الماء القابل للامتصاص ومعدل التبخر للذرة الملقحة بالمايكورايزا

EXTRACTABLE SOIL WATER AND TRANSPIRATION RATE OF MYCORRHIZAL CORN

[1](Abid Al-Agely (PhD) & [2]Ammar Wahbi (PhD)
1. Department of Soil and Water Science, P.O. Box 110290- University of Florida, Gainesville, FL 32611, USA
2. Soil Science Dept., Faculty of Agriculture,University of Aleppo, P O Box 8047- Aleppo, Syria
E-mail: wahbi@scs-net.org

المُلَخَّص

أنجزت العديد من البحوث لمعرفة استجابة النباتات الملقحة بفطور االمايكورايزا لظروف الجفاف. على أية هناك ندرة في المعلومات عن أدوار المايكورايزا المتعايشة مع النبات تحت معدلات مختلفة من التبخر والماء القابل للإتاحة. تفترض هذه الدراسة أن خصائص التربة الفيزيائية لا تكفي لوحدها لتوضيح الزيادة في الماء القابل للإفادة دون معرفة النشاطات الحيوية الفيزيائية لفطور المايكورايزا. تم تنمية النباتات في أصص مع وبدون التلقيح بفطور المايكورايزا. تم قياس استخلاص النباتات لماء التربة بالاعتماد على تحديد فيزيولوجي لنقطة النهاية العظمى والصغرى. لم يلاحظ زيادة في إنتاجية النباتات في كلتا المعاملتين: الملقحة بالمايكورايزا (M+) وغير الملقحة (M-) حتى ارتفعت كمية ماء التربة لمعدل ثلث كمية الماء الممكن نزعها من قبل النباتات والمتبقية في التربة. لم تلاحظ فروقا معنوية في حجم ماء التربة المستخلص عند نقطة النهاية الصغرى بين المعاملتين المجففتين: الملقحة بالمايكورايزا (M+) وغير الملقحة (0.139) (M-) و0.155 m^3m^{-3}) على التوالي. وعلى العكس فإن النباتات الملقحة بالمايكورايزا استخلصت كميات من ماء التربة (معنوية عند P=0.02) أعلى (0.400 m^3m^{-3}) مقارنة بالنباتات غير الملقحة (0.329 m^3m^{-3}) عند نقطة النهاية العظمى. الأهم من ذلك، أن كمية الماء المستخلصة لم تتغير في النباتات الملقحة والمعرضة للاجهاد المائي حتى انخفضت كمية الماء القابلة للاستفادة (FTSW) عن 0.195. في حين تغيرت كمية الماء المستخلصة في النباتات غير الملقحة والمعرضة للاجهاد المائي عندما بلغت كمية الماء القابلة للاستفادة (FTSW) أقل من 0.236. كانت كمية المادة الجافة أعلى دائماً في المعاملات الملقحة بالمايكورايزا سواءً المعرضة للاجهاد المائي أو الرطبة مقارنة مع مثيلاتها من المعاملات غير الملقحة بالمايكورايزا. تنشأ هذه النتائج قاعدة لدراسات مستقبلية لاختبار دور فطور المايكورايزا في النشاطات الأنيزيمية للمجموع الخضري والجذري تحت ظروف الجفاف.

ABSTRACT

Substantial research has been done to describe plant response to mycorrhizal fungi association and drought conditions. There is, however, a lack of information about the roles of mycorrhizal association in plants under various transpiration rates and soil water availabilities. This study assumes that soil physical properties alone are not sufficient to define the amount of extractable water from soil without having knowledge of mycorrhizal fungi biophysical activities.

Plants were grown in pots with or without mycorrhizal fungi. Plant extracting soil water was tested based on physiological definition of the upper and lower end-point. In both mycorrhizal (M+) and nonmycorrhizal (M-) treatments, a decrease in plant productivity was not observed until the soil water decreased to the level where approximately one third of the water that could be extracted by plants remained in the soil. No significant differences were observed in the volume of extractable soil water at the lower end-point between water-stressed mycorrhizal and nonmycorrhizal treatments (0.139 and 0.155 m^3m^{-3}), respectively. In contrast, mycorrhizal plants extracted (significantly at P=0.02) more soil water (0.400 m^3m^{-3}) than did the nonmycorrhizal plants (0.329 m^3m^{-3}) at the upper end-point. More important, extraction soil water in the water-stressed mycorrhizal plants did not change until fraction transpirable soil water (FTSW) was below 0.195. However, extraction of soil water in water-stressed nonmycorrhizal plants starts to change when FTSW was 0.236. Higher dry matter yield were observed for both water-stressed and well-watered mycorrhizal plants than for the nonmycorrhizal plants in the respective treatments. These results form the basis for additional studies to examine the role of mycorrhizal fungi on shoot and root enzymatic activities under drought conditions.

Key words: Extractable soil water, gradual drought, Mycorrhizal, transpiration, corn.

INTRODUCTION

Drought is a major constraint to crop production in arid and semiarid regions of the world. Drought, in general, reduces nutrient and water uptake by roots because of restricted transpiration rates (Kramer and Boyer, 1995). The decline in soil moisture results in a decrease in the diffusion rate of nutrients (particularly P) from soil to the absorbing root surface (Viets, 1972; Pinkerton and Simpson, 1986).

Many investigators found that crop productivity increased when Arbuscular Mycorrhizal Fungi (AMF) established a symbiotic association under drought conditions (Michelsen and Rosendahl 1990; Marshner and Dell 1994; Trimble and Knowles 1995; Al-Karaki and Al-Raddad 1997).

This kind of symbiotic association also appears to provide plants with a higher resistance to drought, through enhanced water absorbance (Ellis et al. 1985; Hardie, 1985; Bethlenfalvay et al. 1988; Davies et al. 1992; Ruiz-Lozano et al. 1995). Plant roots together with the symbiotic association of AM fungi have higher water uptake due to hyphal extraction of soil water (Allen 1982; Bethlenfalvay et al. 1988; Faber et al. 1991; Davies et al. 1992; Ruiz-Lozano et al. 1995), and higher root hydraulic conductivity (Safir et al. 1972; Augé and Stodola 1990) than nonmycorrhizal plants.

Drought impacts on crop productivities are associated with physiological abnormalities, which are controlled by soil moisture. These include changes in respiration, photosynthesis, protein synthesis, mineral nutrition, and hormone relations, together with increased exposure to a variety of phototoxic compounds. In attempt to alleviate the economic and ecological detriment caused by droughts, scientists are focusing on the development of more integrated drought-management policies that will recognize mycorrhizal fungi roles in modeling plant growth and estimating irrigation needs.

The symbiotic association of AM fungi with crops grown under drought conditions need to be better evaluated in order to optimize the beneficial effects of this association on the rate of plant respiration and on the availability of soil water. The present study will presume that the soil physical properties alone are not sufficient to define the amount of extractable water from soil without the specific roles of the

biophysical activities of the symbiotic association of AMF and crop roots. Therefore, the objectives of this research were to estimate the benefits of AMF symbiotic association under drought conditions of corn dry matter yield, the percentages of colonized root length, and to examine AMF effect on soil-water uptake under water stress conditions.

MATERIALS AND METHODS

ENVIRONEMENT:

An experiment was carried out in a controlled-environment growth chamber for two months (at the University of Florida, USA) set at 26°C, 78% relative humidity, and 13 hours of 1000 µmol/m²/s photosynthetic photon flux density (PPFD) a day.

PLANTS:

Sweet corn, local cultivar of *Zea mays* (L.), was used. Seeds were cleaned with water and commercial detergent to remove the fungicide that may affect the mycorrhizal treatments.

SOIL:

Culture media was pasteurized Terra-Lite®. which is an Agricultural mix (#92873, Scotts Company, Mary Sville, and Oh) that is designed and formulated to provide optimum aeration, drainage, enough moisture, and nutritional characteristics. The material is porous and lightweight. "Essentially sterile" medium is a mixture of vermiculite, perlite, and processed bark. The rich mixture of horticulture vermiculite and Canadian sphagnum peat moss promotes plant growth. The three common Terra Lite components are heated to over 10000 °F in the manufacturing process. The pH range after wet out with nutrient solution was 5.0 - 6.4 and dry bulk density 0.128 - 0.160 g/cm^3. Additional chemical analyses are listed in Table 1.

Water release curve was calculated to characterize the relationship between water content by volume and water potential of the media. In the low pressure range (0.3 to 34 kPa) PVC temp cells (Soil moisture Equipment Corp., Santa Barbara, CA) were used. A water column connected to the cells obtained the pressure exerted on the soil. The values for the higher pressures of 490 and 1471 kPa were obtained using a pressure plate apparatus (Soilmoisture Equipment Corp., Santa Barbara, CA). Duplicate samples were tested. The soil media were packed into the pressure apparatuses with approximate bulk densities of 0.11 g cm^{-3}. This bulk density was used to calculate volumetric water content of the soil from the water content by weight extracted during soil pressurization.

Growing containers (10-cm diam. by 10-cm deep) were used. Containers received synthetic cotton -to secure draining holes-

and pasteurized culture media. For the mycorrhizal treatments, each replication received 10-grams of 'soil inoculum' of *Glomus etunicatum*-Like Becker & Gerdemann on the culture media, gently mix and cover with 3-cm of additional culture media. The mycorrhizal inoculum potential (MIP) of the AM fungal isolate was 40%. For the nonmycorrhizal treatments, each replicate received filtrated solution of 10-grams "soil inoculum".

Table 1: Culture media chemical analysis

pH	OM	EC	NO_3-N	P	K	Ca	Mg	Cu	Mn	Zn
	(%)	(dS/m)	mg/L							
5.1	42.8	2.7	103.0	4	241	201.1	273	0.0	2.5	0.2

SOIL CHARACTERIZATION:

The chemical analysis of the culture media is shown in Table (1), which illustrates the very rich nature of this media, evident from the organic matter content (42.8%). The dry bulk density of the culture media is noticeably low (estimated from the water retention measurements 0.11, and provided from the company manufactures between 0.128-0.16 g/ cm^3), these will indeed illustrate the high porosity [1- (bulk density/ particle density)], where particle density suggested for Vermiculite to be 2.3 g/ cm3; then porosity is about 94%. The water release curve of the culture media, as indicated in figure 1, shows high water contents and high losses at low pressures. This suggests that the media retains a high quantity of big size pores.

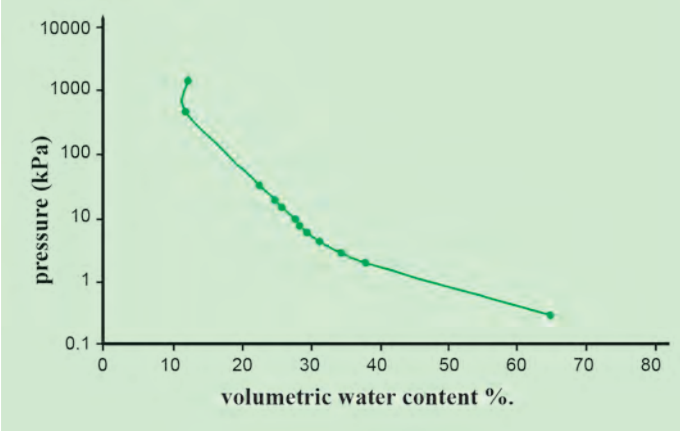

Fig. 1: Soil water release curve plotted as the log of pressure (kPa) against percentages of volumetric water content.

The dehydration treatments were initiated four weeks after sowing, when plants were big enough to lose sufficient amounts of water and enough time for mycorrhizal establishment to occur. Pots were watered to return soil moisture in each to roughly 75% of the extractable soil water, resulting in a wet soil weight of approximately 0.4 kg. Each pot was enclosed with parafilm and sealed around the stem to prevent loss of soil water. Pots were weighed daily around 3:00 pm to estimate the daily water loss and that need to be added for the next day. Four replicates were identified as well watered (control) and maintained at 75% extractable soil water by daily addition of water. Seven replicates were allowed to dehydrate over span of the experiment. To determine the daily relative transpiration rate (TR), the ratio of the daily water loss from each water-stressed replicate relative to the mean water loss (by transpiration) of the control (well-watered plants) was calculated. The dehydration was continued until the plants in each replicate were noted as having reached permanent wilting. In these experiments, permanent wilting was defined as when Fraction Transpired Soil Water (FTSW) reached zero and the TR of each plant decreased to 0.1 of the well-watered plants.

The TR values were further normalized relative to the mean of the TR values of the first few days of the experiment when the soil medium for each plant was still wet and there was no evidence of decreased transpiration rate. Transpirable soil water for each replicate was calculated by subtracting the weight when normalized TR was first less than 0.1 from the drained upper limit weight. FTSW was daily calculated for each replicate. This was done by subtracting the lower limit weight from the daily weight measurement then dividing by the total transpirable soil water of that replicate (Sinclair and Ludlow, 1986).

COLONIZATION TEST:

Roots were removed from the culture media by wet sieving, and their fresh weights were determined. Subsample of 0.5 g from each root system was cleared with 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue in lactophenol as described by Phillips and Hayman (1970). Percent colonization of root length was determined by the gridline-intersect technique (Giovannetti and Mosse, 1980). Calculations based on root fresh and dry weight was used to estimate the dry weight of the subsamples used for AM colonization tests and added to the total root dry weight.

EXPERIMENT PLAN:

The experiment was randomized in complete blocks of two treatments of water supplies (gradual water-stressed and well watered) and two treatments of mycorrhizal inoculation (with M+ and without M-inoculum) replicated four times for the well watered treatments and seven for the water-stressed treatments. Results were statistically analyzed and plotted by PC SAS. TR was plotted against physiological method used for estimating the FTSW. Two shapes of curves were found; one with linear-plateau of 1.0 TR values, and the other with linear-plateau of less than 1.0 TR values by using plateau regression procedures:

TR= A+B*FTSW, when $FTSW < C_p$

TR = 1.0 $FTSW \geq C_p$

Where A, and B are regression coefficients and C_p is the critical value (threshold) of FTSW demarcating the two stages of the model. In this case, a subscript denotes that C_p is for the extractable soil water based on physiological defined end-points as described by Sinclair et al in their work at 1998. The value of C_p is defined by the regression coefficients in the following equation: $C_p = (1-A)/B$

This usual relation found by several scientists (for more information refer to Sinclair et. al., 1998).

Mycorrhizal dependency (MD) or response to mycorrhizal colonization was calculated using the following formula (Plenchette et al. 1983):

MD (%) = Plant biomass (M+) – Plant Biomass (M-) / Plant Biomass (M+) * 100

RESULTS

MYCORRHIZAL COLONIZATION:

Percent colonization was correlated positively with the amount of extractable soil water. The water-stressed plants had 52.07 ± 4.3 percentages colonized while the well-watered plants had 68.02 ± 6.7 (increased of 31%). This indicates, that dehydration had a marked effect on mycorrhizal colonization, but varies with the type of dehydration, which the plants face.

WATER EXTRACTION:

Soil water uptakes by mycorrhizal and nonmycorrhizal plants were expressed by relative transpiration rate as a function the fraction of transpirable soil water based on physiological values. Mycorrhizal plants maintained transpiration rates of 1.0 until the critical threshold (Cp) of FTSW values reached 0.195. However, the transpiration rate of the nonmycorrhizal plants that maintained a 1.0 value declined when the FTSW critical threshold was 0.236 (Figure 2).

No significant differences were observed in the volume of extractable soil water at the lower limit point between dehyradted mycorrhizal and nonmycorrhizal treatments. However, mycorrhizal plants were reported to extract significantly more soil water than

did nonmycorrhizal plants when measurements were taken at the upper limit point ($P = 0.02$) or at the transpirable soil water ($P = 0.004$) (Table 2).

Mycorrhizal plants under water-stress efficiently used soil water to maintain transpiration rates of 1.0 for longer time than nonmycorrhizal plants. Even at the lower limit, mycorrhizal plants normalized TR were 0.48 while the nonmycorrhizal plants normalized TR value was 0.14 (Figure 3). After the first day of dehydration conditions,

nonmycorrhizal plants displayed a reduction in the normalized TR below the 1.0 value.

On the contrary, mycorrhizal plants maintained normal transpiration rates paralleling the well-watered control plants. It was not until the half waypoint of the experiment that the mycorrhizal plants began to decline below 1.0 (Figure 3).

BIOMASS CHARACTERIZATION:

Because of increased water-use-efficiency by

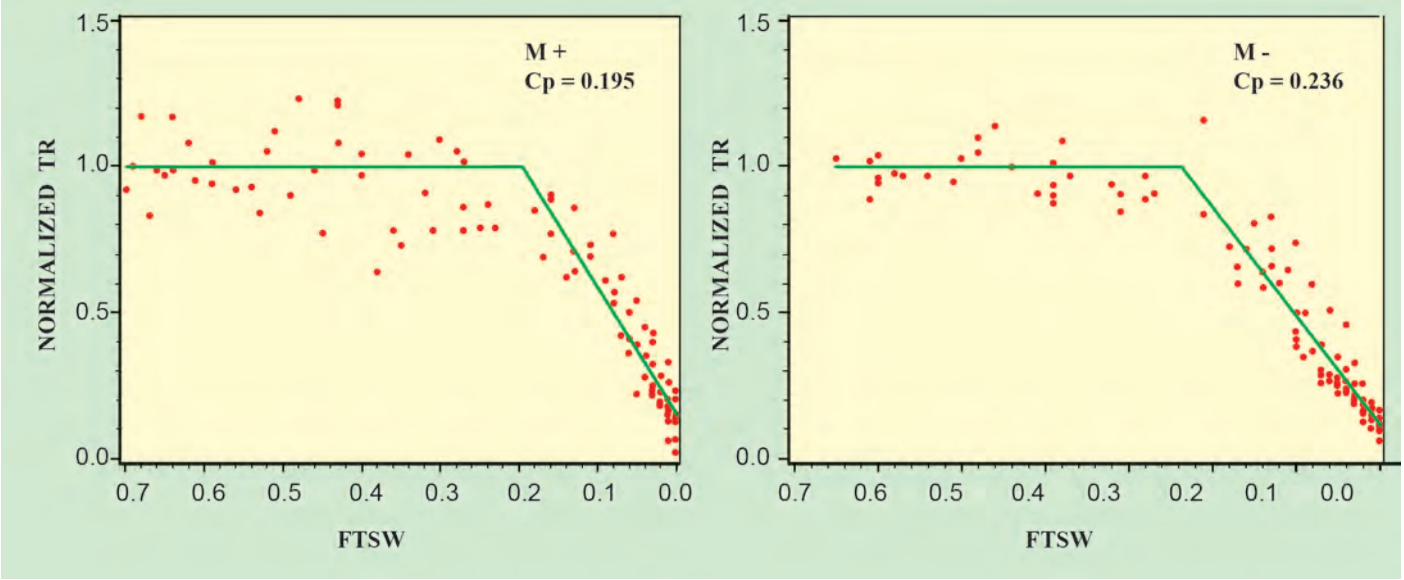

Figure 2: Relationship between normalized TR and FTSW for mycorrhizal (M+) and nonmycorrhizal (M-) plants.

Table 2: Soil water extraction of mycorrhizal and nonmycorrhizal treatments as calculated based on physiological measurements for the upper and lower limits.

Variable	Extractable Soil Water	
	Mycorrhizal Treatment	Nonmycorrhizal Treatment
	----------m^3 m^{-3}----------	
Upper Limit Drained	0.400a	0.329b
Lower Limit Permanent Wilting	0.139a	0.155a
Soil Water ExtractionTranspirable Soil Water	0.261a	0.174b

Means followed by different letters differ significantly at $LSD_{0.05}$

mycorrhizal plants in both water-stressed and well-watered treatments, mycorrhizal plants show shoot dry biomass mycorrhizal dependency of 12% in water-stressed treatment and 16% in the well-watered treatment when contrasted with the nonmycorrhizal plants in the respective treatments (Figure 3). However, in the root biomass, the contrary results were evident, the nonmycorrhizal plants in both water-stressn and well-watered treatments show an increase in root dry biomass (10% and 20%, respectively) compared to the mycorrhizal plants (Figure 3).

The ratio of shoot to root dry biomass in the mycorrhizal treatment was significantly higher (P = 0.01) than the nonmycorrhizal treatment (Table 3). No difference was observed between mycorrhizal treatments in P content, however, difference was

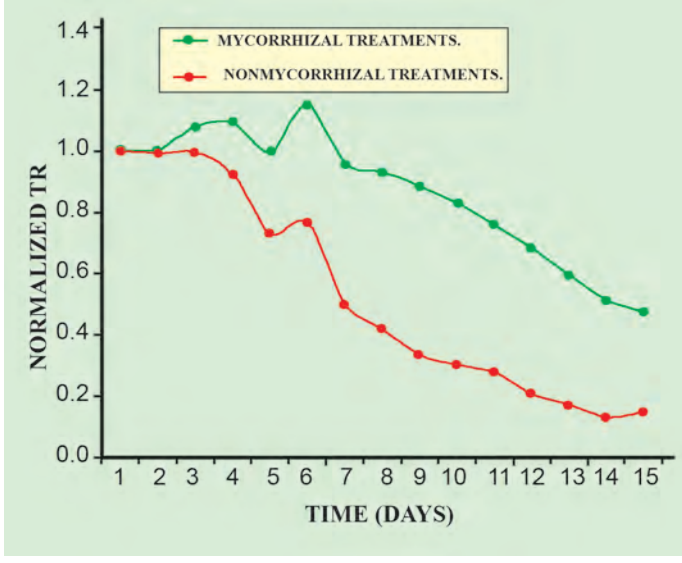

Figure 3: Relation between normalized TR and time for mycorrhizal and nonmycorrhizal treatments.

evident for water treatments at P=0.01 (Table 3).

Within the mycorrhizal treatments, dehydration caused a 13.9% reduction in shoot dry biomass and a 5.7% reduction in root dry biomass when compared to the well watered plants. In the mean time, dehydration within the nonmycorrhizal treatments caused a 11.4% reduction in the shoot dry biomass and a 17.1% reduction in the root dry biomass (Figure 4).

DISCUSSION

Findings in this research may add a new dimension to the water sink models described by Cowan (1965),

Table 3: Effects of water status and mycorrhizal colonization on selective biological measurements and their statistical analyses.

Water Status	Mycorrhizal Colonization	Shoot Dry Biomass (g)	Root Dry Biomass (g)	Shoot/Root	P Content (mg/Plant)	Colonize Root Lenght %
Dehydration	M+	3.30±0.39	1.75±0.26	1.90±0.21	2.80±1.11	52.07±11.38
	M-	2.89±0.47	2.11±0.81	1.50±0.45	2.25±1.14	0.00
Well-Watered	M+	4.40±0.45	1.96±0.15	2.26±0.31	4.00±0.56	68.00±13.48
	M-	3.69±0.45	2.98±0.93	1.32±0.40	3.75±0.62	0.00
LSD (0.05)	Water Status	0.41	0.58	0.33	0.93	7.98
	Mycorrhizae	0.39	0.56	0.32	0.92	7.68
Significant	Water Status	**	NS	NS	**	*
	Mychorrhizae	**	*	**	NS	**
	Water*Mycorrhizae	NS	NS	NS	NS	*

(*, **, and NS mean significant at $P = 0.05, 0.01$ and no significant respectively).

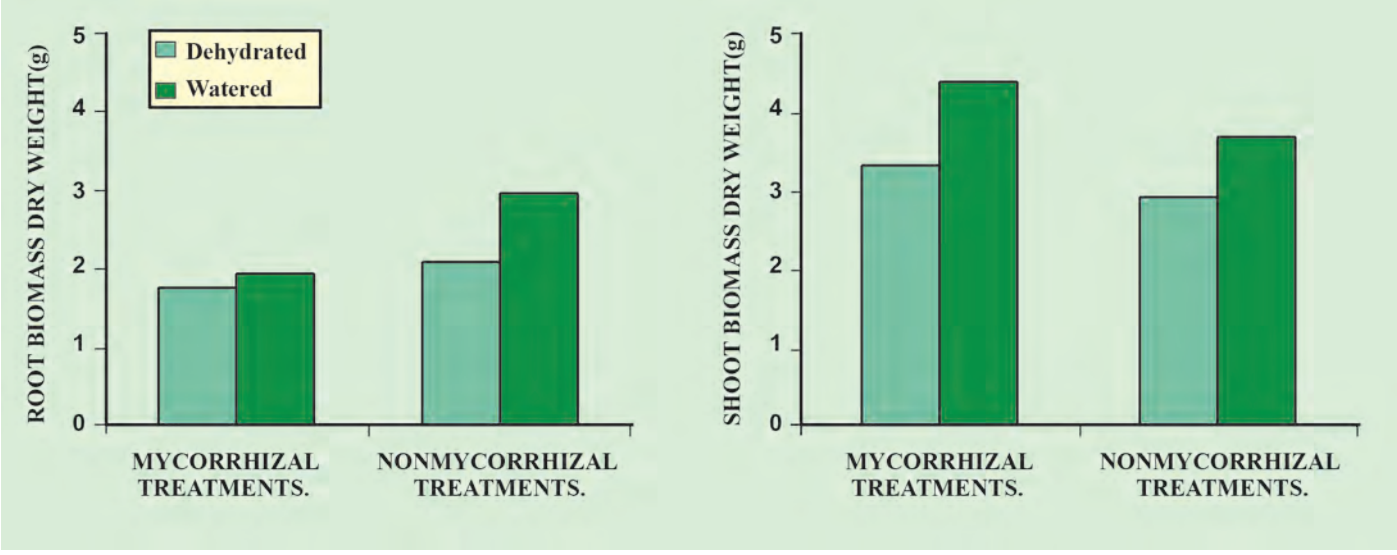

Figure 4: Shoots and root biomass dry weight in the different water and mycorrhizal status.
Error bars represent ± standard error.

Federer (1979), and Martin (1990). Mycorrhizal association is clearly an important component of a model that integrates water uptake by roots as part of the broad cycle of water in soil. Mycorrhizal fungi were demonstrated to be the link in the interface interaction of plant-root-soil-atmosphere system that effectively vacillated water extraction and the following transpiration that acts as a sink for soil water.

Extractable soil water in the nonmycorrhizal treatment did not deviate from the consistent pattern in the response of plant transpiration relative to the volumetric soil water content. Normalized TR in the water-stressed nonmycorrhizal plants did not change until 0.236 of the fraction of transpired soil water (FTSW) remained in the soil. This value came very close to what Sinclair *et al* (1998) found in their work on soybean plants on a loamy sandy soil. However, the mycorrhizal plants were able to extract more soil water until FTSW was below 0.195. It is very clear that plants with mycorrhizal fungi were able to access more water in the soil micro pores; this water is not available to plants without mycorrhizal fungi.

The results reported here clearly showed that while transpiration rate generally decreased as dehydration progressed, mycorrhizal plants maintained higher transpiration rates than the nonmycorrhizal plants particularly as the volume of soil water content declined. Others have also found higher transpiration in mycorrhizal than nonmycorrhizal plant (Augé *et al* 1986; Bildusas *et al* 1986; Bryla & Duniway 1997; and Augé 2000).

The physiological changes in the mycorrhizal plants that lead to the increase in the transpiration rates and to the increase in plant dry biomass are the forces behind pulling up more water and keeping stomata open for longer periods. Therefore, more gas exchange lead to more carbohydrate production, which may offset the cost of having the symbiotic association of the mycorrhizal fungi. This conclusion may contradict some of the suggestion that made early by Dunsiger et al (2003) that AM fungi influence their host in direct signal communication that result in stomatal closure under water stress conditions. However, the questions of how and why are still unclear and need further anatomical, physiological, and chemical studies.

This study provides an aid to understand the role of AM fungi in plant root function, which is the field that recently recognized as a gap in our knowledge of rhizosphere academy. The results from this study are practical demonstrations of the roles that mycorrhizal fungi play in the change of the volumetric soil-water in any particular field condition. This work proves that the expected changes in plant physiology and mycorrhizal colonization have a significant impact on soil water content. Other studies may be expected to answer the question of the effect of soil physical and chemical characteristics such as soil temperature, aeration, root competition, root density, fertilizer, microorganism composition, and soil chemical processes on the ability of mycorrhizal fungi to uptake soil-water.

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