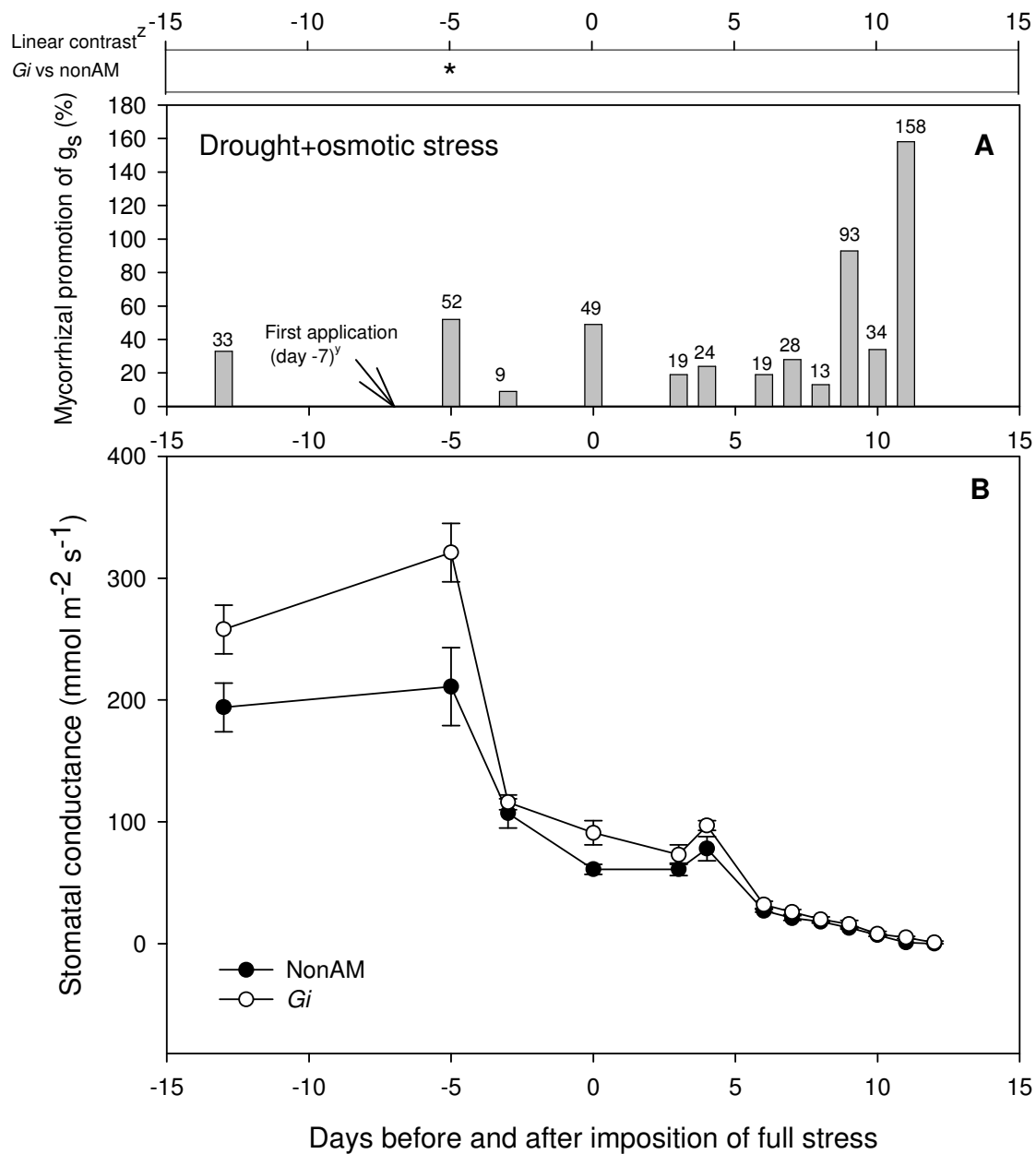


Fig.5. Exp.1. Mycorrhizal promotion of stomatal conductance (g_s) by *Glomus intraradices* (Gi) relative to nonAM plants (A), and changing g_s of Gi and nonAM sorghum plants exposed to drought and osmotic stress (B). Vertical bars mean standard error (SE) calculated from 18 replications for each measurement.

^zThe symbol (*) indicates a significant difference between treatments based on linear contrast ($P < 0.05$).

^yFirst application; half strength NaCl (40 mM) or macronutrient solution (-0.4 MPa) on day -7, second application; full strength NaCl (80 mM) or macronutrient solution (-0.8 MPa) on day 0.

It took 18 and 20 days for the nonAM and *Gi* plants, respectively, subjected to NaCl solution to reach lethal point, which were 3 to 5 days less than those for plants subjected to osmotic stress. There was a significant difference between *Gi* and nonAM plants receiving no saline application in the number of days needed to reach stomatal closure, but the difference disappeared when saline stress and drought were combined. When drought and saline stresses were both applied to the plants, there was no significant difference in the number of days needed to reach stomatal closure and lethal point between *Gi* and nonAM plants (Table 5).

Leaf Water Relations

Leaf water potential may be a direct parameter to detect the water status of leaves subjected to drought and salt stresses. The 1st and 2nd saline applications lowered the leaf Ψ of sorghum plants significantly (Table 6) and this suggests that the available water in the leaves subjected to salt and osmotic stress should be temporarily lower than control plants (without any salt solutions). As soil drought developed with applications of salt solutions, leaf Ψ rapidly dropped (more negative) and ranged -3.86 to -2.42 MPa at stomatal closure and -9.21 to -4.02 MPa at the lethal point throughout whole measurement period (Table 6). It was interesting that sorghum plants subjected to drought alone showed much lower lethal leaf Ψ (-6.88 MPa in nonAM and -9.21 MPa in *Gi* plants) than salt-treated plants ranged -5.71 to -4.02 MPa (Fig. 6). Lethal leaf Ψ of NaCl-treated and osmotic-stressed sorghum plants was similar.

Table 5. Exp.1. Days to reach stomatal closure and lethal point for sorghum plants under salt and drought stresses.

Mycorrhizal treatment	Application	Stomatal closure	Lethal point
NonAM	Drought	9.3 d ^z	14.0 d
	Drought+NaCl	11.3 ab	17.8 c
	Drought+ osmotic stress ^y	10.0 cd	23.3 a
<i>Gi</i>	Drought	11.2 ab	15.0 d
	Drought+NaCl	11.8 a	20.0 b
	Drought+ osmotic stress	10.5 bc	22.8 a
Linear contrast ^x			
<i>Gi</i> vs. nonAM		**	*
Drought vs. Drought+saline solutions ^w		*	**
NaCl vs. osmotic stress		***	***
<i>Gi</i> drought vs. nonAM drought		**	NS
<i>Gi</i> drought+saline vs. nonAM drought+saline		NS	NS

^z Mean separation by Duncan's multiple range test at 5% level. Same letters indicate no significant differences between treatments

^y Osmotic stress was imposed by macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 19 mM NH₄NO₃ (Augé et al., 1992)

^x Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^w NaCl and osmotic stress together were considered to be saline treatment for the linear contrast analysis.

Table 6. Exp.1. Changes of leaf water potential (leaf Ψ) in mycorrhizal and nonmycorrhizal sorghum plant subjected to saline and drought stresses at each experimental stage (MPa).

Mycorrhizal treatment	Application	1 st application ^z	2 nd application	Stomatal closure	Lethal point
NonAM	Drought	-0.66 ab ^y	-1.32 a	-3.86 b	-6.88 c
	Drought+NaCl	-0.84 b	-1.67 bc	-2.50 a	-5.71 ab
	Drought+osmotic stress ^x	-0.81 b	-1.81 c	-2.86 a	-4.79 a
<i>Gi</i>	Drought	-0.52 a	-1.40 ab	-2.66 a	-9.21 d
	Drought+NaCl	-0.80 b	-1.58 abc	-2.42 a	-4.36 a
	Drought+osmotic stress	-0.83 b	-1.75 c	-2.93 a	-4.02 a
Linear contrast ^w					
<i>Gi</i> vs. nonAM		NS	NS	NS	NS
Drought vs. Drought+saline solutions ^v		**	**	*	***
NaCl vs. osmotic stress		NS	NS	NS	NS
<i>Gi</i> drought vs. nonAM drought		NS	NS	**	**
<i>Gi</i> drought+saline vs. nonAM drought+saline		NS	NS	NS	NS

^z1st application; half strength NaCl (40 mM) and macronutrient solution (-0.4 MPa), 2nd application; full strength NaCl (80 mM) and macronutrient solution (-0.8 MPa)

^y Mean separation by Duncan's multiple range test at 5% level. Same letters indicate no significant differences between treatments

^x Osmotic stress was imposed by macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 19 mM NH₄NO₃ (Augé et al., 1992).

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^v NaCl and osmotic stress together were considered to be saline treatment for the linear contrast analysis.

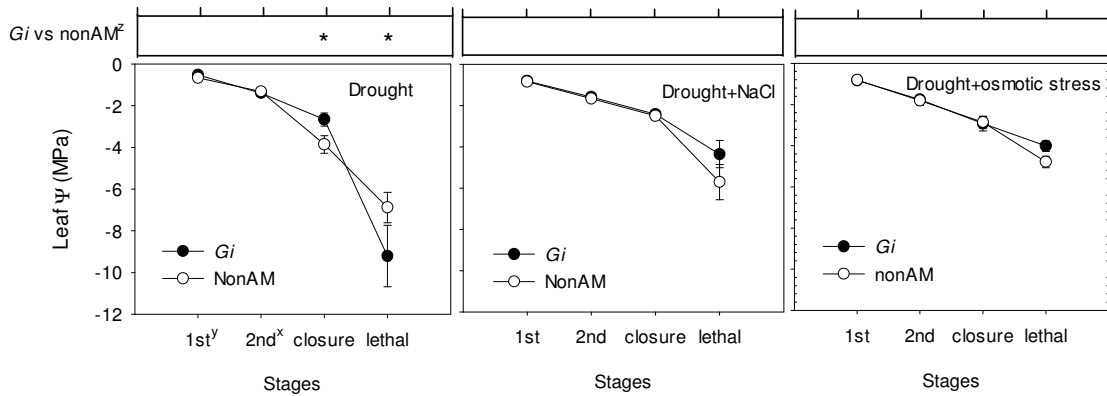


Fig.6. Exp.1. Changes in leaf water potential (leaf Ψ) of mycorrhizal and nonmycorrhizal sorghum plants exposed to drought alone (left), drought with NaCl (middle) and drought with osmotic stress (right) at each experimental stage. Vertical bars mean standard error (SE) calculated from 6 replicates. “Closure” refers to point of stomatal closure, and “lethal” refers to point at which most foliage had died.

^z The symbol (*) indicates significant differences between treatment resulting from linear contrast ($P < 0.05$).

^y First application; half strength NaCl (40 mM) or macronutrient solution (-0.4 MPa).

^x Second application; full strength NaCl (80 mM) or macronutrient solution (-0.8 MPa).

Based on mean separation or linear contrasts, *Gi* sorghum plants had a significantly higher leaf Ψ (-2.66 MPa) than nonAM plants (-3.86 MPa) when subjected to drought alone at stomatal closure. Contrary to that, *Gi* plants showed much lower leaf Ψ (-9.21 MPa) than nonAM plants (-6.88 MPa) at lethal point (supporting hypothesis 1). When combined with either NaCl or osmotic stress, the significant differences in leaf Ψ between nonAM and *Gi* plants disappeared at both stomatal closure (not supporting hypothesis 2). At lethal point, lower lethal leaf Ψ was shown in nonAM plants than in *Gi* plants, but not significant.

When pre-tested before applications (day -13), the nonAM and *Gi* sorghum leaves had similar osmotic potential at full turgor (leaf Ψ_{π}^{100}) after rehydration. However NaCl and

osmotic stress combined with drought altered leaf Ψ_{π}^{100} significantly (Fig. 7). The first application did not change leaf Ψ_{π}^{100} in each treatment (data not shown).

However as the soil dried to the lethal point, leaf Ψ_{π}^{100} of the sorghum leaves subjected to macronutrients decreased -1.48 MPa (2nd application) and -3.09 (lethal point) in nonAM plants and -1.37 MPa (2nd application) and -2.81 MPa (lethal point) in *Gi* plants. Much more osmotic adjustment has occurred in the leaves subjected to osmotic stress than treated with NaCl solution. For control plants (drought alone), little had changed in leaf Ψ_{π}^{100} between before the treatments were applied and lethal point. There were no significant differences in leaf Ψ_{π}^{100} between nonAM and AM plants (Table 7).

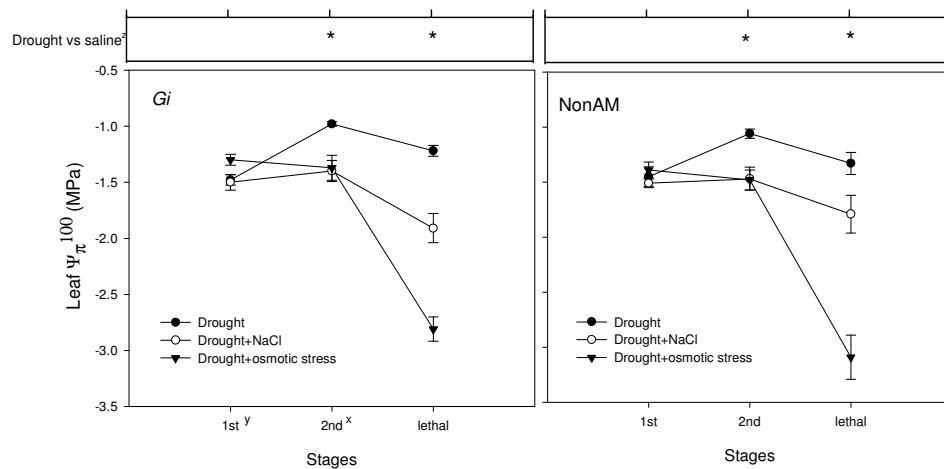


Fig.7. Exp.1. Changes in leaf osmotic potential at full turgor (leaf Ψ_{π}^{100}) of mycorrhizal (left) and nonmycorrhizal (right) sorghum plants exposed to drought alone, drought with NaCl, and drought with osmotic stress at each experimental stage. Vertical bars mean standard error (SE) calculated from 6 replicates. “Lethal” refers to point at which most foliage had died.

^z The symbol (*) indicates significant differences between treatments resulting from linear contrast ($P < 0.05$).

^y First application; half strength NaCl (40 mM) or macronutrient solution (-0.4 MPa).

^x Second application; full strength NaCl (80 mM) or macronutrient solution (-0.8 MPa).

Table 7. Exp.1. Leaf osmotic potential (leaf Ψ_{π}^{100}) at full turgor and osmotic adjustment of mycorrhizal and nonmycorrhizal sorghum plants before and after exposure to saline and drought stresses (MPa).

Mycorrhizal treatment	Application	Before applications (Day -13)	2 nd application ^z (Day 0)	Lethal point	Osmotic adjustment ^y
NonAM	Drought	-0.82 a ^x	-1.06 a	-1.33 a	0.27 b
	Drought+NaCl	-0.82 a	-1.47 b	-1.79 b	0.32 b
	Drought+osmotic stress ^w	-0.82 a	-1.48 b	-3.09 c	1.61 a
<i>Gi</i>	Drought	-0.75 a	-0.98 a	-1.22 a	0.24 b
	Drought+NaCl	-0.75 a	-1.40 b	-1.91 b	0.51 b
	Drought+osmotic stress	-0.75 a	-1.37 b	-2.81 c	1.44 a
Linear contrast ^v					
<i>Gi</i> vs. nonAM		NS	NS	NS	NS
Drought vs. Drought+saline solutions ^u		NS	***	***	***
NaCl vs. osmotic stress		NS	NS	***	***
<i>Gi</i> drought vs. nonAM drought		NS	NS	NS	NS
<i>Gi</i> drought+saline vs. nonAM drought+saline		NS	NS	NS	NS

^z Full strength NaCl (80 mM) and macronutrient solution (-0.8 MPa)

^y Osmotic adjustment was calculated by subtracting Ψ_{π} at lethal point from Ψ_{π} before application.

^x Mean separation by Duncan's multiple range test at 5% level. The values with the same letters indicate no significant differences between treatments.

^w Osmotic stress was imposed by macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 19 mM NH₄NO₃ (Augé et al., 1992).

^v Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^u NaCl and osmotic stress together were considered to be the saline treatment for the linear contrast analysis.

Therefore mycorrhizal colonization did not affect the changes in leaf Ψ_{π}^{100} under drought and/or saline stresses (not supporting hypothesis 1 and 2). Ambient leaf osmotic potential (leaf Ψ_{π}) is an important function of the amount of solutes present in the leaf tissue and showed similar alteration patterns as leaf Ψ_{π}^{100} . No significant differences were observed before and after the 1st application among treatments. A lower value of leaf Ψ_{π} was found in NaCl and osmotic stressed leaves after the 2nd application (Table 8). There were no differences in osmotic adjustment either between nonAM and *Gi* plants or between control and NaCl treated plants.

The lowest value of leaf Ψ_{π} was recorded in the leaves receiving osmotic stress under drought at stomatal closure and lethal point (Fig.7). At stomatal closure, plants subjected to drought and osmotic stress together were clearly observed to have more decreasing leaf Ψ_{π} than plants under drought alone or plant subjected to NaCl and drought. After the day of stomatal closure, the opportunity to check the proper lethal point of control plants was missed. The leaves were cut and immediately put into a water-filled bottle, but it was of no use because the leaves of control plants (treated with water) were too dry to rehydrate and measure leaf Ψ_{π} . Based on linear contrasts, there were strong differences in leaf Ψ_{π} between the control (drought alone) and NaCl-treated plants and between the NaCl and osmotic stressed plants after development of soil drying (Table 8). However based on the mean separation by Duncan's multiple range tests and linear contrasts, mycorrhizal colonization did not affect the alteration of leaf Ψ_{π} during the experimental period (not supporting hypothesis 1 and 2). There was an exponential relationship between g_s and leaf Ψ of AM and nonAM sorghum plants during drying episode (Fig. 8).

Table 8. Exp.1. Ambient leaf osmotic potential (leaf Ψ_{π}) at each experimental stage of mycorrhizal and nonmycorrhizal sorghum plants before and after exposure to saline and drought stresses (MPa).

Mycorrhizal treatment	Application	1 st application ^z	2 nd application	Stomatal closure	Lethal point
NonAM	Drought	-0.92 a ^y	-1.29 ab	-1.93 ab	- ^x
	Drought+NaCl	-1.10 b	-1.46 abc	-2.22 b	-3.22 a
	Drought+osmotic stress ^w	-0.97 ab	-1.65 c	-2.66 c	-4.62 c
<i>Gi</i>	Drought	-0.90 a	-1.26 a	-1.84 a	-
	Drought+NaCl	-1.00 ab	-1.39 ab	-1.94 ab	-3.01 a
	Drought+osmotic stress	-0.94 ab	-1.49 bc	-2.60 c	-3.92 b
Linear contrast ^v					
<i>Gi</i> vs. nonAM		NS	NS	NS	*
Drought vs. Drought+saline solutions ^u		NS	***	***	
NaCl vs. osmotic stress		NS	*	***	*
<i>Gi</i> drought vs. nonAM drought		NS	NS	NS	
<i>Gi</i> drought+saline vs. nonAM drought+saline		NS	NS	NS	NS

^z 1st application; half strength NaCl (40 mM) and macronutrient solution (-0.4 MPa), 2nd application; full strength NaCl (80 mM) and macronutrient solution (-0.8 MPa)

^y Mean separation by Duncan's multiple range test at 5% level. The values with the same letters indicate no significant differences between treatments.

^x The leaf samples were too dry to get a drop of sap for measurement of Ψ_{π}

^w Osmotic stress was imposed by macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 19 mM NH₄NO₃ (Augé et al., 1992).

^v Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^u NaCl and osmotic stress together were considered to be saline treatment for the linear contrast analysis.

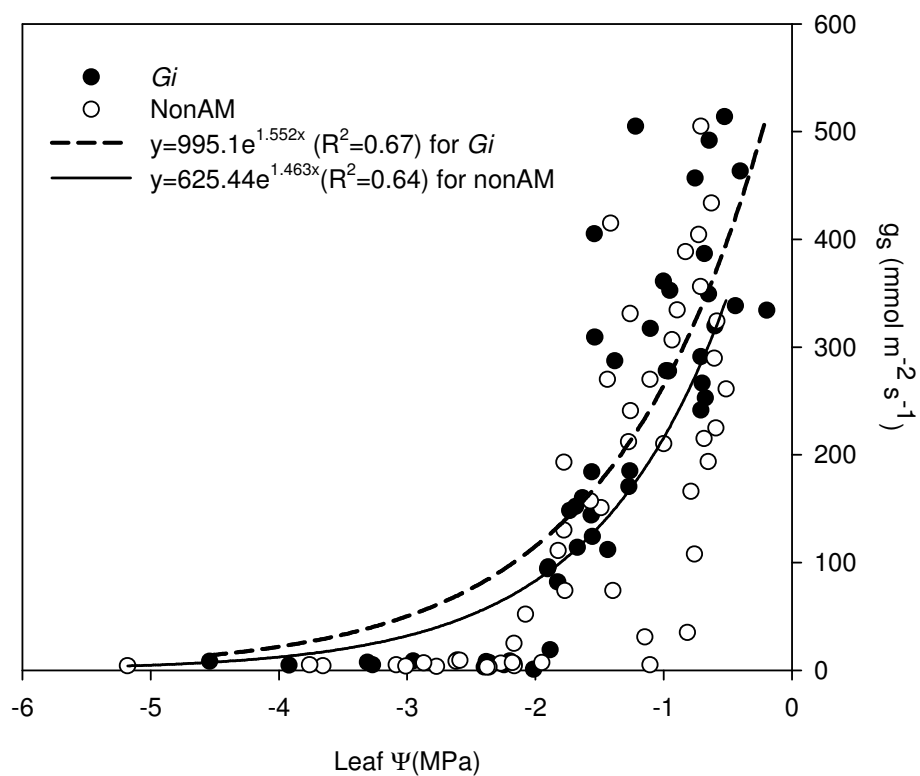


Fig.8. Exp.1. Relationship between leaf water potential and stomatal conductance of mycorrhizal and nonmycorrhizal sorghum plants during experimental period.

Soil Water Relations

The soil water potential (soil Ψ) was lowered (more negative) by applications of saline solution during soil drying. The NaCl application did not alter soil Ψ at first application, but it was significantly lowered after the 2nd application (-0.47 MPa in nonAM and -0.37 MPa in *Gi* plants). Application of osmotic stress lowered soil Ψ much more strongly than NaCl at either application stage. Based on mean separation, there were differences after the 1st and the 2nd applications, but no differences were detected between *Gi* and nonAM plants (Table 9). As soil drying developed to reach the stomatal closure and lethal point, soil Ψ of nonAM plants subjected to drought alone decreased to -5.24 MPa and -10 MPa, respectively. The decreases in soil Ψ of plants subjected to NaCl and osmotic stress also were measured and at lethal point, all the pot soil had very low soil Ψ . Contrary to the initial period (1st and 2nd application), soil Ψ were much higher (less negative) in the plants subjected to NaCl or macronutrient solution than in the plants with drought alone.

Significantly higher soil Ψ was measured for *Gi* plants than nonAM plants when treated with drought alone at the stomatal closure (supporting hypothesis 1), but there were no differences when combined with saline application (not supporting hypothesis 2). At the lethal point, marked mycorrhizal effects were found between *Gi* and nonAM plants subjected to drought and saline together (supporting hypothesis 2). Soil Ψ in the plants subjected to drought alone was too dry to be measured at the end of experiment (lethal point), irrespective of *Gi* or nonAM plants.

Table 9. Exp.1. Changes in soil water potential (soil Ψ) at each experimental stage under salt and drought stresses (MPa).

Mycorrhizal treatment	Application	1 st application ^z	2 nd application	Stomatal closure ^y	Lethal point
NonAM	Drought	0.00 a ^x	-0.01 a	-5.24 b	-
	Drought+NaCl	0.00 a	-0.47 b	-1.88 a	-5.20 b
	Drought+osmotic stress ^x	-0.26 b	-1.00 c	-2.08 a	-5.69 b
<i>Gi</i>	Drought	0.00 a	-0.05 a	-3.49 a	-
	Drought+NaCl	-0.03 a	-0.37 b	-1.38 a	-3.95 a
	Drought+osmotic stress	-0.31 c	-0.87 c	-1.87 a	-3.92 a
Linear contrast ^w					
<i>Gi</i> vs. nonAM		NS	NS	**	*
Drought vs. Drought+saline solutions ^v		***	***	***	-
NaCl vs. osmotic stress		***	***	NS	NS
<i>Gi</i> drought vs. nonAM drought		NS	NS	***	-
<i>Gi</i> drought+saline vs. nonAM drought+saline		NS	*	NS	*

^z 1st application; half strength NaCl (40 mM) and macronutrient solution (-0.4 MPa), 2nd application; full strength NaCl (80 mM) and macronutrient solution (-0.8 MPa)

^y The data were an average of sample soil from the bottom and middle portion of each pot

^x Mean separation by Duncan's multiple range test at 5% level. Same letters indicate no significant differences between treatments.

^w Osmotic stress was imposed by macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 19 mM NH₄NO₃ (Augé et al., 1992).

^v Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^u NaCl and osmotic stress together were considered to be saline treatment for the linear contrast analysis.

The measurement of soil water content was made only at the last two stages of the experiment, because it was assumed that the initial water content was sufficient and similar in each pot. The accumulation of salt ions in soil might dominantly affect soil θ and much more water was remained in the soil of pots receiving osmotic stress in spite of the assumption that the whole pot had the same amount of water from the beginning of the experiment. The sorghum plants under combined drought and osmotic stress, had lower g_s , lower leaf Ψ and lower leaf Ψ_π than control plants (drought alone) even though they had sufficient water in the pot soil. There was more water in the bottom than in the middle portion. The NaCl application also might make the soil water unavailable for sorghum plants. The remaining amount of water in the NaCl-applied pots was more than in control pots. Mycorrhizal colonization did not affect the changes in soil θ at stomatal closure (not supporting hypothesis 1 and 2). At the lethal point, the soil of *Gi* plants had slightly higher soil θ when subjected to NaCl or osmotic stress and these were significant differences detected in the bottom portion (supporting hypothesis 2). There were no differences in soil θ between *Gi* and nonAM plants subjected to drought alone at the lethal point (not supporting hypothesis 1). These results clearly indicate that plants will not uptake enough water under combined salt and drought stress and that the remaining water was unavailable for the sorghum plants (Table 10).

Table 10. Exp.1. Changes in soil water contents (soil θ) at each experimental stage under salt and drought stresses (mg/g dry soil).

Mycorrhizal treatment	Application	Stomatal closure		Lethal point	
		Bottom ^z	Middle	Bottom	Middle
NonAM	Drought	0.72 c ^y	4.07 c	0.53 d	0.88 b
	Drought+NaCl	9.56 b	14.12 b	5.31 c	2.47 b
	Drought+osmotic stress ^x	39.73 a	25.11 a	11.71 b	5.70 ab
<i>Gi</i>	Drought	1.65 c	3.63 c	0.55 d	0.79 b
	Drought+NaCl	16.62 b	13.27 b	6.77 c	4.20 ab
	Drought+osmotic stress	40.73 a	15.97 b	15.95 a	9.03 a
Linear contrast ^w					
<i>Gi</i> vs. nonAM		NS	NS	*	NS
Drought vs. Drought+saline solutions ^v		***	***	***	**
NaCl vs. osmotic stress		***	*	***	*
<i>Gi</i> drought vs. nonAM drought		NS	NS	NS	NS
<i>Gi</i> drought+saline vs. nonAM drought+saline		NS	NS	**	NS

^z Soil was sampled on both middle (14 to 17 cm from top) and bottom-portion (27 to 30 cm from top).

^y Mean separation by Duncan's multiple range test at 5% level. Same letters indicate no significant differences between treatments.

^x Osmotic stress was imposed by macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 19 mM NH₄NO₃ (Augé et al., 1992).

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^v NaCl and osmotic stress together were considered to be the saline treatment for the linear contrast analysis.

CHAPTER V

RESULTS OF EXPERIMENT 2

Shoot Dry Weight, Root Colonization, Root Characteristics and Soil EC

The host plants were colonized with three kinds of AM fungi including nonAM and grown in greenhouse maintaining same environmental and nutritional condition. Before application of salt solution and soil drying, shoot dry weight, leaf [P], soil hyphal density and root colonization rate of extra five pots in each host plant were measured and the results were shown in Table 11 and 12. The plants colonized with Arizona mix (AZ plants) had lowest dry weight (0.8 g/pot) and the whole plant size was also smallest (data not shown) among three host plants after growing for two months. The plants colonized with *Gigaspora margarita* (GM plants) had more dry weight (1.4 g/pot) than nonAM plants (1.2 g/pot), but the difference was not significant. Like experiment 1, the shoot of AM sorghum plants contained a little bit more phosphorus contents (1.8 mg/g DW) than nonAM plants. More soil hyphal density was obtained in AM plants than nonAM plant, but relatively lower value (0.2 and 0.4 m/g dry soil) than those in experiment 1 (1.16 m/g dry soil) (Table 11).

AM plants were confirmed to have good root colonization throughout the counting the number of hyphae, arbuscules and vesicles. The occupation by hyphae and vesicles in roots were higher in the roots of AZ plants (33% and 22%) than in *Gm* plants (24% and 5%). Although vesicles should not be formed in the roots colonized with *G. margarita*, small portion of the roots might be contaminated with other species of AM fungi in the greenhouse during experimental period (Table 12).

Table 11. Exp.2. Shoot dry weight, leaf phosphorus concentration (leaf [P]) and soil hyphal density of mycorrhizal and nonmycorrhizal sorghum plants before applications of salt solution and soil drying. Values represent means of 5 replicates of extra plants for each parameter.

Mycorrhizal treatment	Shoot dry weight (g/pot)	Leaf [P] (mg/g DW)	Soil hyphal density (m/g dry soil)
Non-mycorrhizal (nonAM)	1.2 ab ^z	1.5 b	0.0 b
Arizona Mix (AZ)	0.8 b	1.8 a	0.4 a
<i>Gigaspora margarita</i> (GM)	1.4 a	1.8 ab	0.2 b
Linear contrast ^y			
NonAM vs AM	*	ns	***
AZ vs <i>Gm</i>	Ns	ns	*

^z Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^y Linear contrasts indicate nonsignificance (ns), or significance at 5% (*), 1% (**) or 0.1% (***) levels.

Table 12. Exp.2. Mycorrhizal colonization of roots in mycorrhizal and nonmycorrhizal sorghum plants before applications of salt solution and soil drying. Values represent means of 5 replicates of extra plants for each parameter.

Mycorrhizal treatment	Hyphae (%)	Arbuscule (%)	Vesicle (%)	Root colonization ^z (%)
NonAM	0 c ^z	0 b	0 c	1 c
AZ	33 a	2 a	22 a	36 a
<i>Gm</i>	24 b	1 a	5 b	24 b
Linear contrast ^y				
NonAM vs AM	***	***	***	***
AZ vs <i>Gm</i>	*	*	**	*

^z Root colonized with hyphae, arbuscules or vesicles

^y Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^x Linear contrasts indicate nonsignificance (ns), or significance at 5% (*), 1% (**) or 0.1% (***) levels.

A small portion of mycorrhizal colonization in nonAM roots (1%) might be due to contamination too. According to the linear contrast, there were significant differences in hyphaes, arbuscules, vesicles and root colonization between AZ and *Gm* plants as well as nonAM and AM plants.

The results of root size measurement suggested that the roots of all the host plants in this experiment should not develop enough when compared with experiment 1 (Table 13). Overall values of parameters related with root development were much less than those in experiment 1. Mycorrhizal association did not promote the root development significantly and the roots of plants infected by AZ were even smaller than nonAM plants instead, but *Gm* plants had more surface area density ($0.67 \text{ cm}^2/\text{g dry soil}$), root mass density ($0.5 \text{ mg DW /g dry soil}$) and root length density ($8.63 \text{ cm/g dry soil}$) than nonAM and AZ plants. Colonization with Arizona mix did not benefit the shoot and root growth at all in this experiment. Based on linear contrast, there were no significant differences in all parameters related with root size between nonAM and AM plants. However, the roots of *Gm* plants developed significantly much better than those of AZ plants (Table 13).

The initial soil EC was same in each pot (0.12 dS/m), but it has increased to 1.33 dS/m at most when NaCl solutions were applied to the pot soil (Table 14). On the contrary, in control and heavy leached soil, the average EC was down to $0.05\text{-}0.07 \text{ dS/m}$ at the 2nd application. No mycorrhizal colonization effects on changes in EC were observed. Compared to the EC values (0.25 to 0.31 dS/m) in experiment 1, very low EC values (0.06 to 0.07 dS/m) of water-treated soil were detected in experiment 2 after second application.

Table 13. Exp.2. Root characteristics of mycorrhizal and nonmycorrhizal sorghum plants before applications of salt solution and soil drying. Values represent means of 5 replicates of extra plants for each parameter.

Mycorrhizal treatment	Root surface area density (cm ² /g dry soil)	Root volume density (mm ³ /g dry soil)	Specific root length (cm/mg DW)	Root mass density (mg DW/g dry soil)	Root length density (cm/g dry soil)
NonAM	0.44 b ^z	0.04 a	0.06 a	0.38 b	6.09 b
AZ	0.46 b	0.01 b	0.02 b	0.11 c	5.89 b
<i>Gm</i>	0.67 a	0.05 a	0.07 a	0.50 a	8.63 a
Linear contrast ^y					
NonAM vs AM	NS	NS	NS	NS	NS
AZ vs <i>Gm</i>	*	*	*	**	**

^y Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^y Linear contrasts indicate nonsignificance (ns), or significance at 5% (*), 1% (**) or 0.1% (***) levels.

Table 14. Exp.2. Electrical conductivity (EC) changes of mycorrhizal and nonmycorrhizal pot soil before and after applications of salt solution (dS/m).

Mycorrhizal treatment	Application	Before application	1 st application	2 nd application
NonAM	Drought	0.12 a ^z	0.11 b	0.07 b
	Drought+fewer solute ^y	0.12 a	0.09 b	0.05 b
	Drought+more solute ^x	0.12 a	0.63 a	1.31 a
AZ	Drought	0.12 a	0.12 b	0.06 b
	Drought+fewer solute	0.12 a	0.11 b	0.06 b
	Drought+more solute	0.12 a	0.62 a	1.27 a
<i>Gm</i>	Drought	0.12 a	0.13 b	0.06 b
	Drought+fewer solute	0.12 a	0.09 b	0.05 b
	Drought+more solute	0.12 a	0.62 a	1.33 a
Linear contrast ^w				
AM vs. nonAM		NS	NS	NS
Fewer solutes vs. more solute		NS	***	***
Control vs. more solutes		NS	***	***
AM drought ^v vs. nonAM drought		NS	NS	NS
AM drought+salt vs. nonAM drought+salt		NS	NS	NS

^z Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^y Leached heavily with distilled water several times for simulation remaining fewer solutes in soil solution than control.

^x 1st application (day -7); half strength NaCl solution (40 mM), 2nd application (day 0); full strength solution (80 mM)

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^v Drought in this linear contrast contains normal drought (without any treatments) and fewer solutes (leached with distilled water) together.

Stomatal Behavior

It has been confirmed that the mycorrhizal colonization enhanced stomatal behavior of host plants several times as observed in experiment 1. However the frequency was not much than that in experiment 1. Promotion by *Gm* was more easily checked for the host plants at all stages, but it was hard to find the effect of AZ colonization on promotion of g_s except the last stage of experimental period. There were no significant differences in mycorrhizal promotions of g_s by *Gm* or AZ during day-7 to day 7 in experiment 2. *Gm* colonization promoted g_s of sorghum plants subjected to drought for 8 of the 21 measurement days and the promotion rates were lower than 20% (Fig. 9 and 10). *Gm* plants also hardly promoted g_s when the plants were imposed with NaCl solution withholding water (Fig. 11). Few measurements showed mycorrhizal promotion by AZ colonization during day-7 through day7. The lowest conductance on every measurement day was recorded on the plants colonized with AZ until day 7, but the g_s in AZ plants became higher than other plants from day8 to the stomatal closure. As mentioned, since the initial size of AZ plants was smaller than other plants, AZ plants might maintain more normal g_s than other plants.

As monitoring g_s during the experimental period, *Gm* plants always showed the highest stomatal behavior from the beginning of the measurement. When NaCl solution was applied to the plants under drought, the absolute value of g_s decreased and the difference between nonAM and *Gm* plants was not significant. After the day 7, g_s in AZ plants was temporarily recorded the highest value at all treatment.

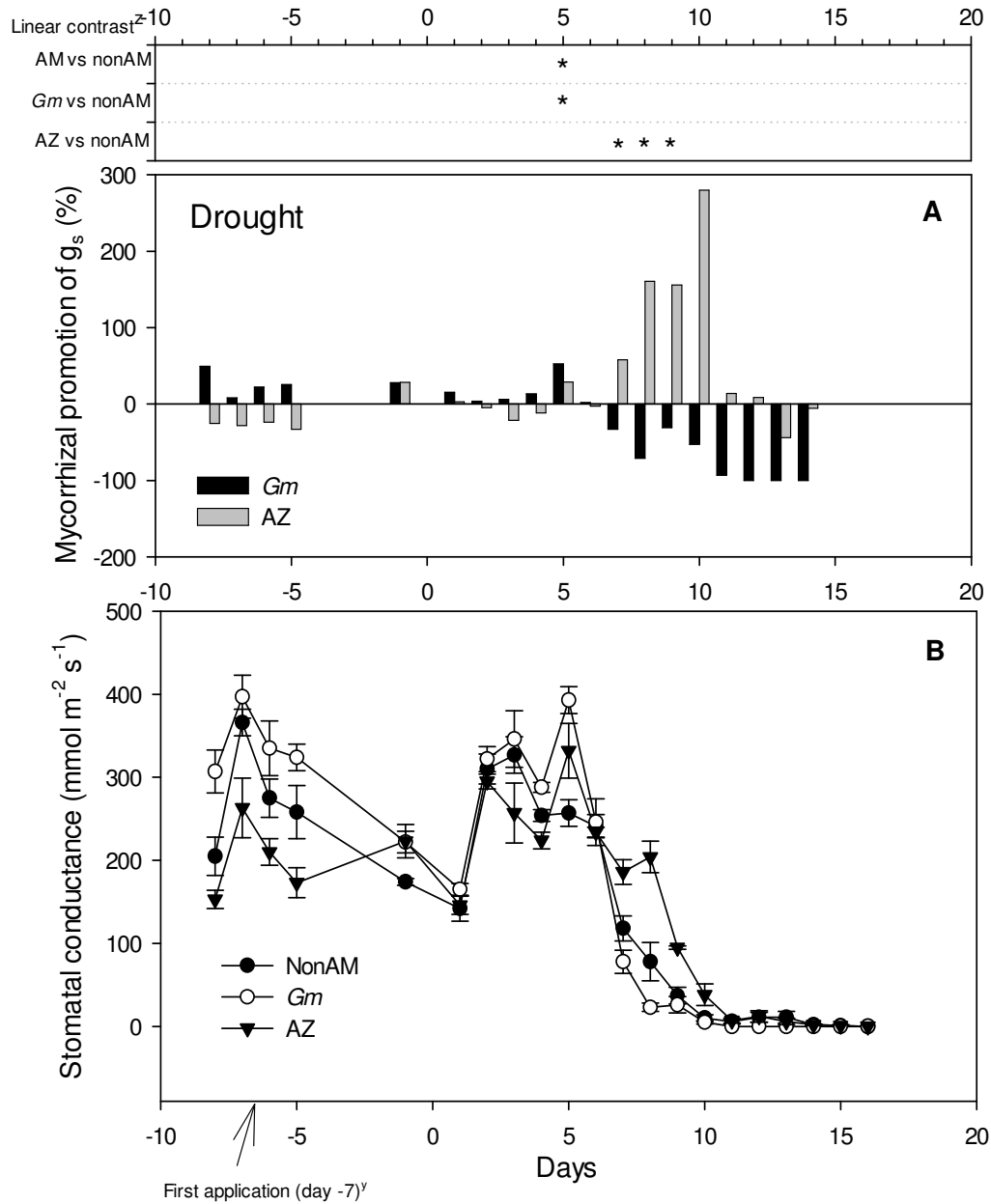


Fig.9. Exp.2. Mycorrhizal promotion of stomatal conductance (g_s) by *Gigaspora margarita* (Gm) or Arizona mix (AZ) relative to nonAM plants (A), and changing g_s of sorghum plants subjected to drought (B). Vertical bars mean standard error (SE) calculated from 18 replications for each measurement.

^zThe symbol (*) indicates a significant difference between treatments based on linear contrast ($P < 0.05$).

^yFirst application; half strength NaCl (40 mM) on day -7, second application; full strength NaCl (80 mM).

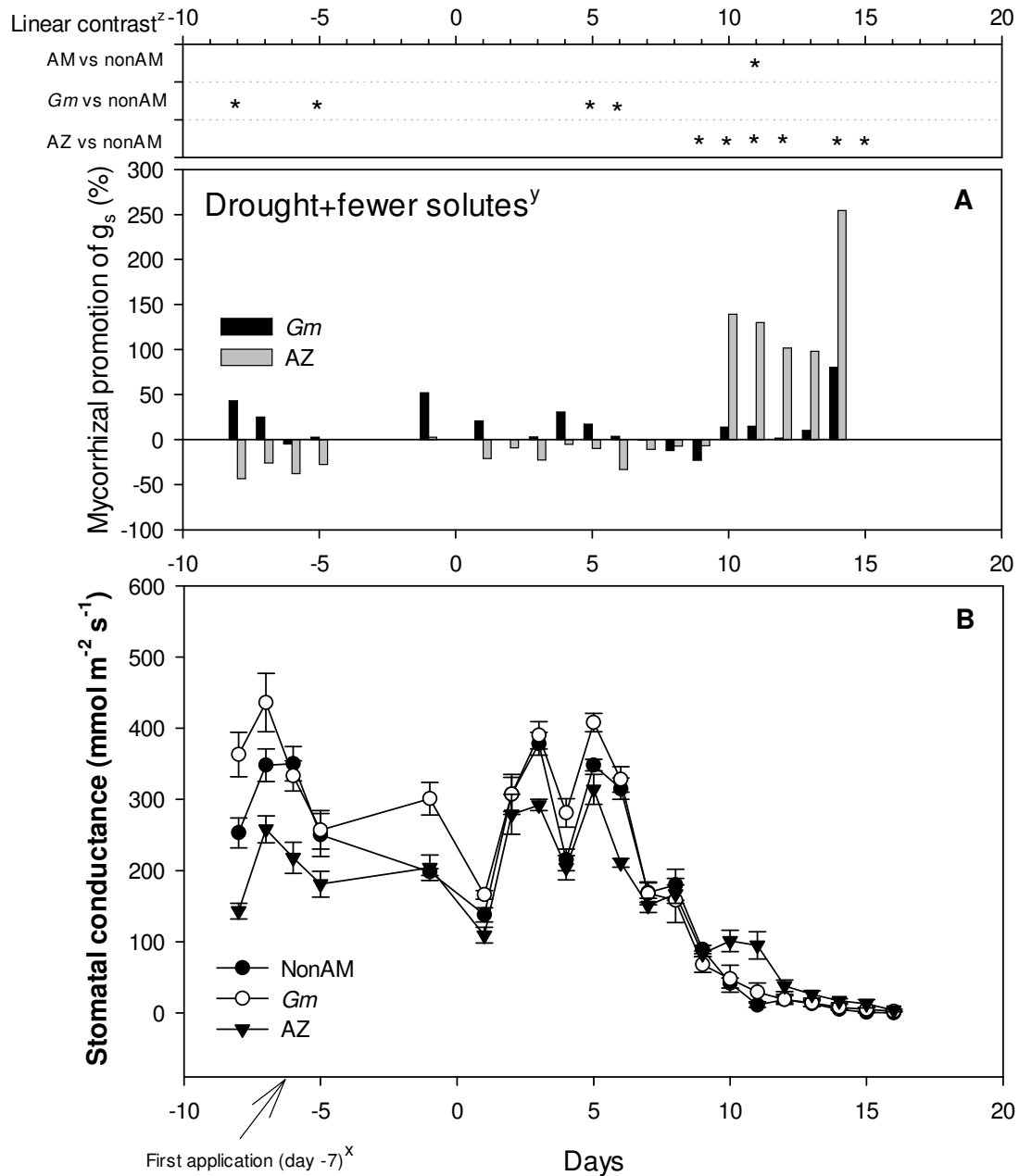


Fig.10. Exp.2. Mycorrhizal promotion of stomatal conductance (g_s) by *Gigaspora margarita* (Gm) or Arizona mix (AZ) relative to nonAM plants (A), and changing g_s of sorghum plants subjected to drought (B). Vertical bars mean standard error (SE) calculated from 18 replications for each measurement.

^zThe symbol (*) indicates a significant difference between treatments based on linear contrast ($P < 0.05$).

^yFirst application; half strength NaCl (40 mM) on day -7, second application; full strength NaCl (80 mM) on day 0.

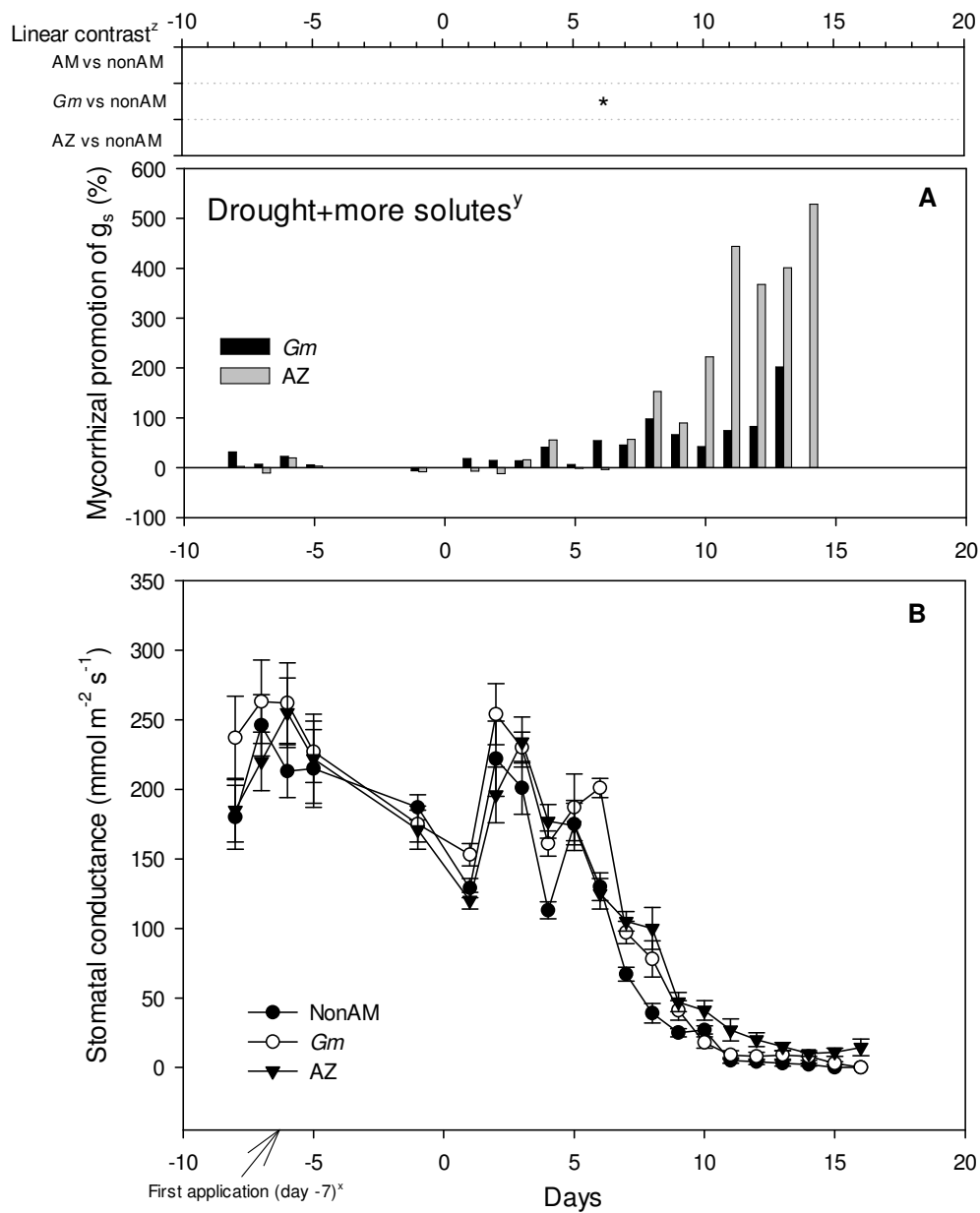


Fig.11. Exp.2. Mycorrhizal promotion of stomatal conductance (g_s) by *Gigaspora margarita* (Gm) or Arizona mix (AZ) relative to nonAM plants (A), and changing g_s of sorghum plants subjected to drought and NaCl (B). Vertical bars mean standard error (SE) calculated from 18 replications for each measurement.

^zThe symbol (*) indicates a significant difference between treatments based on linear contrast ($P < 0.05$).

^yFirst application; half strength NaCl (40 mM) on day -7, second application; full strength NaCl (80 mM).

This experiment was finished at the stomatal closure unlike experiment 1 and the days required to reach at the end of experiment were very variable within each treatment. Control (drought only) plants reached the end point slightly faster (9 to 11 days) than others. When combined with salt solution under drought, the days to stomatal closure became to be longer than those of the plants imposed to drought alone. It took 16 days, the longest days, for AZ plants subjected to NaCl application to reach the stomatal closure. According to the linear contrast and mean separation test, there were significant differences between control plant and NaCl treated plants (Table 15).

Table 15. Exp.2. Days to reach the stomatal closure for mycorrhizal and nonmycorrhizal sorghum plants subjected to saline and drought stresses.

Mycorrhizal treatment	Drought	Drought+fewer solutes ^z	Drought+more solutes ^y
NonAM	10 a ^x	13 a	11 b
AZ	11 a	14 a	16 a
<i>Gm</i>	9 a	12 a	12 b
Linear contrast ^w			
NonAM vs AM			NS
AZ vs <i>Gm</i>			NS
Fewer solutes vs. more solute			NS
Control vs. more solutes			**
AM drought ^v vs. nonAM drought			NS
AM drought+salt vs. nonAM drought+salt			*

^z Leached heavily with distilled water several times for simulation remaining fewer solutes in soil solution than control.

^y 1st application; half strength NaCl solution (40 mM), 2nd application; full strength solution (80 mM)

^x Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

^v Drought in this linear contrast contains normal drought (without any treatments) and fewer solutes (leached with distilled water) together.

Leaf Water Relations

Before salt application and withholding water, the leaf Ψ within each host plants was very similar ranged from -0.85 to -0.93 MPa. Since I have observed the lower leaf Ψ of salt applied plants in experiment 1, decreases in leaf Ψ were routinely expected in experiment 2. However mean separation by multiple range tests showed no significant differences in leaf Ψ among treatments within each host plants during experimental period under drought (Table 16). These measurements were very hard to explain because leaf Ψ did not decrease in spite of two applications of NaCl solution. The only statistical differences by linear contrast were occurred in nonAM vs. AZ plant and nonAM vs. *Gm* plants after 2nd application (Table 17). Integrated across treatment, means of leaf Ψ at stomatal closure were quite similar among AM host species, in which ranged -2.45 to -2.32. Drought directly lowered leaf Ψ , but NaCl treatment didn't do any marked effects on leaf Ψ (Table 17). Fig.12 showed the rapid decrease in leaf Ψ near stomatal closure within different host plants, but there were no statistical differences between host plants or salt treatments (not supporting hypothesis 1 and 2).

Measurement of leaf Ψ_{π} was made from the sap of thawed leaves at room temperature. Before full strengthen application (day 0), no differences in leaf Ψ_{π} were observed within treatments and host plants. After the 2nd application, linear contrast showed the only significant difference in leaf Ψ_{π} between heavy leached (drought plus fewer solutes) and NaCl treated (drought plus more solutes) plants (Table 18). At stomatal closure point, leaf Ψ_{π} in control (drought only) plants was significantly different from those in NaCl treated plants.

Table 16. Exp.2. Changes in leaf water potential (leaf Ψ) of mycorrhizal and nonmycorrhizal sorghum plants subjected to drought and salt stresses (MPa).

Mycorrhizal treatment	Application	Before application	1 st application	2 nd application	Stomatal closure
NonAM	Drought	-0.85 a ^z	-0.94 a	-1.00 a	-2.26 a
	Drought+fewer solutes ^y	-0.91 a	-0.94 a	-0.84 a	-2.61 a
	Drought+more solutes ^x	-0.87 a	-0.93 a	-0.96 a	-2.48 a
AZ	Drought	-0.88 a	-0.93 a	-0.85 a	-2.39 a
	Drought+fewer solutes	-0.93 a	-0.91 a	-0.80 a	-2.03 a
	Drought+more solutes	-0.88 a	-0.97 a	-0.93 a	-2.66 a
<i>Gm</i>	Drought	-0.90 a	-0.99 a	-0.92 a	-2.38 a
	Drought+fewer solutes	-0.93 a	-0.94 a	-0.83 a	-2.46 a
	Drought+more solutes	-0.90 a	-0.94 a	-0.86 a	-2.13 a
Linear contrast ^w					
AM vs. nonAM		NS	NS	NS	NS
Fewer solutes vs. more solute		NS	NS	NS	NS
Control vs. more solutes		NS	NS	NS	NS
AM drought ^v vs. nonAM drought		NS	NS	NS	NS
AM drought+salt vs. nonAM drought+salt		NS	NS	NS	NS

^z Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^y Leached heavily with distilled water several times for simulation remaining fewer solutes in soil solution than control.

^x 1st application (day -7); half strengthen NaCl solution (40 mM), 2nd application (day 0); full strengthen solution (80 mM)

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels

^v Drought in this linear contrast contains normal drought (without any treatments) and fewer solutes (leached with distilled water) together.

Table 17. Exp.2. Average leaf water potential (leaf Ψ) of mycorrhizal and nonmycorrhizal sorghum plants under drought and salt stresses (MPa). To compare the mycorrhizal effect on Ψ_{leaf} , each data of species was combined irrespective of soil solution application.

Mycorrhizal treatment	Before application	1 st application ^z	2 nd application	Stomatal Closure
NonAM	-0.87 b ^y	-0.94 a	-0.94 b	-2.45 a
AZ	-0.90 ab	-0.94 a	-0.86 a	-2.36 a
<i>Gm</i>	-0.91 a	-0.96 a	-0.87 a	-2.32 a
Linear contrast ^x				
NonAM vs Arizona mix	NS	NS	*	NS
NonAM vs <i>Gm</i>	*	NS	*	NS
AZ vs <i>Gm</i>	NS	NS	NS	NS

^z 1st application (day -7); half strengthen NaCl solution (40 mM), 2nd application (day 0); full strengthen solution (80 mM)

^y Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^x Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

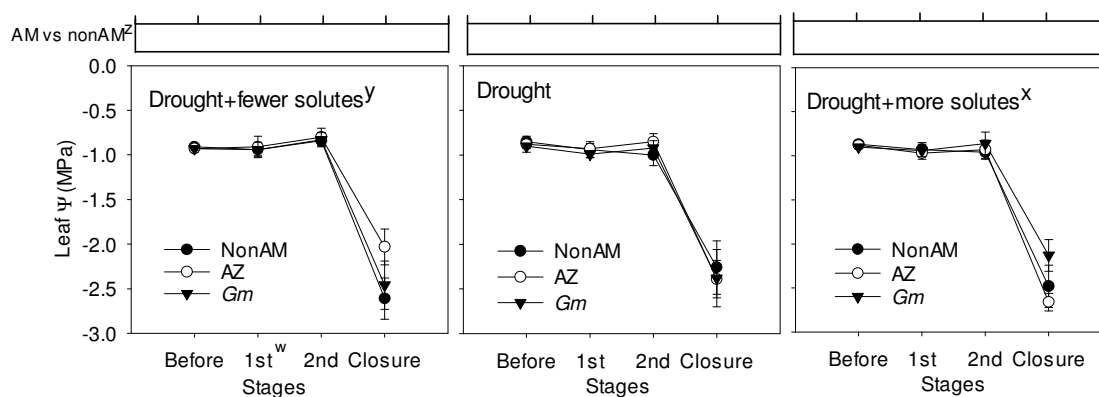


Fig.12. Exp.2. Changes in leaf water potential (leaf Ψ) of mycorrhizal and nonmycorrhizal sorghum plants exposed to drought with fewer solute (left), drought (middle), and drought with more solutes (right) at each experimental stage. Vertical bars mean standard error calculated from 6 replicates.

^zThe results of linear contrast showed no significant differences between AM and nonAM.

^yLeached heavily with distilled water before soil drying.

^xApplied with NaCl solution of 40 mM (1st application) and 80 mM (2nd application).

^w1st application; day -7, 2nd application; day 0, closure; stomatal closure meaning average g_s was under $10 \text{ mmol m}^{-2} \text{ s}^{-1}$ per each pot.

Table 18. Exp.2. Changes in leaf osmotic potential (leaf Ψ_{π}) of mycorrhizal and nonmycorrhizal sorghum plants subjected to drought and salt stresses (MPa).

Mycorrhizal treatment	Application	Before application	1 st application	2 nd application	Stomatal closure
NonAM	Drought	-0.84 bcd ^z	-0.89 ab	-0.84 a	-1.53 ab
	Drought+fewer solutes ^y	-0.90 cd	-0.91 b	-0.91 abc	-1.61 abc
	Drought+more solutes ^x	-0.88 cd	-0.93 b	-1.05 c	-1.82 c
AZ	Drought	-0.79 ab	-0.83 a	-0.90 abc	-1.54 ab
	Drought+fewer solutes	-0.92 d	-0.86 ab	-0.93 bc	-1.49 a
	Drought+more solutes	-0.85 bcd	-0.88 ab	-0.91 abc	-1.85 c
<i>Gm</i>	Drought	-0.81 ab	-0.87 ab	-0.89 ab	-1.63 abc
	Drought+fewer solutes	-0.83 abc	-0.84 a	-0.89 ab	-1.54 ab
	Drought+more solutes	-0.75 a	-0.83 a	-0.89 ab	-1.78 bc
Linear contrast ^w					
AM vs. nonAM		NS	NS	NS	NS
Fewer solutes vs. more solute		NS	NS	**	*
Control vs. more solutes		NS	NS	NS	*
AM drought ^v vs. nonAM drought		NS	NS	NS	NS
AM drought+salt vs. nonAM drought+salt		NS	NS	*	NS

^z Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^y Leached heavily with distilled water several times for simulation remaining fewer solutes in soil solution than control.

^x 1st application (day -7); half strengthen NaCl solution (40 mM), 2nd application (day 0); full strengthen solution (80 mM)

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels

^v Drought in this linear contrast contains normal drought (without any treatments) and fewer solutes (leached with distilled water) together.

Any beneficial effects of mycorrhizal association with host plants on the alteration of leaf Ψ_{π} could not be observed during the whole experimental period (Table 19).

The initial value (before application of drought and soil solution) of leaf Ψ_{π}^{100} in each plant was similar ranging from -0.09 to -0.22. After first application of soil solution, significant differences in leaf Ψ_{π}^{100} were detected between heavy-leached and NaCl-treated plants, and between control and NaCl-treated plants. However the differences were not shown after second application. When the sorghum plants reached the stomatal closure, leaf Ψ_{π}^{100} did not change much like that of experiment 1. After drying episode (at stomatal closure), leaf Ψ_{π}^{100} ranged from -1.14 to -1.18 MPa in the plants exposed to drought alone (water or heavy-leached) and -1.45 to -1.52 MPa in the plants applied with NaCl solution (Table 20). Osmotic adjustment ranged from -1.04 to -1.07 MPa in the plants under drought alone and -1.23 to -1.29 MPa in salt imposed plants. The values in NaCl treated plants were significantly lower than either control or heavy-leached plants. Like in leaf Ψ_{π} , there were not any differences in leaf Ψ_{π}^{100} between nonAM and AM plants (Table 21) (not supporting hypothesis 1 and 2).

As soil solutions were applied and the soil drying developed, leaf Ψ_{π}^{100} decreased. The lowest values of leaf Ψ_{π}^{100} for the plants receiving NaCl solution in each host plant were clearly shown in Fig.13.

Table 19. Exp.2. Average leaf osmotic potential (leaf Ψ_{π}) of mycorrhizal and nonmycorrhizal sorghum plants under drought and salt stresses (MPa). To compare the mycorrhizal effect on leaf Ψ_{π} , each data of species was combined irrespective of soil solution application.

Mycorrhizal treatment	Before application	1 st application ^z	2 nd application	Stomatal closure
NonAM	-0.88 b ^y	-0.91 b	-0.93 a	-1.65 a
AZ	-0.85 ab	-0.86 a	-0.91 a	-1.62 a
<i>Gm</i>	-0.79 a	-0.84 a	-0.89 a	-1.65 a
Linear contrast ^x				
NonAM vs AZ	NS	*	NS	NS
NonAM vs <i>Gm</i>	*	*	NS	NS
AZ vs <i>Gm</i>	NS	NS	NS	NS

^z 1st application (day -7); half strengthen NaCl solution (40 mM), 2nd application (day 0); full strengthen solution (80 mM)

^y Mean separation by Duncan's multiple range test test ($P=0.05$). Same letters indicate no significant differences between treatments

^x Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

Table 20. Exp.2. Changes in leaf osmotic potential at full turgor (leaf $\Psi\pi^{100}$) of mycorrhizal and nonmycorrhizal sorghum plants exposed to drought and salt stresses (MPa).

Mycorrhizal treatment	Application	Before application	1 st application	2 nd application	Stomatal closure	Osmotic adjustment
NonAM	Drought	-0.09 a ^z	-0.10 a	-0.80 a	-1.18 a	-1.07 a
	Drought+fewer solutes ^y	-0.14 a	-0.10 a	-0.91 a	-1.15 a	-1.04 a
	Drought+more solutes ^x	-0.16 a	-0.22 a	-0.94 a	-1.45 b	-1.23 ab
AZ	Drought	-0.12 a	-0.09 a	-0.90 a	-1.14 a	-1.05 a
	Drought+fewer solutes	-0.18 a	-0.13 a	-0.93 a	-1.18 a	-1.06 a
	Drought+more solutes	-0.18 a	-0.25 a	-0.90 a	-1.50 b	-1.25 ab
<i>Gm</i>	Drought	-0.16 a	-0.12 a	-0.90 a	-1.18 a	-1.06 a
	Drought+fewer solutes	-0.20 a	-0.12 a	-0.93 a	-1.16 a	-1.04 a
	Drought+more solutes	-0.22 a	-0.24 a	-0.84 a	-1.52 b	-1.29 b
Linear contrast ^w						
AM vs. nonAM		NS	NS	NS	NS	NS
Fewer solutes vs. more solute		NS	*	NS	*	*
Control vs. more solutes		NS	*	NS	*	*
AM drought ^v vs. nonAM drought		NS	NS	NS	NS	NS
AM drought+salt vs. nonAM drought+salt		NS	NS	NS	NS	NS

^zMean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^yLeached heavily with distilled water several times for simulation remaining fewer solutes in soil solution than control.

^x1st application (day -7); half strengthen NaCl solution (40 mM), 2nd application (day 0); full strengthen solution (80 mM)

^wLinear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels

^vDrought in this linear contrast contains normal drought (without any treatments) and fewer solutes (leached with distilled water) together.

Table 21. Exp.2. Average leaf osmotic potential at full turgor (leaf Ψ_{π}^{100}) of mycorrhizal and nonmycorrhizal sorghum plants under drought and salt stresses (MPa). To compare the mycorrhizal effect on leaf Ψ_{π}^{100} , each data of species was combined irrespective of soil solution application.

Mycorrhizal treatment	Before application	1 st application ^z	2 nd application	Stomatal closure	Osmotic adjustment
NonAM	-0.13 a ^y	-0.14 a	-0.88 a	-1.26 a	-1.12 a
AZ	-0.16 a	-0.16 a	-0.91 a	-1.27 a	-1.12 a
<i>Gm</i>	-0.19 a	-0.16 a	-0.89 a	-1.29 a	-1.13 a
Linear contrast ^x					
NonAM vs AZ	NS	NS	NS	NS	NS
NonAM vs <i>Gm</i>	NS	NS	NS	NS	NS
AZ vs <i>Gm</i>	NS	NS	NS	NS	NS

^z 1st application (day -7); half strengthen NaCl solution (40 mM), 2nd application (day 0); full strengthen solution (80 mM)

^y Mean separation by Duncan's multiple range test test ($P=0.05$). Same letters indicate no significant differences between treatments

^x Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

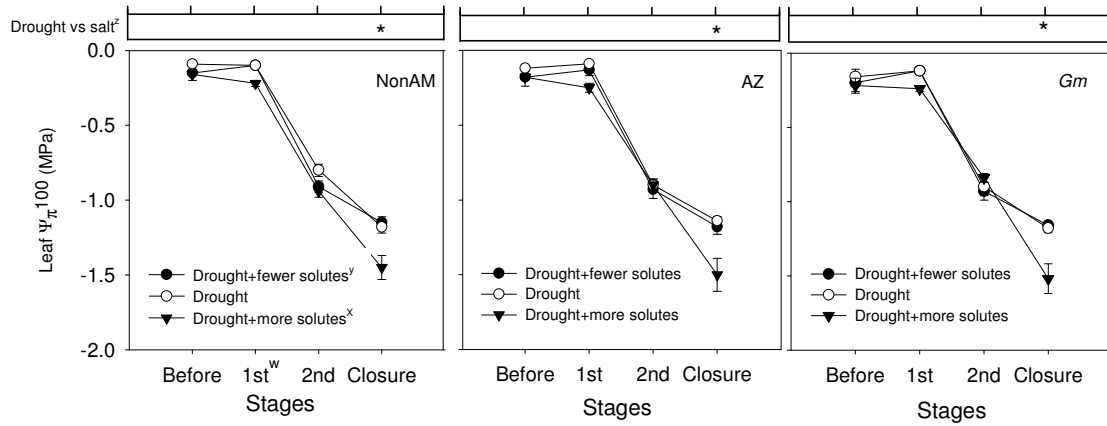


Fig.13. Exp.2. Changes in leaf osmotic potential at full turgor (leaf Ψ_{π}^{100}) of sorghum plants infected by nonmycorrhizal soil (left), Arizona mix (middle) and *Gigaspora margarita* (right) under drought and salt stresses at each experimental stage.

²The results of linear contrast showed no significant differences between AM and nonAM.

^yLeached heavily with distilled water before soil drying.

^xApplied with NaCl solution of 40mM (1st application) and 80mM (2nd application).

^w1st application; day -7, 2nd application; day 0, closure; stomatal closure meaning average g_s was under $10 \text{ mmol m}^{-2} \text{ s}^{-1}$ per each pot.

Soil Water Relations

Unlike other parameters, soil Ψ and soil θ related with soil water status were influenced by application of salt solution in experiment 2. Application of NaCl solution allowed soil Ψ to be significantly lower than control plants after 2nd application. At stomatal closure, host plants subjected to drought alone showed very lower soil Ψ than those of plants exposed to drought and salt stress together (Table 22). Lower soil Ψ was measured in bottom portion of pots than in middle portion of pots at stomatal closure. Based on linear contrast, a significant difference in soil Ψ was detected in the middle portion of pots between AM and nonAM plants when exposed to either drought alone or drought combined with salt stress at stomatal closure (supporting to hypothesis 1 and 2). However mean separation (Duncan's multiple range test) did not showed the differences among mycorrhizal treatments and salt applications. In comparison of mycorrhizal effects on soil Ψ , the sorghum plants colonized with *Gm* showed the lowest soil Ψ among mycorrhizal treatments at stomatal closure (Table 23).

Mycorrhizal plant might uptake more water from pot soil than non-mycorrhizal plants during drying experimental period. The initial water content in the pots (1st and 2nd application) ranged 21.8 to 39.3 (mg/g dry soil) with non-significance among the treatments (Table 24). At stomatal closure point, the water in nonAM pot soil (5.8 mg) remained much more than mycorrhizal pot soil (2.9 mg in AZ and 3.0 mg in *Gm*) in middle portion of the NaCl treated pots. When it was assumed that the water content of all the pots were almost same, it is believed that mycorrhizal-inoculated root took up more water than those of nonAM under saline condition.

Table 22. Exp.2. Changes in soil water potential (soil Ψ) after application of soil solution and at stomatal closure (MPa).

Mycorrhizal treatment	Application	1 st	2 nd	Stomatal closure		
		application	application	Middle ^z	Bottom	Mean
NonAM	Drought	0.0 a ^y	0.0 a	-1.5 a	-4.0 abc	-2.7 abc
	Drought+fewer solutes ^x	-0.1 a	0.0 a	-1.9 ab	-5.2 c	-3.6 cd
	Drought+more solutes ^w	-0.1 a	-0.2 b	-0.9 a	-1.9 a	-1.4 a
AZ	Drought	-0.1 a	0.0 a	-2.2 ab	-5.1 c	-3.6 cd
	Drought+fewer solutes	0.0 a	0.0 a	-1.6 a	-4.5 bc	-3.1 bc
	Drought+more solutes	-0.1 a	-0.2 b	-1.5 a	-2.3 ab	-1.9 ab
<i>Gm</i>	Drought	-0.1 a	0.0 a	-3.6 c	-6.0 c	-4.8 d
	Drought+fewer solutes	0.0 a	0.0 a	-3.0 bc	-4.8 bc	-3.9 cd
	Drought+more solutes	-0.1 a	-0.1 ab	-1.6 a	-1.8 a	-1.7 a
Linear contrast ^v						
AM vs. nonAM		NS	NS	**	NS	NS
Fewer solutes vs. more solute		NS	***	*	***	***
Control vs. more solutes		NS	***	**	***	***
AM drought ^u vs. nonAM drought		NS	NS	*	NS	NS
AM drought+salt vs. nonAM drought+salt		NS	NS	*	NS	NS

^z Soil was sampled on both middle (14 to 17 cm from top) and bottom-portion (27 to 30 cm from top).

^y Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^x Leached heavily with distilled water several times for simulation remaining fewer solutes in soil solution than control.

^w 1st application (day -7); half strengthen NaCl solution (40 mM), 2nd application (day 0); full strengthen solution (80 mM)

^v Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels

^u Drought in this linear contrast contains normal drought (without any treatments) and fewer solutes (leached with distilled water) together.

Table 23. Exp.2. Average soil water potential (soil Ψ) in mycorrhizal or nonmycorrhizal soil after application of soil solution and at stomatal closure (MPa). To compare the mycorrhizal effect on soil Ψ , each data of species was combined irrespective of soil solution application.

Mycorrhizal treatment	1 st application ^z	2 nd application	Stomatal closure		
			Middle ^y	Bottom	Mean
NonAM	-0.06 a ^x	-0.06 a	-1.43 a	-3.70 a	-2.56 a
AZ	-0.01 a	-0.02 a	-1.76 a	-3.96 a	-2.86 a
<i>Gm</i>	-0.05 a	-0.02 a	-2.72 b	-4.18 a	-3.45 b
Linear contrast ^w					
NonAM vs AZ	NS	NS	NS	NS	NS
NonAM vs <i>Gm</i>	NS	NS	**	NS	**
AZ vs <i>Gm</i>	NS	NS	**	NS	**

^z 1st application; half strengthen NaCl solution (40 mM), 2nd application; full strengthen solution (80 mM)

^y Soil was sampled on both middle (14 to 17 cm from top) and bottom-portion (27 to 30 cm from top).

^x Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^wLinear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

Table 24. Exp.2. Changes in soil water content (soil θ) after application of soil solution and at stomatal closure (mg/ g dry soil).

Mycorrhizal treatment	Application	1 st	2 nd	Stomatal closure		
		application	application	Middle ^z	Bottom	Mean
NonAM	Drought	28.35 a ^y	20.18 a	0.92 c	0.55 c	0.74 c
	Drought+fewer solutes ^x	33.96 a	23.22 a	0.92 c	0.33 c	0.63 c
	Drought+more solutes ^w	34.23 a	22.39 a	5.76 a	11.14 a	8.45 a
AZ	Drought	36.51 a	30.59 a	1.19 c	0.54 c	0.87 c
	Drought+fewer solutes	37.36 a	21.36 a	1.68 c	0.68 c	1.18 c
	Drought+more solutes	33.78 a	22.68 a	2.91 b	7.74 b	5.33 b
<i>Gm</i>	Drought	21.81 a	15.70 a	0.65 c	0.52 c	0.59 c
	Drought+fewer solutes	38.81 a	22.92 a	0.87 c	0.45 c	0.66 c
	Drought+more solutes	29.58 a	20.26 a	2.97 b	11.42 a	7.20 a
Linear contrast ^v						
AM vs. nonAM		NS	NS	NS	NS	NS
Fewer solutes vs. more solute		NS	NS	***	***	***
Control vs. more solutes		NS	NS	***	***	***
AM drought ^u vs. nonAM drought		NS	NS	NS	NS	NS
AM drought+salt vs. nonAM drought+salt		NS	NS	**	NS	NS

^z Soil was sampled on both middle (14 to 17 cm from top) and bottom-portion (27 to 30 cm from top).

^y Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^x Leached heavily with distilled water several times for simulation remaining fewer solutes in soil solution than control.

^w 1st application; half strengthen NaCl solution (40 mM), 2nd application; full strengthen solution (80 mM)

^v Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels

^u Drought in this linear contrast contains normal drought (without any treatments) and fewer solutes (leached with distilled water) together.

The soil Ψ was observed a little bit lower in NaCl treated soil in initial stage of the experiment and higher at the stomatal closure point than the pot soil treated with water and heavy leach with non-significant difference between AM and nonAM pot. Due to limited amount of water condition inside the pots under drought stress, the continuous water uptake and transpiration might result in shortage of water and allow the all plants to reach the stomatal closure point in 9 through 14 days since day 0 (the day with full strengthen treatment). Among them, the sorghum plants inoculated with AZ took longer days to reach the point than any others in all treatment. It was assumed that the smallest initial size of AZ plants might attribute to the longer day to reach stomatal closure than other host plants.

There were no evidences to retain more water in nonAM soil at stomatal closure. Based on linear contrast, there were no differences in soil water content between host plants. However, mean separation test showed a significant difference in soil water content between host plants in the middle portion of pot at stomatal closure. NonAM plants had more amount of soil water (2.53 mg/g dry soil) than AZ (1.93 mg) and *Gm* plants (1.50 mg) (Table 25).

Table 26 is a compendium of results of two experiments to show whether the hypothesis 1 and 2 are either approved or not approved by each parameter based on linear contrast.

Table 25. Exp.2. Average soil water content (soil θ) in mycorrhizal or nonmycorrhizal soil after application of soil solution and at stomatal closure (mg/ g dry soil). To compare the mycorrhizal effect on soil θ , each data of species was combined irrespective of soil solution application.

Mycorrhizal treatment	1 st application ^z	2 nd application	Stomatal closure		
			Middle ^y	Bottom	Mean
NonAM	32.18 a ^x	21.93 a	2.53 a	4.01 a	3.27 a
AZ	35.88 a	24.87 a	1.93 b	2.99 a	2.46 a
<i>Gm</i>	32.18 a	21.93 a	1.50 b	4.13 a	3.27 a
Linear contrast ^w					
NonAM vs AZ	NS	NS	NS	NS	NS
NonAM vs <i>Gm</i>	NS	NS	NS	NS	NS
AZ vs <i>Gm</i>	NS	NS	NS	NS	NS

^z 1st application; half strengthen NaCl solution (40 mM), 2nd application; full strengthen solution (80 mM)

^y Soil was sampled on both middle (14 to 17 cm from top) and bottom-portion (27 to 30 cm from top).

^x Mean separation by Duncan's multiple range test ($P=0.05$). Same letters indicate no significant differences between treatments

^w Linear contrasts indicate nonsignificance (ns), or significance at the 5% (*), 1% (**), or 0.1% (***) levels.

Table 26. Tests of hypothesis 1 and 2 for experiment 1 and 2. Effect of mycorrhizal fungi, *Gi*, *Gm* or AZ on tolerance to drought, salt or their combined stress. H₀; There are no significant differences between mycorrhizal inoculation and nonAM (hypothesis 1; when subjected to drought alone, hypothesis 2; when salt stress was combined to drought). Rejection of H₀ means that there was a significant effect of the AM treatment.

Experiment	Hypothesis	Parameter ^z	AM	Water stress / Treatment	H ₀	
					Stomatal closure	Lethal Point
1 ^y	1	Leaf Ψ	<i>Gi</i>	Drought	Reject	Reject
		Leaf $\Psi\pi$			Accept	Accept
		Leaf Ψ_{π}^{100}			Accept	Accept
		Soil Ψ			Reject	Accept
		Soil θ			Accept	Accept
	2	Leaf Ψ	<i>Gi</i>	Drought+NaCl	Accept	Accept
		Leaf $\Psi\pi$			Accept	Accept
		Leaf Ψ_{π}^{100}			Accept	Accept
		Soil Ψ			Accept	Reject
		Soil θ			Accept	Accept
	2	Leaf Ψ	<i>Gi</i>	Drought+osmotics ^x	Accept	Accept
		Leaf $\Psi\pi$			Accept	Accept
		Leaf Ψ_{π}^{100}			Accept	Accept
		Soil Ψ			Accept	Reject
		Soil θ			Accept	Accept
	2	Leaf Ψ	<i>Gi</i>	Drought+both ^w	Accept	Accept
		Leaf $\Psi\pi$			Accept	Accept
		Leaf Ψ_{π}^{100}			Accept	Accept
		Soil Ψ			Accept	Accept
		Soil θ			Accept	Accept
2	1	Leaf Ψ	<i>Gm</i>	Drought	Accept	- ^v
		Leaf $\Psi\pi$			Accept	-
		Leaf Ψ_{π}^{100}			Accept	-
		Soil Ψ			Reject	-
		Soil θ			Accept	-
	1	Leaf Ψ	AZ	Drought	Accept	-
		Leaf $\Psi\pi$			Accept	-
		Leaf Ψ_{π}^{100}			Accept	-
		Soil Ψ			Accept	-
		Soil θ			Accept	-
	2	Leaf Ψ	<i>Gm</i>	Drought+NaCl	Accept	-
		Leaf $\Psi\pi$			Accept	-
		Leaf Ψ_{π}^{100}			Accept	-
		Soil Ψ			Accept	-
		Soil θ			Reject	-
	2	Leaf Ψ	AZ	Drought+NaCl	Accept	-
		Leaf $\Psi\pi$			Accept	-
		Leaf Ψ_{π}^{100}			Accept	-
		Soil Ψ			Accept	-
		Soil θ			Accept	-

^z Leaf Ψ , leaf water potential; leaf $\Psi\pi$, leaf osmotic potential; leaf Ψ_{π}^{100} , leaf osmotic potential at full turgor after rehydration; soil Ψ , soil water potential; soil θ , soil mass water content.

^y For experiment 1 and 2, soil Ψ and soil θ refer to average of values measured at middle of pot and bottom of pot given in table 9, and 22-25.

^x Osmotic stress was imposed with macronutrient solution composed of 40 mM MgSO₄, 90 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 62 mM KNO₃ and 9 mM NH₄NO₃ (Augé et al., 1992)

^w Average values of treatment with NaCl and osmotics.

^v Experiment 2 was finished at stomatal closure.

CHAPTER VI

DISCUSSION

Mycorrhizal Symiosis and Stomatal Behavior under Drought and Salt Stress

Because an immediate response of plants to drought may be a quick change in stomatal behavior (Medrano et al., 2002; Ueda et al, 2003) and plant growth was closely related to stomatal conductance (Ruiz-Lozano et al, 1995), g_s has been considered to be the main mechanism by which plants respond to soil water deficits (Liu and Stützel, 2002). Throughout these two separate experiments, either drought or salt stress reduced g_s and resulted in full closing of stomata in sorghum leaves in 10 to 15 days after imposition of withholding water. When the two stresses were combined, g_s was much lower than for exposure to drought alone.

According to Ludlow (1989), sorghum plants have avoidance strategy of response to water stress, which means they have characteristics that maximize water uptake and minimize water loss. In general, plants having avoidance strategy usually have well-developed root systems to uptake maximum amount of water from soil and control stomatal behavior to reduce water loss (Levitt, 1980). Stomatal closure is one of the primary defense mechanisms protecting plants from severe dehydration (Chapman and Augé, 1994).

Soil hyphae resulting from mycorrhizal colonization could help host plants have drought avoidance by scavenging available water in drying soil (Augé et al., 2003). The sorghum root system associated with *Glomus intraradices* were slightly more developed

than nonAM roots in experiment 1, but Arizona mix and *Gigaspora margarita* did not affect root growth in experiment 2. Perhaps this difference in root development is the reason why the promotion of g_s by *G. intraradices* was more than those by *G. margarita* or Arizona mix.

Higher g_s in AM than nonAM plants has been observed in a number of studies (reviewed by Augé, 2001). However, mycorrhizal colonization did not always enhance g_s and the mycorrhizal promotion of g_s was often unpredictable. The extent of AM promotion of g_s has been observed to vary with temperature and light (Augé et al., 2004) or kinds of fungal species (Ruiz-Lozano et al., 1995). In my experiment, mycorrhizal promotional effects on g_s were observed several times during soil drying, indicating the colonization with either *Glomus intraradices* or *Gigaspora margarita* increased stomatal opening relative to nonAM plants.

I did not observe consistent patterns in AM promotion of g_s when the plants were exposed to either salt or osmotic stress under drought. The g_s data in each measurement day showed increased g_s in AM plant when subjected to salt or osmotic stress, but their promotion rates were often lower than AM plant under drought alone. Even though little is known about stomatal behavior when imposed to salt and drought stress together, the salt or osmotic stress appeared to inhibit the mycorrhizal effect on promotion of g_s . When cowpea was exposed to short-term osmotic stress using either sorbitol or the same macronutrient solution used in my experiment, leaves of AM cowpea had higher g_s than those of nonAM cowpea before and after lowering soil water potential to -0.7 MPa (Augé et al., 1992). On the contrary, g_s was not higher in AM citrus seedlings than nonAM plants when imposed to saline soil solution (Hartmond et al., 1987).

Symbiosis with a mixture of AM fungi isolated from a semi-arid region in Arizona (AZ) did not affect g_s promotion on most measurement days except several days near stomatal closure. Probably it took longer for soil to dry out in the same soil volume because their shoots were smaller (less leaf area). When compared to similar size of nonAM plants, *Phaseolus vulgaris* colonized with Arizona mix showed greater ability to avoid drought (Augé et al., 2003). The AM promotion rate was higher in experiment 1 than in experiment 2. It is uncertain whether the higher AM promotion rate in experiment 1 was due to the specific mycorrhizal fungi or the different period of colonization between two experiments.

Mycorrhizal Symbiosis and Leaf Water Relations under Drought and Salt Stress

Drought and salt stress often occur simultaneously, but their effects on plant development often have been studied separately. Water and salt stress induce similar plant responses initially, yet clear differences have been observed at stomatal closure and lethal point. Leaf Ψ of the leaves imposed to salt solutions were statistically greater (less negative) than water stressed leaves at lethal point in experiment 1. As salt concentration increased, a water stress situation was established in the leaves resulting in a more negative water potential with increasing salt concentration in soil solutions (Sohan et al., 1999). Therefore salt stress accompanied with drought might make the host plants less tolerant to water deficit condition. When osmotic stress was imposed on the plants, leaf Ψ became less negative than imposition of NaCl solutions. Osmotic stress can also slow water uptake by diminishing the water potential gradient between the soil and the root

(Sohan et al., 1999). The reason was uncertain that there were no significant differences in leaf Ψ between treatments in experiment 2.

Leaf Ψ at lethal point has been considered to be the main parameter controlling stomatal behavior during drought (Ludlow, 1989). When exposed to drought alone, both nonAM and *Gi* plants had lower leaf Ψ than those of saline-treated plants at lethal point in experiment 1, which meant more loss of absolute water contents in plants imposed to drought alone.

Because sorghum plants are very sensitive to leaf Ψ (Ludlow, 1989), I expected some measurable differences in leaf water relations between mycorrhizal and non-mycorrhizal plants. However, AM influences on stress tolerance were only sporadic in these two experiments. When the sorghum plants were subjected to drought stress alone, leaf Ψ was significantly higher in *Gi* plants (-2.66 MPa) than in nonAM plants (-3.86 MPa) at stomatal closure experiment 1. At the same point, there were no differences in leaf Ψ between AM and nonAM plants subjected to soil salt solutions. Therefore the result of linear contrast suggested that hypothesis 1 (AM plants might have different value from nonAM plants under drought) should be approved in the light of leaf Ψ at stomatal closure. A significantly higher leaf Ψ was measured in mycorrhizal *Helianthemum almeriense* than nonAM plants by 14% in well-watered and 26% in drought condition (Morte et al., 2000).

When either salt or osmotic stress was applied to the plants under drought, *Gi* plants did not show any marked difference in leaf Ψ relative to nonAM plants in experiment 1. Colonization with *Gm* or Arizona mix also did not affect leaf Ψ at stomatal closure

resulting in disprove of the hypothesis 2. The reasons for inconsistent pattern of leaf Ψ by experiments or measurement stages were unknown.

When the sorghum plants were exposed to saline solutions, leaf Ψ_{π} became more negative at every measurement day in experiment 1 and 2. Salinity reduces the ability of plants to take up water, and quickly causes reduction in growth rate (Munns, 2002; Mohammad et al, 2003). Leaf Ψ_{π} is a function of the amount of solutes present in leaves and the quantity can be markedly affected by osmotic adjustment to water stress or salinity (Bradford and Hsiao, 1982). If mycorrhizal symbiosis should make the host plants tolerant to water and salt stress, the direct mechanism might involve the active development of low osmotic potentials, which characterize many plant species found in more arid environments (Morgan, 1984).

Lowered leaf Ψ_{π}^{100} or osmoregulation can lead to the maintenance of turgor potential and hence continued plant growth. In experiment 1 and 2, the decline of leaf Ψ_{π}^{100} at full turgor was greatest in osmotic stressed leaves, but there were no significant differences between AM and nonAM plants. When exposed to drought alone, mycorrhizal rose plants maintained more normal leaf turgor and g_s than nonAM plants, and had lower leaf Ψ and soil Ψ (Augé et al, 1986). Combined stress might inhibit the mycorrhizal effects on improving tolerance of host plants.

At the lethal point in experiment 1, saline stress significantly lowered leaf Ψ_{π}^{100} and resulted in higher osmotic adjustment relative to plants imposed to drought alone, but there were no differences between nonAM and AM plants at that point. As the soil drying developed, leaf Ψ_{π} at full turgor kept lowering in each treatment. In general, leaf Ψ_{π} was lowered under water and salt stress together, but turgor potential was maintained under

salt stress. That means adaptation to the saline soil might occur and its physiological mechanism was osmotic adjustment or osmoregulation (Ueda et al, 2003).

Instances where turgor potential is almost fully maintained are considered as an evidence of osmoregulation (Morgan, 1984). A kind of osmoregulation might be observed in my experiments. The tolerance against dehydration often linked to osmotic adjustment (Ludlow, 1989) might be an important for given plants because it enables plants to uptake water and maintain growth and stable yield under water deficit (Machado and Paulsen, 2001). Mycorrhizal symbiosis did not improve tolerance mechanism of sorghum plants against combined drought and salt stress in these two experiments.

Mycorrhizal Symbiosis and Soil Water Relations under Drought and Salt Stress

Direct measurement of soil Ψ is widely used to characterize water status in soil and useful for predicting water availability to plants (Campbell, 1988), even though investigations appreciate that soil Ψ varies throughout the soil volume due to the complex distribution of water through the root zone (Jones, 1990). Saline solution and drought clearly affected the soil water relations of sorghum plants, and the mycorrhizal fungi were another factor to determine soil water status under drought and salt stress. However, when the plants were imposed to prolonged soil drying, the decline pattern of soil Ψ in each stress differed. Large, rapid decreases of soil Ψ occurred in the sorghum plants imposed to drought alone in experiment 1 and 2. At the lethal point, the soil was too dry to measure soil Ψ using the chilled mirror hygrometer. On the other hand, the lowering of soil Ψ in the pot receiving salt solution was not much different than that of

the pot subjected to drought alone. At stomatal closure, the salt-treated plants had soil Ψ ranging from -2.0 to -1.5 MPa in spite of 10-12 days of withholding water. The soil Ψ of drought stressed plants ranged from -5.24 to -3.49 MPa in 9-10 days since stopping water in experiment 1. Similar pattern of changing soil Ψ like this was confirmed in experiment 2. These differences suggest that the soil holding saline ions should maintain higher soil Ψ (less negative) than the soil without saline ions under drought.

Shani and Dudley (2001) pointed out that the negative effect of water stress was more than 10 times greater than salt stress at equal water potential when either the soil matric or soil osmotic potential was varied in corn, melon and alfalfa. No wilted leaves were observed when beans were exposed to salt stress using KNO_3 , but 50% of the leaves were wilted when exposed to the similar level of water stress similar lowering of soil Ψ applying with PEG (Jensen, 1981).

The pot soil of sorghum plant subjected to saline solution under drought maintains much more amount water than the plants imposed drought alone on the day of stomatal closure and lethal point. This indicates the sorghum plants could not use or uptake the remaining water in the pot soil imposed to salt stress because of decreasing osmotic potential or unavailable forms of water. Depletion of soil water was faster in sorghum plants subjected to drought alone than in plants exposed to drought and salt stress together. Soil water content was very closely related to g_s in experiment 1 and 2, which was identical to the result of Ray and Sinclair (1998). They found that soil water content was a dominant factor to determine transpirational response to drought in maize and soybean.

A number of studies reported that mycorrhizal plants often had greater tolerance to drought stress than nonAM plants even though somewhat variable according to the fungi species (Al-Karaki and Clark, 1998; Safir et al, 1971; Augé et al, 2001; Simpson and Daft, 1990; Al-Karaki, 1998). Mohammad (2003) reported the reduced plant growth under highly saline soil condition and inoculation of AM fungi decreased sodium concentration level in soil compared to nonAM plants. Through my experiments associated with three kinds of AM fungi, I did not consistently observe mycorrhizal effect on the drought stress alone. The sorghum plants colonized with *Gi* showed significant higher leaf Ψ and soil Ψ than nonAM plants at stomatal closure in experiment 1. *Gm* plants also showed significant higher soil Ψ than nonAM plants at stomatal closure in experiment 2. However AZ plants did not affect tolerance to drought.

When salt solution was applied and the soil was exposed to drought, I expected the mycorrhizal plants should wilt at lower soil Ψ than nonAM plants. However, the results of experiment 1 were contrary to my expectation. Most parameters except soil θ did not show any significant differences between AM and nonAM plants. The influences of multiple stresses (more than one) seem to be more accelerate on plant responses (Shani and Dudley, 2001; Machado and Paulsen, 2001; Jensen, 1981) than that of individual stress.

CHAPTER VII

CONCLUSION

I conducted two experiments to test whether promotional influences by AM fungi, *Gi*, *Gm* and *AZ*, on drought resistance of host plants would be more effective under saline conditions.

AM symbiosis with *Gi* promoted g_s relative to nonAM plants by 10-30% under drought, and 20-60% under salt stress during drought on some measurement days. Promotion of g_s by *Gm* was also observed in host plants exposed to drought, but when salt solution was applied, there were no differences in g_s between *Gm* and nonAM plants. *AZ* did not affect the promotion of g_s relative to nonAM except near stomatal closure.

When host plants were exposed to drought stress alone, AM symbiosis with *Gi* significantly increased leaf Ψ and soil Ψ at stomatal closure. At lethal point, *Gi* plants showed much lower leaf Ψ than nonAM plants when exposed to drought alone (supporting to hypothesis 1). No AM-induced changes in leaf Ψ and soil Ψ were observed when NaCl or osmotic stress was combined with drought (not supporting to hypothesis 2). Influences of *Gm* and *AZ* on leaf and soil water status during drought/salt stress were less than that of *Gi* through two experiments (not supporting to hypothesis 1 and 2).

It was unsure that more colonization and more developed root system of *Gi* plants than *Gm* and *AZ* might make the differences in some parameters between mycorrhizal species. However, the effect of AM symbiosis on drought resistance of host plants was greater in *Gi* than *Gm* and *AZ* in function of parameters from two experiments. Under combined stress of drought and saline soil, AM symbiosis did not show any beneficial

effect on tolerance of host plants to stresses. In order to verify the mycorrhizal effects on the tolerance of host plants to combined drought and salt stress, further studies required for other host plants colonized with varied mycorrhizal fungi.

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