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Quantification of water uptake of hyphae contributing to barley subjected to drought conditions

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Contents

1 General introduction

1.1 The concept and definition of drought

Drought is a normal and recurrent feature of climate, although many erroneously consider it a rare and random event. It occurs in virtually every climatic zone, but its characteristics vary significantly from one region to another. Drought is a temporary aberration; it differs from aridity, which is restricted to low rainfall regions and is a permanent feature of climate.

Drought is an unseen hazard of nature. Although it has several definitions, it originates from a deficiency of precipitations over an extended period of time, usually one season or more. This deficiency results in a water shortage for some activity, group, or environmental area. Drought should be considered relative to some long-term average condition of balance between precipitation and evapotranspiration (i. e., evaporation + transpiration) in a particular area, a condition often perceived as "normal". It is also related to the timing (i. e., principal season of occurrence, delays in the start of the rainy season, occurrence of rains in relation to principal crop growth stages) and the effectiveness (i. e., rainfall intensity, number of rainfall events). Other climatic factors such as high temperature, high wind, and low relative humidity are often associated with drought in many regions of the world and can significantly make its more serious severity.

Drought should not be viewed as only a physical phenomenon or natural event. Its impacts on society result from the interplay between a natural event (less precipitation than excepted resulting from natural climatic variability) and the demand of people for water supply. Human beings often exacerbate the impact of drought. Recent droughts in both developing and developed countries and the resulting economic and environmental impacts and personal hardships have underscored the vulnerability of all societies to this "natural" hazard (TWDB, 2004).

1.2 Expression and classification of water stress in plants

Because of the general aspects of plant/water relations, there is no single index of water supply by the environment (soil water content; bulk air humidity etc.) that can be used to quantify the degree of water deficit stress (or water stress) to which a plant is subjected. In the absence of an *environmental* index, it is the convention to quantify water stress in terms of the extent to which tissue water content has fallen below that at full torpor (i.e. below the optimum water content for growth and function). The principal index is tissue water potential, although relative water content (RWC: water content as a percentage of the fully hydrated content), torpor (Jones and Cornett, 1992) and water deficit can be of value in some circumstances (see below). Since photosynthetic uptake of CO_2 *via* open stomata is definitely associated with water loss to the atmosphere, and some loss of torpor, nearly all plants are exposed to some degree

of water stress throughout their life cycle especially during the daily period of lighting. Tolerance to water stress is a *routine* aspect of plant life, not simply a feature of species adapted to dry habitats. The leaves of desert plants can survive water potential as low as –11.5 MPa (Mega Pascal), with photosynthesis continuing at –5 to – 8 MPa. On the other hand, species adapted to the under storey of moist frosts are rarely exposed to (or equipped to deal with) values lower than –1 MPa (Fitter and Hay, 2002). It is, therefore, misleading to refer to "typical" levels of water stress. Nevertheless, in reviewing the effects of water stress on plant growth and function, Hsiao (1973) found it convenient to use three, loosely defined, degrees of water stress, in relation to a "typical mesophyte" (probably best represented by the crop and weed species of temperate agriculture):

Mild stress: ψ_{cell} slightly lowered, typically down to –0.5 MPa at most;
Moderate stress: ψ_{cell} in the range –0.5 to –1.2 or -1.5 MPa
Severe stress: ψ_{cell} below –1.5 MPa

Lawlor (1995) has proposed an alternative, but broadly compatible, classification for mesophytes, based on RWC: values down to 90% are associated with effects on stomata and cell expansion; 80-90% with effects on photosynthesis and respiration; and below 80% (corresponding to water potentials of –1.5 MPa or lower) with the cessation of photosynthesis and the disruption of cell metabolism.

Interpretation of the effects of different degree of drought or water stress on plant physiology can be complicated by the fact that responses can be brought up at the organ tissue, cell or molecular level. For example, the stomata of mesophytic plants start to close at leaf water potentials in the range –0.5 to –1.0 MPa (or possibly even higher under the influence of intraplant signals), thereby reducing the flux of CO_2 from the bulk air to the photosynthetic mesophyll. Thus, the rate of photosynthesis may be reduced by a whole leaf response before there are significant effects of water stress on individual cells, chloroplasts, membranes or biochemical reactions (Lawlor, 1995; Tezara et al., 1999).

The primary effect of dehydration on plants is loss of turgor. The action of mild water stress is associated with a fast or rapid reduction in turgor pressure, which continues at a declining rate per unit of water potential under moderate stress. Severe water stress or drought involves a complete loss of turgor ($\psi = 0$), and leaf wilting. The exposure of cells to severe water stress, therefore, impacts on mechanical stress as well as dehydration, which bring reactive molecules closer together. Loss of turgor has a range of influence for plant leaves. On the other hand, the rates of cell division, and the duration of leaf expansion, are both relatively unaffected by mild to moderate stress, although both will be curtailed under severe stress.

In addition to slowing growth, a lowering of leaf water potential by less than -0.5 MPa (mild stress) is associated with some break up of biosynthetic activities including the generation of cell wall components, chlorophyll and etc. Under moderate stress there is further reduction in turgor, leading to narrowing of stomatal aperture and a progressive reduction in photosynthetic activity. Increased respiration may also play a part in stomatal closure owing to an increase in CO_2 concentration within the leaf air space. With the onset of severe stress, photosynthetic exchange of CO_2 ceases and a general disruption of metabolism is manifested by high rates of respiration and the build up of against stress solutes in tissue; in plants resistant to drought such accumulation of organic solutes, leading to osmoregulation, can occur at lower stress.

In summary, exposure of plants to even mild water stress can affect growth, and disturb metabolic processes. Depending on their severity, such effects can reduce the ability of plant to survive and reproduce. Consequently, it is important for terrestrial species either to avoid water stress, or to slowly progressing to morphological or physiological adaptations, which lead to the tolerance of water stress.

1.3 Effects of drought on crop growth

The terms crops or agricultural drought are often used to link various characteristics of meteorological (or hydrological) drought to agricultural impacts, focusing on precipitation shortages, differences between actual and potential evapotranspiration, soil water deficits, reduced ground water or content levels, and so forth. Plant water demand depends on prevailing weather conditions, biological characteristics of the specific plant, its stage of growth, and the physical and biological properties of the soil. A good definition of agricultural drought should be able to account for the variable susceptibility of crops during different stages of crop development, from emergence to maturity. For example, deficient topsoil moisture at planting may hinder germination, leading to low plant populations per hectare and a reduction of final yield. However, if topsoil moisture is sufficient for early growth requirements, deficiencies in subsoil moisture at this early stage may not affect final yield if subsoil moisture is filled up as the growing season progresses or if rainfall meets plant water needs. In order to ease the critical affects of environmental stress on crops, agriculture researchers should consider using natural and artificial methods to advice farmers for better taking care of crops under harsh environmental conditions. Crop yields are most likely to suffer if dry periods occur during critical developmental stages such as reproduction. In most grain crops, flowering, pollination, and grain-filling are especially sensitive to water stress. Figure 1 provides an overview of a number of cellular functions that are altered by decreasing water potential. There is hardly a physiological process which is not effected by water stress or drought; this section nevertheless outlines some overly apparent effects which perhaps are instrumental to causing further harmful changes which may take place at somewhat later stages (Beyla et al., 1997c).

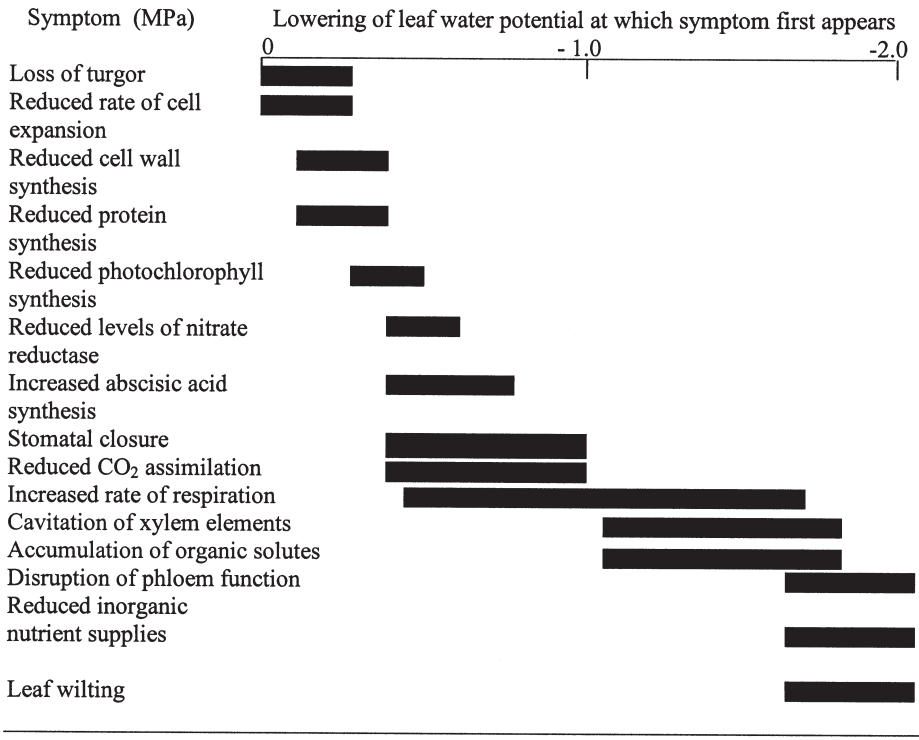

Figure 1. The influence of water stress on the physiology of mesophytic plants. The horizontal bars are indicated to the level of stress at which the relevant symptoms first occur. The lowering of leaf water potential is in relation to a well-watered plant under mild evaporative demand (updated from Hsiao et al., 1976)

1.4 Vesicular arbuscular mycorrhizal fungi

Association of vesicular arbuscular mycorrhizae (VAM) with plant roots is the most common underground symbiosis. They are formed in the roots of an enormously wide variety of host plants by aseptate, obligatory symbiotic fungi in the order *Glomales* (Zygomycotina). The plants include angiosperms, gymnosperms and pteridophytes, which all have true roots, as well as the gametophytes of some mosses, *Lycopods* and *Psilotales*, which do not (Pocock and Duckett, 1984, 1985; Peterson et al. 1981). It seems highly likely that the fungi had their origins between 353 and 462 million years ago and that the symbiosis is similarly ancient and was probably important in the colonization of land by vesicular plants (Simon et al. 1993). The name 'vesicular-arbuscular' is derived from characteristic structures, the arbuscular which occur within the cortical cells, and vesicles, which occur within or between them. A VAM has three important components: the root itself, the fungal structures within the cells of the root and an extraradical mycelium

in the soil. The last may be quite extensive under some conditions but does not form any vegetative pseudoparenchymatous structure comparable to the fungal sheath. Until relatively recently the causal organisms of VAM were classified in the family Endogonaceae of the order Endogonales. The regular association of the very large spores and sporocarps of members of this family with VAM roots was established long ago by Peyronel (1923). However, some species do form sporocarps with limited amounts of sterile mycelium. The majority (about 80%) of described VAM form both arbuscules and vesicles.

The range of potential host plants for VAM fungi is extremely wide and has been responsible for the often statement (Gerdemann, 1968) that it is so easy to find that 'it is easier to list the plant families in which it is not known to occur than to compile a list of families in which it has been found'. Records of VAM are to be found in all the orders from which plants have been examined and are about equally frequent in Dicotyledonae and Monocotyledonae. Consequently, it can be said that about 95% of the present-day species of plants belong to families that are characteristically mycorrhizal.

1.5 Contribution of mycorrhizae to drought tolerance of host plants

Vesicular arbuscular mycorrhizal (VAM) symbiosis often results in altered rates of water movement into, through and out of host plants, with consequent effects on tissue hydration and leaf physiology. Water relations of plants are modified in some ways by the mycorrhizal interactions. Mosse and Hayman (1971) observed that mycorrhizal onions did not wilt when transplanted, but that non-mycorrhizal plants did. Subsequently, several similar observations have been made (Busse and Ellis, 1985; Huang et al., 1985) and there is no doubt that mycorrhizal colonization does affect that water relations of plants. The mechanisms are difficult to determine, but most of the effects have been so far attributed to changes in nutritional status of plants (Gianinazzi-Pearson and Gianinazzi, 1983). There is also evidence for actual water transport via the fungal hyphae or for alterations in root or shoot hydraulic properties or water potentials that are independent of increased P uptake or of changes in growth as a results of this (Smith and Read, 1997). As with other aspects of the physiology of mycorrhizal plants, it is relevant to distinguish direct effects of fungal colonization from indirect effects resulting from changes in plant size. The subject is complex and there are many inconsistencies in the literature, not all of which can be easily explained (Fitter, 1988; Koide, 1993; Nelsen, 1987).

Stomatal conductance or transpiration: VAM and non-VAM plants often display different transpiration rates and stomatal conductance to water vapour (see Auge, 2001 review). However, several investigators found no differences between VAM and non-VAM plants in stomatal conductance or transpiration. An experimenter can expect to find at least occasional differences in stomatal conductance among plants with different

mycorrhizal treatments, especially if stomatal conductance is monitored several times in an experiment, if plants are exposed to a variety of environmental conditions (e.g. varied light or CO_2), or if VAM and non-VAM plants differ in size. Yet we cannot predict with any certainty under which circumstances AM and non-VAM plants are most likely to differ in stomatal conductance (e.g. Read and Boyd, 1986; Nelsen, 1987; Smith and Gianinazzi-Pearson, 1988; Gupta, 1991; Koide, 1993; Sanchez-Diaz and Honrubia, 1994; Smith and Read, 1997; Auge, 2000). Stomatal conductance and leaf ψ are linked functionally: changes in one usually drive changes in the other. Thus, when VAM symbiosis hastens or postpones leaf dehydration, this would naturally be associated with altered stomatal behaviour. The rates at which VAM and non-VAM plants dry soil frequently differ and this typically occurs without altering the functional relationship between stomatal conductance and leaf ψ. In some instances, however, stomatal parameters have been altered by VAM symbiosis without altering leaf hydration (Allen and Boosalis, 1983; Stahl and Smith, 1984; Allen and Allen, 1986; Auge et al., 1986b; Sanchez-Diaz et al., 1990; Osundina, 1995).

Photosynthesis: VAM plants often show higher photosynthetic rates than their experimental non-VAM counterparts, which is consistent with VAM effects on stomatal conductance like stomatal conductance and transpiration, photosynthesis is stimulated by VAM symbiosis about as frequently under well watered as under drought conditions. As with stomatal conductance, different VAM fungi have different effects on photosynthesis during drought, even when plants are of similar size (e.g. Dixon et al., 1994).

Leaf hydration: Tissue hydration or water status is typically quantified by measuring ψ or its components, or water content. Leaf ψ of well-watered (non-stressed) plants has usually not been affected by VAM symbiosis (e.g. Allen et al., 1981; Allen 1982; Nelsen and Safir, 1982a; Levy et al., 1983b; Auge et al., 1986a, 1994; Ramakrishnan et al., 1988b; Drüge and Schönbeck, 1992; Osonubi et al., 1992; Davies et al., 1993; Ebel et al., 1994; Osonubi, 1994; Goicoechea et al., 1996, 1997a, b, 1998; Bryla and Duniway, 1997a, c). On some occasions, leaf ψ has differed in well-watered (non-stressed) VAM and non-VAM plants (Nelsen and Safir, 1982a; Dixon et al., 1994; Gemma et al., 1997). Because of their frequently different photosynthetic rates, leaves of well-watered or non-stressed VAM and non-VAM plants might be expected to develop dissimilar symplastic solute pools and consequently different leaf osmotic potentials, even when total leaf ψ is similar (e.g. Goicoechea et al., 1997b). Lower full turgor osmotic potentials of well-watered or non-stressed VAM plants have been observed in leaves of alfalfa (Goicoechea et al., 1997b) or rose (e.g. Auge et al., 1986b). However, leaf osmotic potential has generally not differed in VAM and non-VAM plants when water is not limiting (Henderson and Davies, 1990; Faber et al., 1991; Auge et al., 1992a, 1995; Davies et al., 1993; Ebel et al., 1996; Bryla and Duniway, 1997c), nor has leaf turgor potential (Auge et al., 1992a; Davies et al., 1992, 1993; Bryla and Duniway, 1997 a, c). Adjustments

in leaf osmotic potential and stomatal conductance are often related (e.g. Ludlow, 1989) and VAM-induced alteration of leaf osmotic potential may explain VAM-induced promotion of stomatal conductance (e.g. Auge et al., 1986b). VAM symbiosis has postponed declines in leaf ψ during drought stress (Huang et al., 1985; Davies et al., 1992; Dixon et al., 1994; Subramanian et al., 1995, 1997; El-Tohamy et al., 1999), even at similar bulk soil moisture around VAM and non-VAM roots for *Glomus deserticola* (Allen and Allen, 1986; Auge et al., 1987a; Duan et al., 1996; Gemma et al., 1997). Leaf ψ has also been reported to return to control levels more quickly in VAM than non-VAM plants after relief of drought (Subramanian et al., 1997). Leaf osmotic potential may differ in VAM and non-VAM plants during drought (Auge et al., 1986b; 1987a: Goicoechea et al., 1997b), but most investigators observed no VAM effects on leaf osmotic potential of droughted plants (Auge and Stodola, 1995; Henderson and Davies 1990; Faber et al., 1991; Auge et al., 1992a; Bryla and Duniway, 1997a, c; Goicoechea et al., 1997b) or osmotically stressed plants (Ramakrishnan et al., 1988b; Auge et al., 1992a). Not surprisingly, osmotic potential tends to be higher when total ψ is higher in leaves of VAM than non-VAM plants during drought, suggesting that VAM plants are not as strained by the drought stress (e.g. Auge et al., 1987a; Davies et al., 1992). Leaf turgor potential has been increased (Auge et al., 1986b; Davies et al., 1992, 1993; Osundina, 1995) or not affected (Bryla and Duniway, 1997a, c; Goicoechea et al., 1997b) by VAM symbiosis during drought. Leaf water content or relative water content has been compared much less frequently in VAM and non-VAM plants than leaf ψ. VAM symbiosis may postpone declines in leaf relative water content in droughted wheat (Panwar, 1993), change shoot water content relationships (Bethlenfalvay et al., 1990), and allow leaves to maintain stomatal opening to lower leaf relative water content (Auge et al., 1986b). As might be expected, when leaf ψ was unchanged by VAM symbiosis, leaf relative water content was also unchanged (e.g. Hendreson and Davies, 1990; Auge et al., 1992a, 1995: Davies et al., 1992; Ebel et al., 1996, 1997).

Hydraulic conductivity and hyphae water transport: VAM hyphae were reported to enhance water uptake in sunflower and cowpea (Faber et al., 1991). Ruiz-Lozano and Azcon (1995) observed that hyphae of *Glomus deserticula* and *Glomus fasiculatum* differed in their influence on water uptake, despite similar intra-and extraradical hyphae extension. When calculated rather than measured, hyphae water transport rates have generally been negligible (Graham and Syvertsen, 1984; Fitter, 1985; George et al., 1992; Koide, 1993). However, VAM root systems were also reported to dry soil more slowly than non-VAM root systems in split-root experiments (Auge et al., 1994, 1995) or single pot experiments (Subramanian et al., 1997), even thought the VAM plants were larger than non-VAM plants in the latter work.

Growth and nutrient uptake during drought: VAM symbiosis has usually increased host growth rates during drought by affecting nutrient acquisition and possibly hydration (Auge, 2001). In experiments designed to detect the influence of VAM symbiosis on growth,

growth of VAM plants was consistently less inhibited by non-hydraulic signals of soil drying than growth of non-VAM plants (Auge et al., 1994, 1995; Ebel et al., 1994, 1996). VAM effects on plant water relation and metabolism during drought have been associated with morphological and phenological effects. VAM Acacia (Osonubi et al., 1992) in rose (Henderson and Davies, 1990) showed more leaf abscission during drought than non-VAM plants, while VAM wheat showed less leaf drop (Ellis et al., 1985) and less leaf necrosis (Bryla and Duniway, 1997c). VAM maize had relatively more green leaf area than non-VAM maize after drought (Subramanian et al., 1995) and VAM symbiosis delayed leaf senescence in droughted alfalfa (Goicoechea et al., 1997a). Leaf movements were greater in VAM than in non-VAM *leucaena* (Huang et al., 1985). When VAM and non-VAM plants with similar leaf areas have been compared, VAM symbiosis has generally not affected stomatal density (number of stomata per leaf area: Allen et al., 1981; Auge et al., 1986a; Henderson and Davies, 1990; Drüge and Schönbeck, 1992), even when transpiration or stomatal conductance differed.

1.6 Aim of this study

The general aim of this study was to investigate whether mycorrhizal colonization of plants affects their drought tolerance directly by changing the plant's water relations or through some indirect way, which may be independent of increased water uptake by mycorrhizal plants. Therefore we concentrated this study to i) quantify water uptake by extraradical hyphae in plants with VAM in split-root-hyphae system under simulated water stress (drought) conditions, and ii) to investigate the interactions between mycorrhizal fungi and drought on several physiological parameters affecting the growth of VAM plants.

1.7 Outline of study

This study consists of three parts:

Part I. *Differential effects of two vesicular arbuscular mycorrhizal fungi on growth, leaf water relations and nutrient uptake of barley under well-watered and drought conditions.* Experiment was carried out in normal pots and the effects of two VAM species (*Glomus intraradices* and *Glomus mosseae*) on water relations under drought condition were studied. In this comparative experiment we studied a) the parameters of plant morphology such as shoot height, number of tillers, and leaf area; b) physiological parameters of plants such as relative leaf water content, leaf water potential, leaf osmotic potential and leaf turgor pressure; c) growth and yield components such as fresh and dry weight, shoot fresh and dry weight and spikes, number of spikes per plant and finally; and d) degree of root maycorrhization rate of VAM plants.

Part II. *Quantification of water uptake by hyphae in barley with split-root-hyphae system under drought conditions*. Plants were grown in split-root-hyphae chambers system and the following parameters were studied in non-mycorrhizal (non-VAM) and

mycorrhizal (VAM) plants grown under well-watered condition or water stress (drought): a) morphological parameters such as leaf elongation rate and total leaf length, shoot height and number of tillers; b) physiological properties such as leaf photosynthesis and respiration; c) water relation components such as stomatal conductance, relative leaf water content, leaf water potential, leaf osmotic potential and leaf turgor pressure; and d) quantity of water uptake up by mycorrhizal hyphae from the hyphae compartment, and e) plant growth and the yield components similar to Part I.

Part III. *Dynamics of water uptake by VAM hyphae for barley determined with capacitance sensors under drought conditions*. Eight capacitance sensors were mounted in both compartments of split-root-hyphae system (plant and hyphae compartments). Every ten minutes computer collected the difference between the dielectric constants of water in the wet soil and that of dry soil and data were recorded in data logger. The data were then converted to water content corresponding to water uptake by roots and hyphae from each compartment. In this experiment we studied a) kinetics of transport of water from hyphae chamber to plant chamber via hyphae under drought conditions; b) change in the water content in plant compartments (PC) of drought stressed (D) VAM and non-VAM plants measured by capacitance sensors; and c) change in the water content in hyphae compartments (HC) of drought stressed (D) VAM or non-VAM plants measured by capacitance sensors.

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2 Differential effects of two vesicular arbuscular mycorrhizal fungi on growth, leaf water relations and nutrient uptake of barley under well-watered and drought conditions

2.1 Abstract

Association of vesicular arbuscular mycorrhizal fungi (VAM) with higher plants have been shown to alter plant's response to drought (water stress) conditions. Our general objectives in this study were: i) to assess the contribution of VAM in improving drought tolerance, and ii) to measure the effects of VAM on physiological parameters and water relations in barley. We studied the effects of colonization of barley (*Hordeum vulgaris* L. var. Scarlett) roots with two VAM fungi (*Glomus intraradices* and *Glomus mosseae*) on leaf water relations, growth, yield components and acquisition of nutrients under simulated drought or well-watered conditions in a greenhouse. Barley plants were grown in soil with relatively high level of soil nutrients such as phosphorus and nitrogen, subjected to eight drying cycles. Soil water potential was kept below –0.08 MPa throughout each drying cycle until the end of taselling (90 days after sowing). We observed mild effects of the two mycorrhizal fungi on leaf water relation between VAM and non-VAM drought stressed plants but no specific effects on yield parameters or plant nutrients uptake. We noted, however, that drought conditions surprisingly increased root colonization by VAM. Under drought condition, only *G. intraradices* changed the leaf area of plants slightly but statistically significant. Leaf water potential was slightly higher in plants colonized by *G. mosseae*, and leaf osmotic potential was lower in the plants colonized by *G. intraradices* as compared with non-VAM plants. We also noted some differences between *G. intraradices* and *G. mosseae* in their effect on nutrient uptake by barley under same growth conditions.

2.2 Introduction

Water stress is one of the most important environmental stresses affecting agricultural productivity around the world and may result in considerable yield reductions (Boyer, 1982; Ludlow and Muchow, 1990). Plant's ability to grow and reproduce satisfactorily under drought conditions is termed its drought resistance, and its ability to slowly modify its structure and function to water deficit so that it can better tolerate drought is termed its drought acclimation (Turner, 1986). Apart from the effect of drying soil on the transport of nutrients in soil towards to plant roots, the morphological and physiological mechanisms involved in cellular and whole plant responses to water therefore generate considerable interest and are frequently reviewed (Hsiao, 1973; Levitt, 1980; Blum, 1988; Davies and Zhang, 1991; Smith and Griffiths, 1993; Close and Bray, 1993; Kramer and Boyer, 1995; Neumann, 1995; Turner, 1997).

Although the abilities of specific-fungus-plant associations to tolerate drought are of great interest (Ruiz-Lozano et al., 1995), the exact role of VA mycorrhizal fungi in drought

resistance is not very clear (Auge et al., 1992a). The only conclusive information that has been suggested is that more studies is needed to determine the direct or indirect mechanisms which control plant water relations in VAM plants symbiosis. Although the effects of VAM fungi on plant water status have been ascribed to the improved host nutrition (Graham and Syverten, 1984; Nelsen and Safir, 1982; Fitter, 1988), there are reports that drought resistance of VA-mycorrhizal plants is somewhat independent of plant P nutrition status of plants (Sweatt and Davies, 1984; Auge et al., 1986a; Bethenfalvay et al., 1988). For example, Vivas (2003) reported that the increased metabolically active fungal biomass in co-inoculated plants was irrespective of P level and was not related to P uptake from the inter soil-less substrate. Baon et al. (1993) reported that different cultivars of barely were not only colonized to different extents by *G. intraradices*, but the extent of colonization was variably sensitive to P additions.

The objective of this investigation was to examine the degree of improvement in water relations of mycorrhizal plants in a soil with high P-content under simulated drought conditions.

2.3 Materials and methods

2.3.1 Experimental design and statistical analysis

The experiment consisted of a randomised complete block design. Treatments consisted of factorial combinations of three mycorrhizal treatments (*Glomus intraradices* Schenck & Smith and *Glomus mosseae* Nicol. & Gerd and non-mycorrhizal plants) with two water supply conditions (well-watered and simulated drought). Five replications of each treatment were tested which gave rise to total of 30 experiments units (pots). Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS institute Cary, USA, 1988) and followed by LSD multiple range tests. Terms were considered significant at $P < 0.05$.

2.3.2 Soil and biological materials

Soil from the horizon (0-15 cm) of a loamy-silt soil belonging to Research Station-Dürnast, Institute of Plant Science, Chair of Plant Nutrition, Technical University of Munich, was used in this study. It consisted of 23% clay, 48% silt and 29% sand 1.66% organic matter (Table 1).

Table 1. Status of greenhouse potting soil used for experiment

pH	EC	P_2O_5-CAL	C/N analysis	NH_4-N
	(dS m^{-1})	(mg $100g^{-1}$)	(TM%)	(mg $100g^{-1}$)
6.7	0.6	22	0.14	1.47

The soil was first air-dried, ground, passed through a 5-mm mesh screen and then sterilized by autoclaving at 120 °C and 1.3 bar pressure. The initial gravimetric soil water content (23% on dry soil basis) was achieved by adding distilled water and thoroughly mixing. The soil bulk density was obtained at 1.4 g cm^{-3}. For the mycorrhizal treatments, inocula of VAM fungi (consisting of roots and hyphae from pot culture) were banded 2-3 cm below the soil surface in plant containers (pots), which contained 8 kg of sterilized soil. This amount of inoculum was selected in preliminary tests as the optimum to produce a good colonization level for the amount of soil in each pot. Seeds of barley (*Hordeum vulgaris* L. *var*. Scarlett) were first sterilized by 0.5 % NaClO solution for 15 min, and then washed three times in sterile water in petri dishes. Seeds were then allowed germinating in the same petri dishes for 15 hours and then sown in the chambers. Seven days later, the number of plants per pot was reduced to 15. The control treatment (non-VAM) was prepared in the same manner but without inoculum.

2.3.3 Plant growth conditions

Plants were grown in greenhouse with 65 / 70 % relative humidity, day / night temperatures of 20-24 °C / 15-18 °C and photoperiod of 14 h at photosynthetic photon flux density of 800 µmol m^{-2} s^{-1} under high intensity incandescent light. Soil moisture (water potential) was measured with tensiometers (DWG 2120, Dr. V. Ballmoos, Switzerland). Water was supplied daily to maintain constant initial soil water content (23% gravimetric soil water content). After 3-week establishment period (when plants were 21 days old) half the plants were acclimated by eight drying cycles till harvest, which took place at 90 days after sowing. At the end of each drying cycles plants received about 400ml with 0.2 g l^{-1} NH_4NO_3 to replenish to 23% soil water content. For well-watered treatments, the water loss was replaced by adding tap water if necessary during the experiment.

2.3.4 Parameters measured

Biomass production: At harvest (90 d after sowing), the shoot and root systems were separated; their fresh weight (FW) was immediately measured. Plants parts were then dried in hot-air oven at 70 °C for 2 d and dry weights (DW) were recorded.

Yield production: At harvest, spikes were separated from the plant shoot and their number and their fresh weight were determined immediately. Their dry weights were recorded after drying at 70 °C for 2 d.

Symbiotic development: The percentage of root colonization by mycorrhizal fungi was estimated by visual observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v / v), according to Phillips and Hayman (1979). Mycorrhizal colonization was determined in 25 random samples of 1-cm long root segments from each of seven plants (n =175) and percentage of mycorrhizal arbuscular, vesicles and hyphae were measured. The extent of mycorrhizal

colonization was calculated according to quantification method of Furlan and Fortin (1977).

Morphological parameters: Plant height, number of tillers and leaf area rate were determined at the end of each drying cycle.

Physiological parameters (water relations):

Physiological parameters were measured during peak stress (11:00-13:00) on the youngest, fully expanded leaf of each treatment. A pressure chamber (Scholander et al., 1964) was used to measure leaf water potential (ψ_s). Osmotic potential (ψ_s) was determined by an osmometer (VAPROtm Model 5520, Wescor Inc. Germany) on leaves that were cut from plant and were sealed in nylon envelopes immediately after cutting, frozen at –20 °C for 24 hours and thawed for 15 min at 22 °C. Turgor (ψ_p) was calculated as the difference between ψ_w and ψ_s.

The relative leaf water content (RWC) was ascertained by measuring the fresh weight, rehydrated weight on distilled water and dry weight (80 °C for 2 d) (DW) and using the following formula (Turner 1986):

$$RWC = (FW\text{-}DW / TW\text{-}DW) \times 100$$

The FW was determined by immediately weighing one fully expanded young leaf, which was allowed to rehydrate for 4 h by floating 1-cm from the cutting part into a covered beaker with distilled water. The rehydrated leaf was weighed to determine saturate mass and then the leaf was dried at 70 °C for 24 h to determine dry weight.

Determination of shoot P and K status: The dried samples were powdered using a Wiley mill and analysed for P and K. For P concentration, dried tissues (300 mg) were digested in $HClO_3$-H_2O_2 (v/v 5.3: 3.5) for 45 minutes using a microwave (MDS-2100 W/T. C., Matthews, North Carolina 28106, CEM, USA). Digested samples were diluted to 50 ml with distilled water and the P content determined using spectronin (501-Mizton Roy Company, Unterfoehring, Germany). Shoot K content was determined with Flame photometer (Eppendorf, ELEX 6361-Eppendorf-Nethele, Hinz GmbH –Hamburg, Germany) by using the same extract used for P measurement. Nutrient content was calculated by multiplying the mineral concentrations by the dry masses of shoots.

2.4 Results

2.4.1 Gravimetric soil water content

The gravimetric soil water content of the growth medium at the end of each drying cycle is shown in Figure 2. A clear difference was observed between the soil water content in pots containing VAM and non-VAM plants from the end of the third drying cycle (41 days after sowing). This difference persisted throughout the rest of the experiment till harvest.

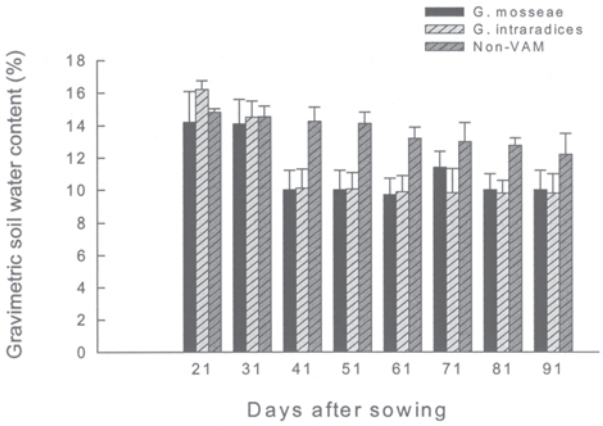

Figure 2. Gravimetric soil water content in the plants with or without vesicular arbuscular mycorrhizal fungi (*G.intraradices* & *G. mosseae*; Non-VAM) at the end of each drying cycle (Error bars represent standard deviation).

2.4.2 Effects of VAM on root mycorrhization

Percentage of root mycorrhization in plants inoculated with *G. mosseae* were 48.0%, 51.5% and in plants inoculated with *G. intraradices* were 27.3% and 62.2% under well-watered and drought conditions respectively. Surprisingly the rate of root mycorrhization was relatively higher in plants subjected to drought conditions than those grown under well-watered conditions (Figure 3).

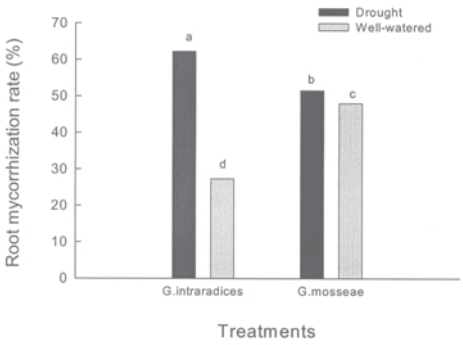

Figure 3. Root mycorrhization rate in the plants with two vesicular arbucular mycorrhizal fungi (*G.intraradices* & *G. mosseae*) under well-watered and drought conditions.
Bars followed by the different letters are significantly different by ANOVA and LSD multiple range test ($P < 0.05$).

2.4.3 Effects of VAM on plant growth

Prior to the initiation of drought cycles, both VAM or without VAM plants were fertilized with 400 ml of 0.25 g l^{-1} NH_4NO_3 regularly to aid them in attaining comparable size (number of leaves, leaf area, shoot height). At the end of the final drying cycle (drought treatment) there were no significant differences in the shoot and root dry weight of plants subjected to different treatments at $P < 0.05$ (Table 2). Also the root / shoot ratio was not affected by the mycorrhizal or drought treatments. Under well-watered conditions, the shoot and root dry weights in VAM and non-VAM plants were similar (Table 2). Small growth depression was observed in the dry weight of shoots and root of VAM as compared with the non-VAM plants, which could possibly be due the competition for photosynthesis between host and fungus (Abbott and Robson, 1984).

Table 2. Effects of two vesicular arbuscular mycorrhizal fungi on biomass, yield and certain morphological parameters of plants with or without vesicular arbuscular mycorrhizal fungi (VAM; non-VAM) plants subjected to well-watered (WW) and drought conditions (D).

- **Means within each row followed by different letters are significantly different at (*P < 0.05*) according to LSD multiple range test.**
- **Means within each row followed by same letters are not significantly different at (*P < 0.05*) according to LSD multiple range test.**

	Well-watered			Drought		
	G. intraradices	G. mosseae	Control	G. intraradices	G. mosseae	Control
Shoot height (cm)	75.1 a	77 a	71.8 a	63.1 b	64.2 b	62 b
Tiller number (per plant)	7.5 a	7.7 a	7.2 a	6.3 a	6.7 a	5.5 a
Shoot dry weight (g plant $^{-1}$)	1.75 a	1.74 a	1.67 a	1.31 b	1.34 b	1.24 b
Root dry weight (g plant $^{-1}$)	2.4 a	2.2 a	2.0 a	1.58 b	1.5 b	1.4 b
Spike dry weight (g plant $^{-1}$)	8.1 a	7.7 a	7.2 a	4.8 b	5.5 b	4.5 b
Leaf area (cm^2 plant $^{-1}$)	19.0 a	19.1 a	18.3 a	19.5 a	16.4 b	16.0b
Root / shoot ratio	1.4 a	1.3 ab	1.2 b	1.2 b	1.2 b	1.1 b

Under drought condition, leaf area of plants inoculated with *G. intraradices* was significantly larger than that in plants inoculated with *G. mosseae* or non-VAM plants. No significant differences were found in the leaf area of VAM and non-VAM plants under well-watered condition.

2.4.4 Effects of VAM on leaf water relations

In plants subjected to water stress (drying cycle), relative leaf water content were 68.7%, 55.1% and 49.2% in plants inoculated with *G. mosseae*, *G. intraradices* and non-VAM plants, respectively (Table 3). Leaf water potential in plants-inoculated with *G. mosseae* was slightly (but not significantly) higher (less negative) (-1.9 MPa) than in plants inoculated with *G. intraradices* (-2.3 MPa) during the last drying cycle. There was, however, a small but statistically significant difference in the osmotic and turgor potentials of plants inoculated with *G. intraradices* and control plants (Table 3). These results show that the two endophytes mycorrhizal species tested had negligible impacts on the leaf water relations in barley.

Table 3. Effects of two vesicular arbuscular mycorrhizal fungi on water relation parameters of plants with or without vesicular arbuscular mycorrhizal fungi (VAM; non-VAM) subjected to well-watered (WW) and drought conditions (D).

- **Means within each row followed by different letters are significantly different at (*P < 0.05*) according to LSD multiple range test.**
- **Means within each row followed by same letters are not significantly different at (*P < 0.05*) according to LSD multiple range test.**

	Well-watered			Drought		
	G. intraradices	G. mosseae	Control	G. intraradices	G. mosseae	Control
RWC %	91.60 a	93.23 a	94.8 a	55.1 c	68.7 b	49.2 d
Leaf water potential (MPa)	-1.42 a	-1.31 a	-1.33 a	-2.3 c	-1.9 b	-2.5 c
Leaf osmotic potential (MPa)	-1.33 a	-1.4 a	-1.35 a	-1.35 b	-1.22 c	-1.10 c
Leaf turgor pressure (MPa)	-0.01 a	0.01 a	0.02 a	-0.95 c	-0.68 b	-1.4 c

2.4.5 Effects of VAM on plant nutrients uptake

Under well-watered conditions, there were no significant differences in the contents of P or K in the VAM and non-VAM plants (Figures 4 & 5). Under water stress conditions, although *G. mosseae* did not affect either P or K contents in plants when compared to control (non-VAM) plants, *G. intraradices* did. Thus under water stress condition, plants inoculated with *G. intraradices* contained significantly higher P and K contents than those inoculated with *G. mosseae* or control plants. Interestingly, the concentration of P in the water-stressed plants, which were inoculated with *G. intraradices*, was equal to the average P concentration in the well-watered plants irrespective of their VAM status (3.43 and 3.2 mg 100 g^{-1} in the *G. intraradices* and *G. mosseae* respectively). The result is shown in Figure 4.

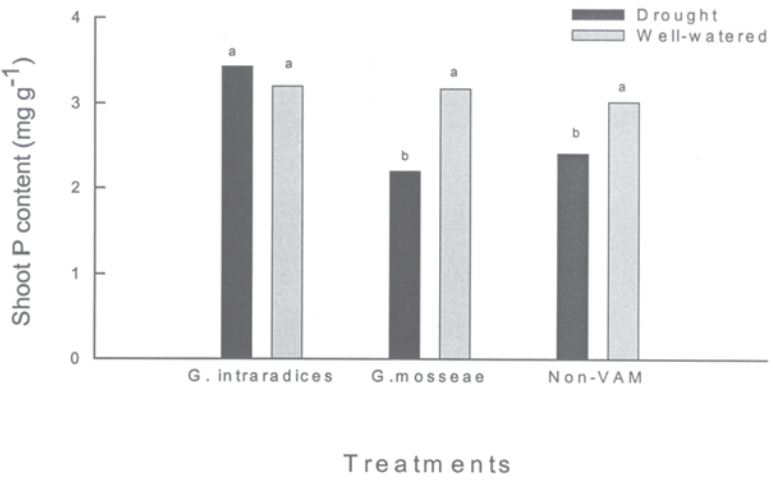

Figure 4. Shoot P content in plants with or without vesicular arbuscular mycorrhizal fungi (*G. intraradices* & *G. mosseae*; Non-VAM) under well-watered and drought conditions.

- **Bars followed by same letters are not significant different by ANOVA and LSD multiple range test (*P < 0.05*).**
- **Bars followed by the different letters are significantly different by ANOVA and LSD multiple range test (*P < 0.05*).**

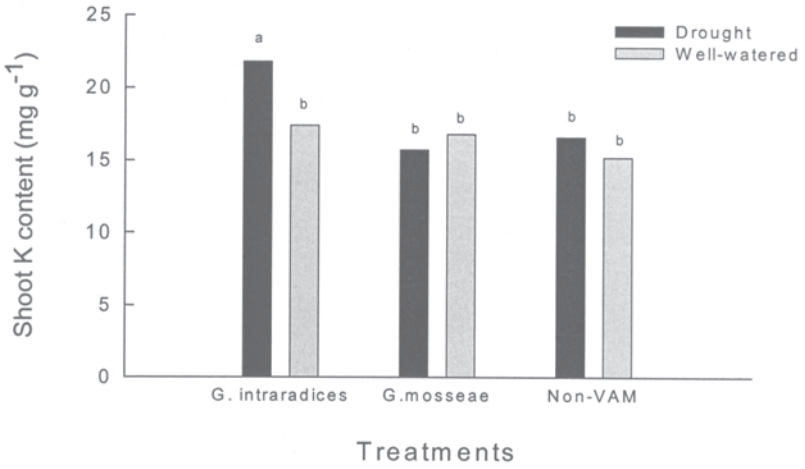

Figure 5. Shoot K content in plants with or without vesicular arbuscular mycorrhizal fungi (*G. intraradices* & *G. mosseae*; Non-VAM) under well-watered and drought conditions.

- **Bars followed by same letters are not significant different by ANOVA and LSD multiple range test (*P < 0.05*).**
- **Bars followed by the different letters are significantly different by ANOVA and LSD multiple range test (*P < 0.05*).**

2.4.6 Correlations between root mycorrhization and leaf water relations

A correlation between calculated inflow via the fungi and percentage of the root length colonized has been observed in some but not all investigations (Smith and Read, 1997). It may be due to the progressive death of the fungus within the root, reduction in the contribution of arbuscules to the colonized length and / or death or destruction of the extraradical hyphae. The consequence would be reduction in the ability of mycorrhizal roots to absorb water or nutrients (Fitter and Merryweather, 1992).

In this experiment, the roots of plants were collected at the end of the study and the colonization of their roots by mycorrhizal fungi was measured. It was noted that under water stress condition, there was no significant correlation between the rate of root colonization by either *G. intraradices* or *G. mosseae* and water relation parameters (RWC, leaf water potential and leaf osmotic potential) (Figures 6 and 7). This might have been due to the relatively low rate of root colonization by these fungi under our experimental conditions as compared to those reported by others (Davies et al., 1992; Busse and Ellis, 1985).

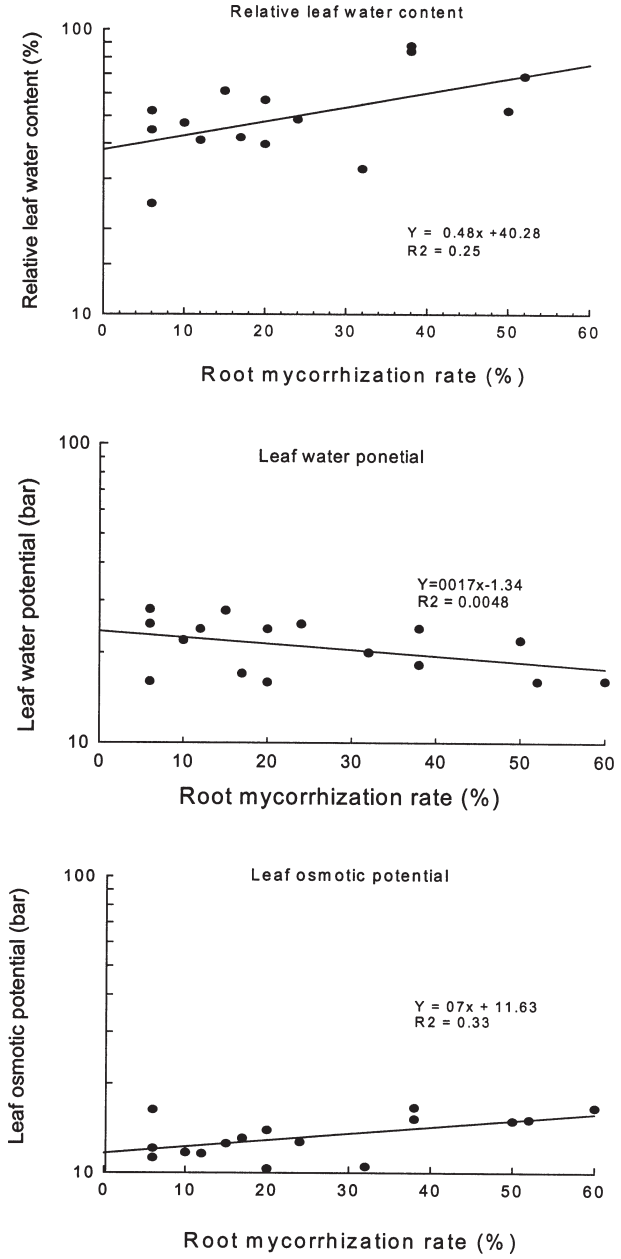

Figure 6. Correlation between root mycorrhization rates of plant with vesicular arbuscular mycorrhizal fungi (*G. mosseae*) and water relation parameters (relative leaf water content, leaf water potential and leaf osmotic potential) under drought conditions.

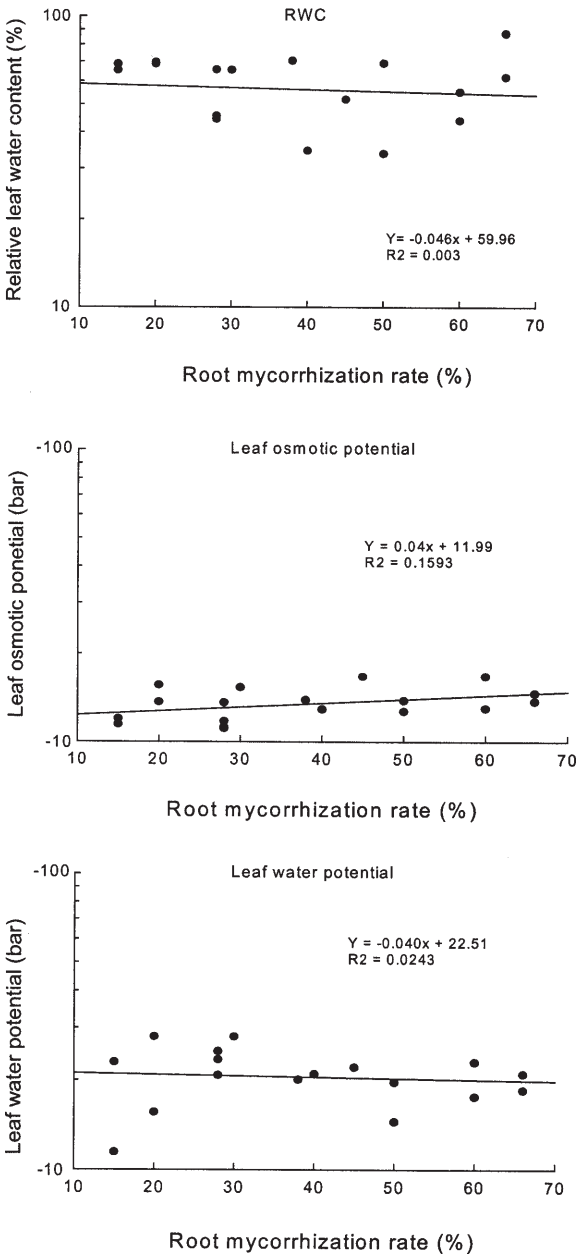

Figure 7. Correlation between root mycorrhization rate of plant with vesicular arbuscular mycorrhizal fungi (*G. intraradices*) and water relation parameters (relative leaf water content, leaf water potential and leaf osmotic potential) under drought conditions.

2.5 Discussion

Plants colonized by mycorrhizal fungi are observed to deplete soil water more thoroughly than non-mycorrhizal (Auge, 2001). One reason for this is the fact that the shoots of VAM-plants usually have a larger biomass (more evaporative leaf surface area) than non-VAM plants (Fitter, 1988; Nelsen, 1987). Also the root systems of VAM-plants are often more finely divided (more water absorptive surface are) (Allen et al., 1981; Busse and Ellis, 1985; Ellis et al., 1985; Huang et al., 1985; Sharma and Srivastava, 1991; Osonubi et al., 1992; Osonubi, 1994; Okon et al., 1996). Furthermore, roots of VAM-plants are noted to dry the soil more quickly than non-VAM plants when both plants were of similar size (e, g. Beyla and Duniway, 1998).

In our experiments, VAM and non-VAM plants grown under water stress condition had equal number of tillers, shoot height and shoot dry weight. It is possible that drought stress imposed was not severe or long enough, or was introduced too late during the plant growth, to significantly alter growth.

The observation that neither *G. intraradices* nor *G. mosseae* influenced shoot dry weight under drought conditions indicates these fungi had no beneficial effects on the photosynthesis processes in the host plant. The observation that barley plants inoculated with *G. intraradices* had a significantly larger leaf area rate as compared with *G. mosseae* or non-VAM plants under drought conditions is, however, worth special attention. In fact the leaf area in plants inoculated with *G. intraradices* and grown under drought condition was similar to that of plants grown under well-watered conditions (Table 2). This observation seems to indicate that *G. intraradices* could mitigate the adverse effect of water stress on the physiology of leaf area in this plant.

Biomass (shoot and root dry weights) of VAM-plants was not significantly different as compared to non-VAM plants neither under water stress nor under well-watered conditions. Hardie (1985) reported that the benefits of VAM infection are observed particularly when P availability is low and are manifest mainly as growth responses and increased internal P status. The observation that, under the water stress condition imposed on barley in this study, plants inoculated with *G. intraradices* had similar leaf areas (Table 2) and similar P content in their leaves (Figure 4) leads us to conclude that *G. intraradices* (but not *G. mosseae*) mitigated the adverse effect of water stress on leaf growth rate by improving the P-nutrition and improved photosynthesis process in barely plants subjected to water stress. This, however, did not translate itself in higher biomass or tiller number perhaps because the nutrient medium we used supplied the plants with sufficient P so that VAM-plants were not benefiting greatly in terms of P uptake from the extra surface area provided by extraradical hyphae.

The observation that under drought conditions, *G. intraradices* or *G. mosseae* did not benefit the drought tolerance or biomass production are in contrast to findings of others (Nelsen and Safir, 1982; Fitter, 1988; Sylvia et al., 1993; Subramanian and Charest 1997),

which were conducted under poor soil phosphorus conditions and often had greater root mycorrhization rates than observed in this study. Our results are in agreement with those reported by Busse and Ellis (1985) and Hardie (1985). The root mass and root / shoot ratios were similar in VAM and non-VAM plants and seem not to have any relationship to P concentration in these plants especially if we consider that plants inoculated with *G. intraradices* had smaller root mass but had supplied the plants significantly higher P as compared to *G. mosseae* or non-VAM plants. The root / shoot ratio or root / leaf weight ration may be increased (Bethlenfavay et al., 1988; Graham et al., 1987), decreased (Hardie and Leyton, 1981), or unaffected (Auge et al., 1986b) by mycorrhizal fungus. A high root / shoot ratio is a frequent response to water stress (Kramer, 1983). In our study, root / shoot ratio was not affected by VAM or by drought treatments. In this study, VAM influenced the water potential rather late under drought conditions which may be due to the time necessary for the extraradical hyphae to grow long and far enough into the soil matrix to become effective enough in water uptake and transfer to make a measurable difference.

We measured osmotic potential at the end of each drying cycle. It was observed that under water stress conditions, leaf osmotic potential was significantly higher in the plants inoculated with *G. intraradices* than that in plants inoculated with *G. mosseae* or in non-VAM plants (Table 3). Under well-watered conditions, however, leaf osmotic potential was the same in VAM and non-VAM plants. Measurement of K, which is an osmotic important nutrient, in the leaves showed that under water stress condition, plants inoculated with *G. intraradices* had significantly higher K than those inoculated with *G. mosseae* or control plants. Hardie (1981, 1985) reported similar findings in the red clover inoculated with two VAM fungi. Our results of higher leaf K content (Figure 5), lower osmotic potential (Table 3) in plants inoculated with *G. intraradices* under water stress conditions leads us to speculate that these are related events which are observed for *G. intraradices* but not for *G. mosseae*.

The water relations in the plants subjected to water stress, and in particular the determination leaf water potential with Scholander bomb, varied somewhat from drying cycle to drying cycle. This was perhaps due to differences in temperatures, or time of day when measurements were conducted. Despite the above, the improved water relations under drought conditions observed in those barley plants inoculated with VAM could not be solely attributed to improved P nutrition. This is agreement with reports of Sweatt and Davies (1984), Auge et al., (1986a), and Bethlenfavay et al., (1988). Although P content in leaf tissue in plants inoculated with *G. intraradices* was slightly higher than those inoculated with *G. mosseae* or in non-VAM plants, there was, however, no difference in the water relations in plants subjected to different treatments. Contrary to our findings, Nelsen and Safir (1982), Graham and Syvertsen (1984) and Fitter (1988) attributed improved water relations of mycorrhizal plants to increased tissue nutrition, particularly P. Although mycorrhiza can promote P uptake, and P uptake is reduced under drought

(Begg and Turner, 1976; Vietz, 1972), it is not clear how increased P could improve water relations or drought resistance. Drought resistance of the VAM plants may have occurred because drying cycles promoted more growth of extraradical hyphae of fungi which in turn increased the water uptake, a mechanism supported by the work of Allen (1982) and Hardie (1985). In a non-mycorrhizal study, McCoy et al. (1984) concluded that increasing root density created smaller root-to-soil water potential gradients and less negative root water potentials for a given daily transpiration loss. This might be a plausible explanation if mycorrhizal hyphae explore the soil volume in a manner analogous to increasing root density in which case mycorrhizal roots could have higher water potential than would have occurred in non-VAM roots and this in turn should promote higher Y leaf.

In this study the rate of root mycorrhization showed very small positive correlation with leaf water potential in plants inoculated by *G. mosseae* but not by those inoculated by *G. intraradices*. The very slight improvement in leaf water potential brought about by *G. mosseae* under drought condition, on the one hand, and no significant differences in yield (shoot and root biomass) of plants inoculated with either of the two VAM tested suggests that under our experimental condition mycorrhizal improvement of water uptake by the plants was not enough to translate itself into any measurable yield difference.

Some VAM fungi can adapt to physical and chemical changes in soil, the amount, and possibly type, of external hyphae of some species of VAM fungi is affected by other microorganisms, root exudates, pests, clay content, soil pH, organic matter, fungicides, pesticides and the phosphorus content of the plant, and possibly the charges of the surfaces of the hyphae and of the clay (Gianinanzzi-Pearson et al., 1989; Tisdall, 1991). It might be argued that a nutritional influence of VAM symbiosis on host water balance can never really by excluded from any experiment with complete confidence, given the integral effect of VAM fungi on P acquisition and plant growth. As often noted (e.g. Bethlenfalvay et al., 1988) and demonstrated (e.g. Faber et al., 1991), P supplementation for producing proper controls is a conscious compromise, as P-supplemented non-VAM plants do not conform to the desired criteria of root and leaf compatibility with VAM plants. It is very difficult, perhaps innately impossible with some host species, to produce VAM and non-VAM plants similar in every respect that might account for and control nutritional or size effects on host water relations. Nonetheless, many experiments that produced VAM and non-VAM plants of similar size and with physiologically comparable P concentrations have still reported VAM-induced changes in host water relations or drought responses. Almost half of the instances of VAM-induced increase in stomatal conductance or transpiration have involved similar-sized and nourished VAM and non-VAM plants. Moreover, in some VAM studies, P fertilization and leaf P concentration have been shown to have no effect on transpiration or the other leaf water relation parameters under study, and yet VAM and non-VAM plants have differed in these parameters (e.g. Auge et al., 1987a; Auge, 1989). Still others have observed higher rates of gas exchange by leaves with significantly lower P concentrations than those from non-VAM controls (e.g. Brown

and Bethlenfalvay, 1987). Larger plants, or plants having leaves with higher P concentration, do not always show higher gas exchange parameters than smaller plants or plants with lower concentrations. For example, amply watered VAM *Bromus inermis* plants had higher photosynthetic rates than non-VAM plants, even though the VAM plants were smaller (Bildusas et al., 1986). Amply watered *Glomus deserticola*-colonized rose plants whose fed less P and having lower leaf and root P concentrations, had higher stomatal conductance than *Glomus intraradices*-colonized roses whose fed more P and demonstrated higher P concentrations in the leaf and root (Auge et al., 1986a). A strictly nutritional or size mechanism of VAM influence on host water balance does not appear to explain many of the published data. It appears that under the investigated level of soil nutrients especially P, VAM association did not benefit the host plants subjected to drought stress. Under such conditions mycorrhizal colonization of plants may be more to the benefit of the fungi (parasitism) than to the mutual benefit of plants and fungi (symbiosis) (Daniels Hetrick et al., 1984).

2.6 Conclusions

Numerous factors may affect the host and the mycosymbiont. Our results showed that the two VAM fungi (*Glomus intraradices* & *Glomus mosseae*) did not significantly improve yield and nutrients uptake of their host (barley plant) under drought conditions. However, we noted some difference on water relations of host plants as compared to uninoculated plants. Lower rate of root mycorrhization observed in this study as compared to similar studies (Busse and Ellis, 1985; Daniels Hetrick et al., 1984) was probably due to high availability of P in this experimental soil. High soil P and other nutrients level are known to prevent the symbiosis event between mycorrhizal fungi and plants root (Smith and Read, 1997; Daniels Hetrick et al., 1984; Busse and Ellis, 1985; Auge, 2001). Considering the equal production of biomass by VAM and non-VAM plants after 60 days under drought-stress suggests that the improvement observed in the leaf water content and slight changes in some components of leaf water potential were probably due to other unknown effects of mycorrhizal fungus on plants hormones and/or membrane properties.

2.7 References

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3 Quantification of water uptake by hyphae in barley with split-root-hyphae system under drought conditions

3.1 Abstract

Water availability limits crop production in many regions of the world. We subjected barley plants (*Hordeum vulgaris* L. *var*. Scarlet) to simulated drought and studied the effects of symbiosis with vesicular arbuscular mycorrhizal fungi (*Glomus intraradices*) on water uptake and elongation of plant leaves. The plants were sown at one-week intervals in split-root chambers consisting of plant and hyphae compartments. Ninety days after sowing, the initial gravimetric soil water content was reduced by about 2-4 % in the hyphae compartments of drought stressed plants with vesicular arbuscular mycorrhizal fungi (VAM plants) as compared to that in the plants without vesicular arbuscular mycorrhizal fungi (non-VAM plants). Leaf osmotic potential were lower in VAM plants. Relative leaf water content and leaf turgor pressure were all higher in VAM plants than in the non-VAM plants, but at the end of drying cycle leaf 5 on the mainstem of drought stressed VAM plants was 33% longer than in the non-VAM plants. The 2-4% decrease in the gravimetric soil water content in the hyphae compartment is attributed to water uptake by the extraradical hyphae and its transport to the drought stressed VAM plants. We suggest, however, that the improved leaf water relations, longer leaf, and faster leaf elongation rate in the drought stressed VAM plants compared with the non-VAM plants, might have been due to the impact of VAM on plants which were independent of the higher contribution of VAM hyphae to water uptake by drought stressed VAM plants.

3.2 Introduction

Limited water conditions (drought) are considered to be one of the most critical abiotic parameters that limits plant growth and yield (Kramer and Boyer, 1997). Vesicular arbuscular mycorrhizal fungi (VAM) symbiosis and its association with plants are known to reduce the impact of harsh environmental conditions on plants (for review see Auge, 2001; Ruiz-Lozano, 2003). Under drought conditions mycorrhizal fungi may modify water relations in the host plants (Nelsen, 1987) so that stomatal conductance, transpiration and leaf water potential are often higher in VAM plants due to a hyphae-mediated higher water uptake by plants (Auge et al., 1987a; Duan et al., 1996; Subramanian and Charest, 1995). This allows plants, which are in association with VAM (VAM plants) to maintain higher rate of net photosynthesis and higher leaf water content than those in non-VAM plants (Auge, 2001). In about 80% of studies on the effect of mycorrhizal fungi on plant growth under drought, VAM plants were reported to be larger than non-VAM plants, which seem to suggest an important role for VAM fungi in promoting the drought resistance of their hosts (Auge, 2001). Safir et al., (1971, 1972) were among the first who reported that mycorrhizal soybean plants had lower resistance to water transport than uncolonized plants, and that most of the difference was attributable to changes in

root resistance, since shoot resistances were small and did not differ in the VAM and non-VAM plants. Safir et al., (1972) concluded that the effect was probably due to improved nutrition, because the differences could be eliminated if nutrients or fungicide were applied. The extraradical mycelial network increases the nutrients uptake surface of the host plant and allows a more efficient extraction of phosphorus, nitrogen and certain micronutrients (Smith and Read, 1997). High phosphorus supply, however, strongly reduces the extent of infection in roots of soybean plants by the VAM fungus (Wyss et al., 1991). Also fertilizing soils with phosphorus had no effects on root or shoot dry weight in similar experiments (Bruce et al., 1994). Koide (1985) found no intrinsic differences between well-watered mycorrhizal and nonmycorrhizal sunflower when plant water relations parameters were adjusted for plant size and P status. For comparing mycorrhizal and nonmycorrhizal plants to ascertain differences in water relations, one difficulty is distinguishing the secondary effects of mycorrhizae on water relations such as altering plant architecture, physiology, or other plant characteristics from a possible direct role of mycorrhizae on water absorption.

Leaf growth is influenced by genotype (Volenec and Nelson, 1981) developmental stage (Schnyder et al., 1990; Meiri et al., 1992), light (Schnyder and Nelson, 1989), salinity (Bernstein et al., 1993 and Hu et al., 2000) and VAM fungi association (Ebel et al., 1994; Auge et al., 1995). Recently, some studies have focused on other aspects of VAM symbiosis. The results of these studies have shown an altered response of sorghum leaves to non-hydraulic signals of soil drying and that VAM symbiosis had eliminated drying-induced decline in the total leaf length (Auge et al., 1995).

Modified growth chambers have been employed to distinguish between the variables, which affect water uptake, and nutrients of VAM plants. Klemedtsson et al., (1987), Ames et al., (1983), Haystead (1988), Rhodes and Gerdemann (1978a), and Cooper and Tinker (1981) have all grown plants in devices which allowed hyphae to cross a barrier so that hyphae could be fed independently from roots. In these systems, however mass flow and diffusion of nutrients through the soil matrix across the screen barriers (i.e., not necessarily through the VAM hyphae) may make it difficult to assess the true contribution of hyphae to nutrient and water transport extent to the plant roots. The system presented here overcomes much of this problem by introducing an air gap between the two layers of 30 ìm-mesh-nylon. In this paper, the physical role that hyphae play in water uptake is examined using such a split-root-hyphae chamber in which the hyphae in plant compartment can pass the membrane (nylon) and extent to hyphae compartment with 23% soil water content. The plant roots and water are not able to passing the membrane because of air gap and very small pore on membrane. With the air gap in place the mass flow and diffusion of substances from the hyphae compartment is eliminated. Thus, if plants inoculated with VAM receive any excess water it must have been transported only by extraracical hyphae from the hyphae compartment. The questions we tried to answer with this research were: 1) Do extraradical hyphae contribute to water uptake by plants

under drought conditions? 2) Does VAM always benefit the plants growth? 3) Does water uptake by hyphae improve plant water relations under drought conditions? To test these, we investigated the interacting effects of VAM and drought on leaf water relations, nutrients status and leaf elongation rate during the linear growth phase of barley leaves.

3.3 Materials and Methods

3.3.1 Experimental design and statistical analysis

Experiments were a factorial design with five replications. Analysis of variance (ANOVA) was achieved by the statistical analysis system (SAS Institute, Cary, USA, 1988). Critical differences at the 5% level of significance were tested using LSD range test.

3.3.2 Construction of split-root-hyphae system chamber

Split-root-hyphae system was made with plexiglass and consisted of two compartments: the hyphae compartment (H x L x W: 30 x 19 x 3 cm) and the plant compartment (30 x 19 x 5 cm). Two layers of nylon net with a pore size 30 ìm and an air gap of about 5 mm between the two nylon nets separated root and hyphae compartments. The air gap of 5 mm is believed to be sufficient to prevent water diffusion and mass flow between the plant and hyphae compartments (Figure 8). In order to avoid water loss by evaporation from the hyphae compartment, the soil surface of the hyphae compartment was covered with a perforated plastic film during the entire experiment.

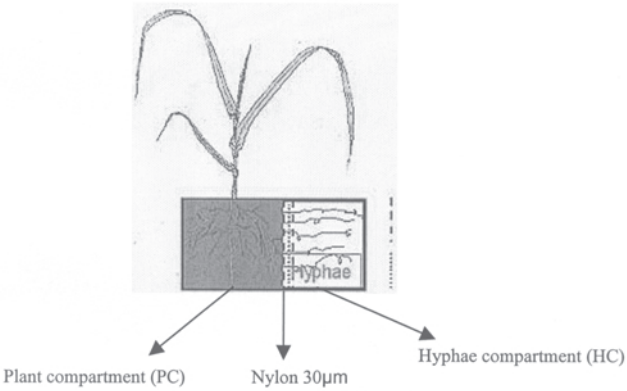

Figure 8. Split-root-hyphae system for quantifying the acquisition of water through VAM

3.3.3 Plant growth conditions

Soil from the horizon (0-15 cm) of a loamy-silt soil belonging to the Research Station-Dürnast, of the Chair of Plant Nutrition, Technical University Munich, was used in this study (see explanation in 2.3.3). The soil was air-dried, ground, and passed through a 5-mm mesh screen. Before the soil was filled into both compartments, it was sterilized by autoclaving at 120 ^{o}C and 1.3 bar pressure. The initial gravimetric soil water content of the soil (23% on dry soil basis) was achieved by adding distilled water and thoroughly mixing. The soil bulk density was 1.4 g cm^{-3}.

Seeds of a commercial variety of barley (*Hordeum vulgaris* L. *var.* Scarlet) were surface sterilized using a 0.5 % NaClO solution for 15 min, then washed three times in sterile water, and pre-germinated in petri dishes and then transferred to the chamber. About 25 g of each inoculated or non-inoculated peat was applied per container uniformly as 1-cm layer so about 2-3 cm below the seeds in each container prior to sowing. Based on the preliminary tests, this amount of inocula was selected to produce an optimum colonization level. Inoculums were banded 2-3 cm below the surface in plant chamber containers of 4 kg sterilized soil. This amount of inoculums was selected in preliminary tests as the optimum to produce a good colonization level for a total amount of soil in the pot. The number of plants per chamber was reduced to six at seven days after sowing.

Plants were grown in a controlled growth chamber at 14 / 10 h photoperiod, PPFD (Photosynthetic Photon Flux Density) of 450-ìmol m^{-2} s^{-1} (day/night) light also the air temperature was 20 / 18 ^{o}C with 65 % humidity during the germination and later regulated to 15/15 ^{o}C with 70% humidity for the whole period of plant growth.

There were total of four factorial treatments, plants well-watered (WW) or subjected to drought (D) and plants which were with or without vesicular arbuscular mycorrhizal fungi (VAM; non-VAM, respectively). All treatments were replicated four times. Slight nutrient deficiency was apparent in some experiments; therefore all plant compartments (PC) were fertilized at 20, 40 and 60 days after sowing (DAS) with a 0.2 g l^{-1} solution of NH_4NO_3. After each application of fertilizer solution, the volumes of irrigation water required to achieve 23% gravimetric soil water content were assessed by daily gravimetric weighing (data not shown) and necessary amounts of water were added every two days. For well-watered treatments, the water loss was replaced by adding tap water if necessary during the experiment. Drought treatment consisted of withholding irrigation to plants starting the 21st day after sowing. The water was withheld for one week (one drying cycle), which reduced the gravimetric soil water content in the plant compartment to around 10-12% at the end of each drying cycle. After each drying cycle plants were watered once to bring the gravimetric soil water to 23%. To prevent algal growth and surface evaporation, and to ensure gradual depletion of substrate moisture, both plant and hyphae chambers were covered with sand and nylons.

3.3.4 Parameters measured

Plant morphological and physiological parameters

Plants were grown in plexiglass chambers (split-root-hyphse system) for 3 weeks in about 23% gravimetric soil water content. Throughout the experiment, water loss from the chambers was determined daily by weighing the chambers and it was replenished by adding deionised water to the chambers to bring the soil water content to the original value of 23%. After 3 weeks of growth, plants were subjected to water stress by withholding water for a period of seven days after which plants were watered and the soil water content was re-established at 23%.

At weekly intervals, plant height and number of tillers in each chamber were measured and its average per plant was calculated. Plants were harvested 90 days after sowing and shoot and root fresh weights were determined immediately after harvesting and the dry weight of the aboveground biomass and roots was determined after plant parts were dried at 65 ^{o}C for 48 hours. The soil gravimetric water content in hyphae compartment for all treatments was determined after harvesting the plants.

For the physiological studies, the youngest, fully expanded leaf was used. All measurements were done on at least three leaves from different plants.

Water relations: leaf water potential (ψ_w) was determined using a Scholander bomb (Scholander et al., 1964), also the relative leaf water content (RWC) was ascertained by measuring the fresh weight, rehydrated weight on distilled water and dry weight (80 ^{o}C for 2 days) and the RWC was determined by using the formula (Turner, 1986):

$$RWC = (FW\text{-}DW / TW\text{-}DW) \times 100$$

Where FW is fresh weight, DW is leaf dry weight and TW is leaf turgid weight. One leaf was also cut, sealed in nylon bag, and immediately frozen in deep freezer at –20 ^{o}C. Osmotic potential (ψ_s) was measured with an osmometer (VAPROtm Model 5520, Wescor Inc. Germany). Leaf net photosynthesis rate and stomatal conductance were measured with a porometer (Lci Console ADC Bioscientific Limited, England).

Determination of the length of hyphae in compartments

A simple inserted membrane technique (IMT) for sampling mycorrhizal extra radical mycelium (ERM) was used in both compartments. Three halves of cellulose acetate/ cellulose nitrate membrane filter (MF-Millipore, Millipore Corporation, 47 mm diameter, 0.45 ìm pore size) wetted with deionised water were installed vertically into the soil in both hyphae and plant compartments at the beginning of the experiment. Membrane filters were placed into zone where either the mycorrhizosphere or the hyphosphere were predicated to develop. After plants were harvested at day 90 after sowing, the membranes with adhering hyphae were carefully removed and gently washed with deionised water. All membranes were then placed into small petri dishes (50 mm diameter), flooded with 10 ml of a solution of trypan blue (Balaz and Vosatka, 2001).

Membrane slides were used to obtain hyphae images. Using the Digitales Farbsystem D x 30 Kamerasteuerung and KAPPA Image Base Software enabled us to determine the total hyphae length per $1mm^{-2}$-membrane area (HEDWIG PFARRHERR, Vertrieb Mikroskope-Zubehör-System - Germany). Fully 50 random images were obtained from each slide at the magnification x100. Total hyphae length was measured from all images with 4 replications using a software program (rhizotron root measurement software program, WinRHIZO Tron, Regent Instruments Inc. Made in Canada, www. Regentinstruments.com).

Root colonisation studies

Colonization of roots by VAM fungi was determined by clearing washed roots in 10% KOH and staining the preparation with 0.05% (vol. / vol.) trypan blue in lactophenol as described by Phillips and Hayman (1979). Present mycorrhizal colonization was determined by sampling 25 1-cm root segments from each of seven plants (n =175) and determining the percentage that contained mycorrhizal fungus arbuscules, vesicles and hyphae. The extent of mycorrhizal colonization was calculated according to the quantification method of Furlan and Fortin (1977).

Instantaneous measurements of leaf elongation rate (LER) and leaf length

Instantaneous measurements of leaf growth were made by linear variable differential transformers (LVDT) when leaf 5 of the main stem was 1-2 cm long (about one day after leaf emergence) in all treatments. The tip of the leaf was connected with the LVDT by a fishing line (0.22 mm diameter), which was attached to the leaf tip using a small clamp cushioned with mounting rubber to avoid damaging the leaf. The force on the fishing line was 10g to eliminate oscillations in the LVTD output resulting from slippage and friction in the measurement system. This force did not affect leaf elongation rates during measurements. A reading was taken from each transducer at 30 min time interval. Over this period of 30 min, six values were averaged and this single value was stored by a logger (Delta-T Device, Cambridge, UK). The measurements of leaf elongation rate were made for eight days. Leaf elongation rate was calculated by dividing the increase in length by the time interval. All measurements of the leaf elongation rate were performed with four replications. Total leaf length was calculated as the integral of LER. Once the measurement for each leaf was finished, a ruler also recorded the final leaf length and results were compared with those from the LVTD method.

Determination of biomass and Shoot P and K status

At the end of each experiment, harvested shoots and roots were oven-dried at 70 ^{o}C for at least 48 h and dry masses determined. The dried samples were powdered using a Wiley mill and analysed for P and K. For the determination of P, dried tissues (300 mg) were digested in $HClO_3$-H_2O_2 (v/v 5.3: 3.5) mixture for 45 min in a microwave (MDS-2100 W/T. C., Matthews, North Carolina 28106, CEM, USA). The digested samples were diluted to 50 ml with distilled water and P content was determined using spectronin

(501-Mizton Roy Company, Unterfoehring, Germany). Shoot K status was determined with Flame photometer (Eppendorf, ELEX 6361-Eppendorf-Nethele, Hinz GmbH 22331-Hamburg-Germany) using the same extracts as for P measurement. The nutrient content was calculated by multiplying the mineral concentrations by the dry masses of shoots.

3.4 Results

3.4.1 Gravimetric soil water content in plant/hyphae compartments

The amount of gravimetric soil water in the plant compartment during a period of eleven drying cycles is shown in Figure 9. Ninety days after the start of experiments, the gravimetric soil water content in the hyphae compartments was determined by taking soil samples from different parts of that compartment. The result is shown in Figure 10. It was observed that under drought condition, the amount of the initial gravimetric soil water content in the hyphae compartment of VAM plants was lower by only 2-4 % than in the similar compartment of Non-VAM plants. This 2-4% soil water in the chambers corresponds to 37-74 ml of water during 90 days growth. If we assume that all of this water loss was due to the transport of water from the hyphae compartment to the plant compartment, this means that during the entire 60 days of experiment and on the average, plants in association with VAM received only 25-75 ml more water than the corresponding non-VAM plants. If we consider than on the average, each hyphae compartment received a total of 430 ml of water at the beginning the entire experiment (to obtained 23% gravimetric soil water content), the 25-75 ml excess water corresponds to 2-4 % more water during the 60 days of growth.

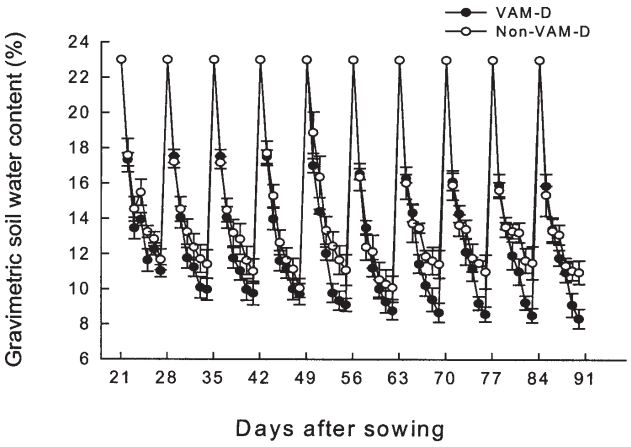

Figure 9. Gravimetric soil water content of plant compartments in drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; non-VAM) (Error bars represent standard deviation).

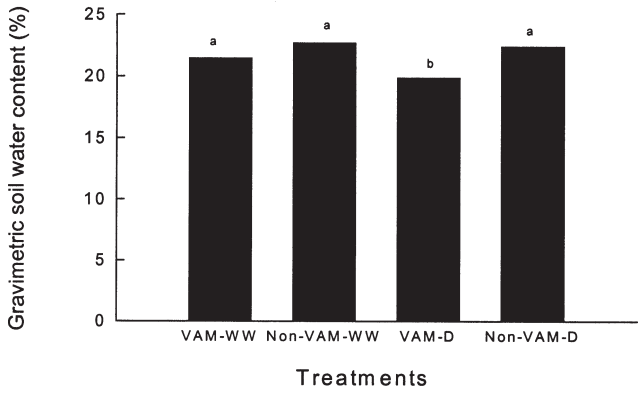

Figure 10. Gravimetric soil water content in hyphae compartments in well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) (Different letters indicate significant differences).

3.4.2 Extent of root colonization by mycorrhizae

Roots of VAM plants were well infected by mycorrhizal fungus as shown by the presence of intraradical hyphae in the stained roots (Photo 1). The percentage of total root colonization by

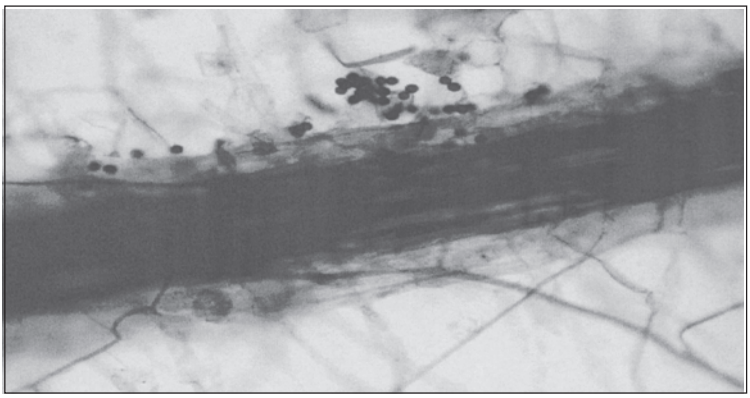

Photo 1. Intraradical hyphae in stained roots of VAM host plant.

mycorrhizal fungus was slightly higher in plants subjected to drought (43 %) as compared with those, which were well-watered (38%).

3.4.3 Contribution of hyphae to root water uptake and total hyphae length

Under drought condition, leaves of VAM plants had significantly ($P < 0.05$) higher relative water content (RWC) as compared with those of non-VAM plants (Figure 11). Under well-watered condition, however, there were no significant differences between the RWC in the leaves of VAM and non-VAM plants.

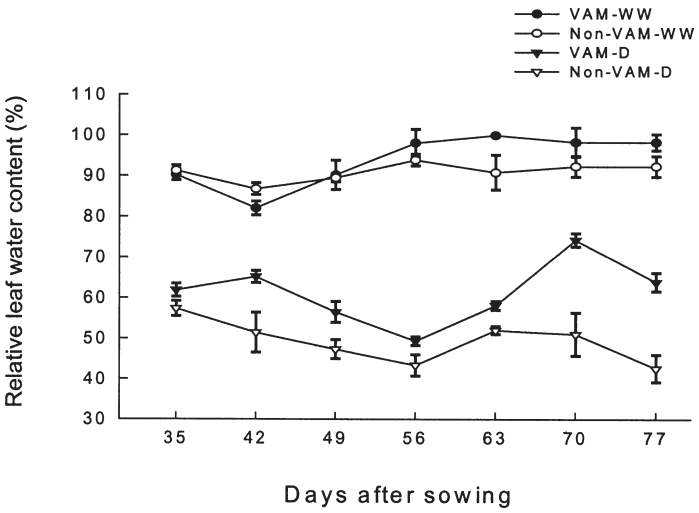

Figure 11. Relative leaf water content in the well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) (Error bars represent standard deviation).

Leaf water potential in plants with or without VAM under well-watered and drought conditions is shown in Figure 12. Irrespective of mycorrhizal status of plans, water potential in leaves of well watered plants was higher (less negative) under well-watered as compared with plants grown under drought conditions. However, under drought condition and particularly

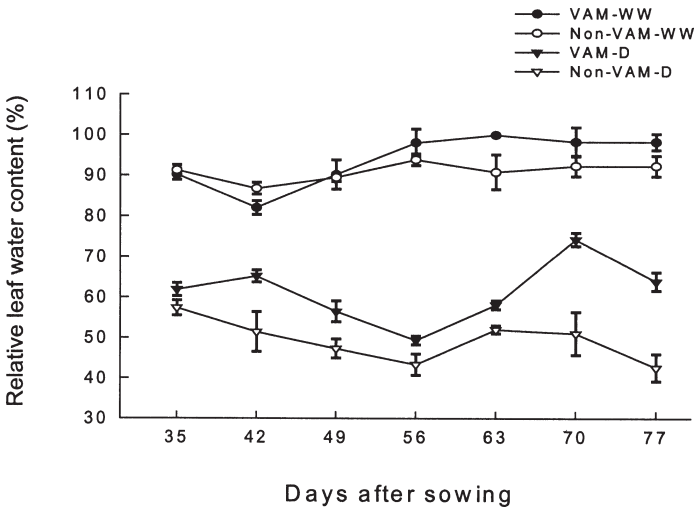

Figure 12. Leaf water potential in the well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM)(Error bars represent standard deviation).

During the last four drying cycles, leaves of VAM plants showed significantly higher water potential than the leaves of non-VAM plants. Results of leaf osmotic potential and turgor pressure are illustrated in Figures 13 and 14. Turgor values of drought stressed VAM plants indicated a positive pressure slightly above zero in VAM plants particularly in the last weeks of growth period as compared with non-VAM plant. There were significant differences observed in leaf osmotic potential and turgor pressure in well-watered VAM and non-VAM plants. Total length of hyphae, measured in both plant and hyphae compartments at the end of experiment, showed that there were significantly more extraradical hyphae under drought conditions than under well-watered conditions. Total length of extraradical hyphae in the hyphae compartment, for example, were 5.24 and 1.14 mm mm^{-2} under drought and well-watered conditions, respectively (Figure 15).

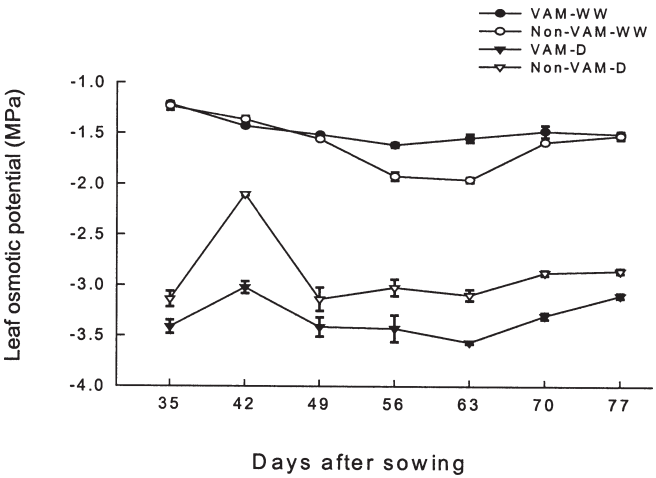

Figure 13. Leaf osmotic potential of well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) (Error bars represent standard deviations).

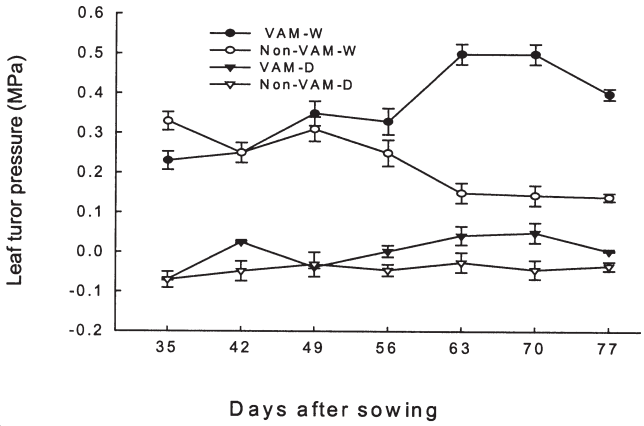

Figure 14. Leaf turgor pressure of well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) (Error bars represent standard deviations).

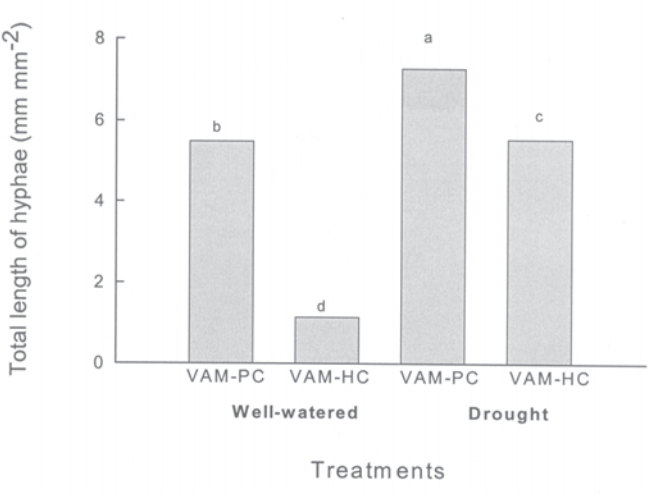

Figure 15. Total length of hyphae on both plant compartment (PC) and hyphae compartment (HC) of well-watered and drought stressed plants with vesicular arbuscular mycorrhizal fungi (VAM).

- **Bars followed by the different letters are significantly different by ANOVA and LSD multiple range test (*P < 0.05*).**

3.4.4 Biomass

Under drought condition, VAM plants exhibited significantly higher number of tillers and shoot dry weight than non-VAM plants (Table 4). Number of spikes per plant, which is an important yield component, was significantly higher in VAM than non-VAM plants under drought conditions but not under well-watered condition (Table 4). Under well-watered condition, however, there were no significant differences in biomass or yield components in VAM and non-VAM plants. (Table 4).

Table 4. Effects of vesicular arbuscular mycorrhizal fungi on water relations parameters of plants with or without vesicular arbuscular mycorrhizal fungi (VAM; non-VAM) subjected to well-watered (WW) and drought conditions (D).

- **Means within each row followed by different letters are significantly different at (P < 0.05) according to LSD multiple range test.**
- **Means within each row followed by same letters are not significantly different at (P < 0.05) according to LSD multiple range test.**

	Well-watered		Drought	
	VAM	Non-VAM	VAM	Non-VAM
Shoot height (cm)	54.21 a	56.81 a	33.8 b	32.1b
Number of tiller (per plant)	8.1 c	8.8 c	16.7 a	11.8 b
Shoot dry weight (g plant $^{-1}$)	2.91 a	2.98 a	1.98 b	1.03 c
Root dry weight (g plant $^{-1}$)	3.39 a	3.32 a	1.88 b	1.56 b
Number of spike (plant $^{-1}$)	7.27 a	7.77 a	2.88 b	0.22 c

3.4.5 Leaf elongation rate (LER) and total leaf length under well-watered and drought conditions

Leaf elongation rate and total leaf length are shown in Figures 16, 17 and 18. In all four treatments, plants exhibited similar pattern of leaf elongation rate during their growth. Leaf elongation rate remained steady for a few days before decreasing in all treatments. With the exception of the first day after emergence, LER under drought condition was considerably higher in VAM plants as compared with those of non-VAM plants.

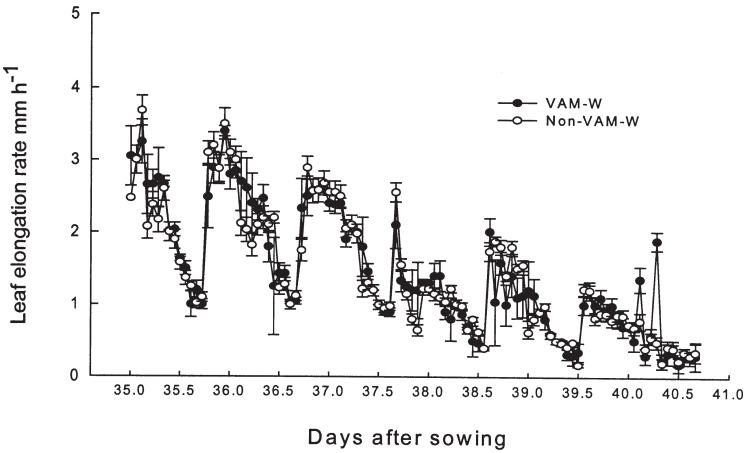

Figure 16. Leaf elongation rate of leaf 5 of the mainstem of well-watered (W) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM).

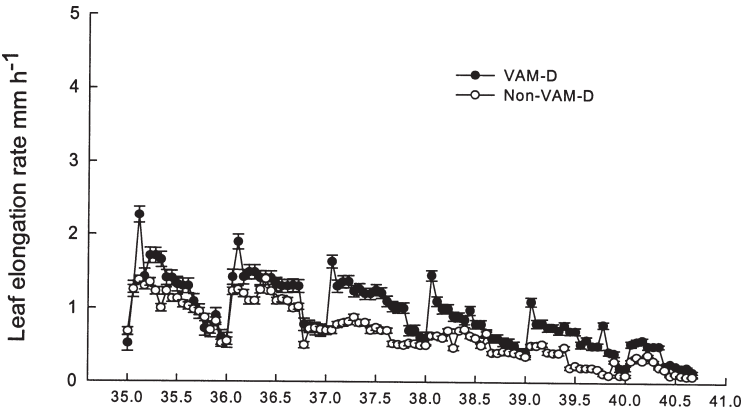

Figure 17. Leaf elongation rate of 5 of the mainstem of drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM).

For example, 40 days after sowing, LER at 12:30 o'clock was 1.27 and 0.58 mm h^{-1} in drought stressed VAM and non-VAM plants, respectively (Figure 16). As a result, under drought conditions leaves of VAM plants were significantly longer than non-VAM plants. Under well-watered condition, however, there was no significant difference between the leaf length of VAM and non-VAM plants (Figure 16).

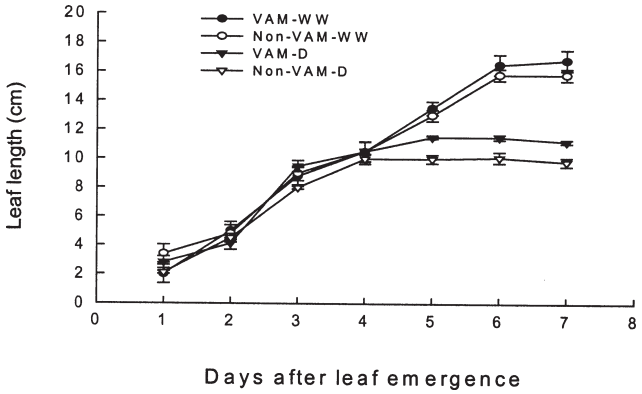

Figure 18. Leaf length of leaf 5 of the mainstem of well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) (Error bars represent standard deviation).

3.4.6 Effects of VAM on stomatal conductance and photosynthesis rate

Effects of VAM on leaf stomatal conductance $g_{(s)}$ and leaf net photosynthesis rate (A) under well-watered and drought conditions were measured at the end of each drying cycle when plants subjected to drought were under maximum water stress. Under drought condition, stomatal conductance and leaf net photosynthesis rate were both significantly higher in VAM than in non-VAM plants (Figures 19 and 20). Under well-watered conditions, stomatal conductance was significantly higher in VAM plants as compared with non-VAM only during the early part of the experiment when plants were young. This difference, however, disappeared, as the plant got older. There were no significant differences between the net photosynthesis rate of well-watered plants with or without VAM and non-VAM (Figure 20).

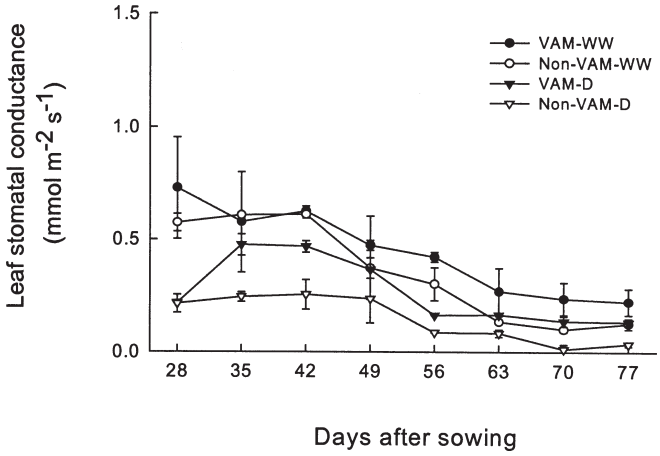

Figure 19. Leaf stomatal conductance of well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) (Error bars represent standard deviations).

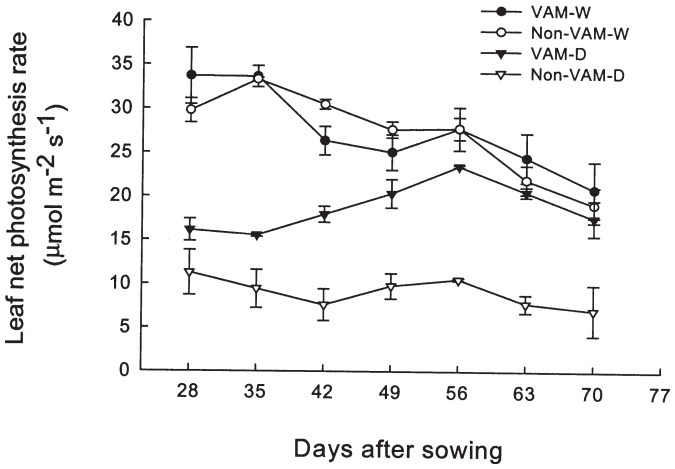

Figure 20. Leaf net photosynthesis rate of well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) (Error bars represent standard deviation).

3.4.7 Shoot nutrient status

Although plants subjected to well-watered conditions had higher concentrations of both P and K in their shoots than those, which were drought stressed, there were no effects of VAM on the concentrations of P under well-watered or under drought conditions but shoot K content was slightly higher in drought stressed VAM plants as compared with non-VAM plants (Figures 21 and 22).

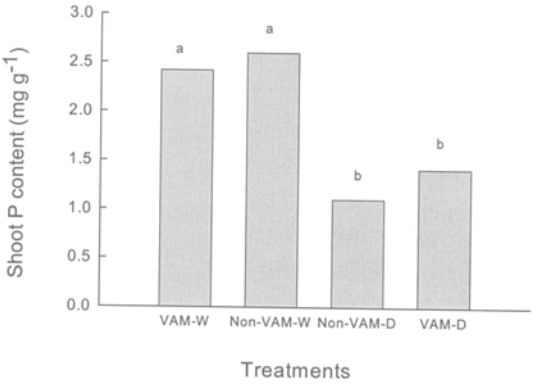

Figure 21. Shoot P content of well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM).

- **Bars followed by same letters are not significantly different by ANOVA and LSD multiple range test (*P < 0.05*).**
- **Bars followed by the different letters are significantly different by ANOVA and LSD multiple range test (*P < 0.05*).**

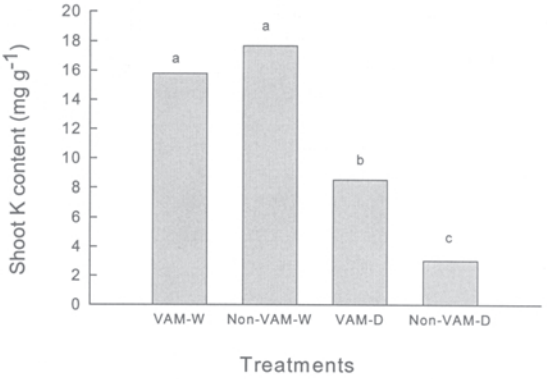

Figure 22. Shoot K content of well-watered (W) and drought stressed (D) plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM).

- **Bars followed by same letters are not significantly different by ANOVA and LSD multiple range test (*P < 0.05*).**
- **Bars followed by the different letters are significantly different by ANOVA and LSD multiple range test (*P < 0.05*).**

3.5 Discussion

Mycorrhizal fungi are known to influence water uptake and water use efficiency in host plants (Allen, 1982). Vesicular arbuscular mycorrhizal (VAM) symbiosis has been shown to increase plant tolerance to water deficit although the exact mechanisms involved are still not very clear (Auge, 2001; Ruiz-Lozano, 2003). In this experiment, we investigated the contribution of external hyphae of *G. intraradices* on some morphological and physiological aspects in barley (*Hordeum vulragis v*. Scarlett), which are believed to be sensitive (responsive) to plant water relations under drought conditions. The results showed that in plants subjected to drought, at the end of ten drought cycles, the gravimetric soil water content in the hyphae compartment in VAM plants was lower by 2-4% than that in the corresponding compartment in non-VAM plants. This is attributed to the water taken up from this compartment by the mycorrhizal hyphae, which had crossed the air gap and entered the hyphae compartment from the root compartment.

Drought stress significantly reduced shoots and root dry weight of both VAM and non-VAM plants at the end of the drying cycles (90 days after sowing). However, higher number of tillers and shoot dry weights observed in drought stressed plants with VAM symbiosis than that in non-VAM plants confirms the idea that VAM may improve drought tolerance in the plants subjected to water deficiency. This is in agreement with other results obtained by others (Ruiz-Lozano and Azcon, 1995). Our results also showed that under well-watered conditions, there was no influence of VAM on shoot and root dry weights. This is in contrast to some reports (Faber et al., 1990; Ruiz-Lozano et al., 1995) but in agreement with the results of Davies et al., (1992) who also found no significant difference between shoot and roots dry weights in plants with or without VAM under well-watered conditions.

In this study, VAM altered water relations in barley plants subjected to drought but not in well-watered plants. These results suggests that under limited water supply (drought) association (symbiosis) of barley plants with VAM may improve water relations in plants by increased water uptake through external hyphae. This effect is, however, inconsequential to plant water relationship under adequate water supply (well-watered conditions). Under well-watered conditions, the leaf water potential was relative high (-1.7 MPa) in VAM plants. Drought stressed VAM plants consistently showed higher relative leaf water content during the experiment than non-VAM plants. Thus, if we consider that the extra amount of water transported to the host plant by the VAM hyphae from the hyphae compartment was very small (37-75 ml), the observed improved plant growth and water potential under drought condition in VAM plants leads us to suspect that these effects may have been due to some unknown influence of VAM on the physiological parameters of host plant other than their mere effect on increased water uptake. This means that VAM may have improved plant water relations in a way independent of water uptake. In our experiments, as the soil was subjected to drought and gravimetric soil water content decreased, water potential and stomatal conductance in

both drought stressed VAM and non-VAM barley plants declined, a condition which should have affected photosynthesis rate in both VAM and non-VAM plants by somewhat similar extents. However, the results showed that photosynthesis rates in the leaves of well-watered VAM and non-VAM plants were very similar, while there were considerable differences between photosynthesis rates in VAM and drought stressed non-VAM plants. This suggests that under well-watered conditions photosynthetic process in barley plants may not benefit from the presence of VAM while under drought conditions, it does.

The extent of outward spread of the extraradical VAM hyphae from the root surface depends on the fungal species, environmental conditions (van Bruggen et al., 2000; Smith et al., 2000) and soil phosphorus concentration (Abbott et al., 1984; Abbott and Robson, 1985). The results of mycorrhizal experiments conducted with well-watered plants, may not reveal (conceal) some effects of symbiosis of VAM on plants probably because all plants had received adequate nutrients from the nutrient solutions applied (Fitter, 1988; Sylvia et al., 1986; Subramanian and Charest, 1997; Busse, 1984). Furthermore association of VAM with host plants under well-watered conditions are often more to the benefit of the fungi which receives metabolites from the host and is in essence in a parasitic association with the host (Johnson et al., 1997; Busse, 1984). Subramanian et al., (1997) reported that improved nutritional status may assist VAM plants to exploit available soil moisture and maintain higher leaf RWC and consequently, higher leaf stomatal conductance, net photosynthesis rate and turgor pressure under moderate drought conditions. Our results, however, showed that improved water status of plants due to the presence of mycorrhizal fungi during drought situations was independent of host plant's P status. This is in agreement with the results of some studies (Davies et al., 1993; Azcon and Tobar, 1998). Concentrations of K in plants shoots were significantly higher in drought stressed VAM plants as compared with the non-VAM plants. This suggests that the observed improvement in the leaves osmotic adjustment and consequently higher leaf turgor pressure in the VAM plants might have been due to higher content of the osmotically active nutrients such as K (Auge and Stodola, 1990; Frey and Schüepp, 1992).

Our results also showed that mycorrhizal association increased the length of leaves. Leaf growth and leaf elongation rate has been attributed directly to leaf turgor pressure or indirectly to the leaf osmotic potential. The observed increase in water uptake and the improved nutrient status of VAM plants may have altered leaf elongation rate, a process that requires positive leaf turgor pressure (Tang and Boyer, 2003). Auge (1995) demonstrated increased leaf length and leaf elongation rate in VAM-colonized wheat, which they attributed to enhanced water uptake by the hyphae under drought conditions. These results are in agreement with other reports (Auge et al., 1986a). In our experiments, despite the eleven drying cycles imposed on plants, leaf RWC, stomatal conductance, net photosynthesis rate, turgor pressures all remained higher in drought stressed VAM plants than in non-VAM plants. The very small volume of water taken up by the hyphae of

VAM plants, leads to conclude that the effects observed were either due to 1) increased root or leaf hydraulic conductivity which in turn caused higher stomatal conductance and leaf net photosynthesis rate in VAM plants than in non-VAM plants under drought conditions, or 2) some unknown mechanisms.

3.6 Conclusions

Using a split-root-hyphae system, we have observed that the external mycelium of VAM fungi (*Glomus interaradices*) may improve leaf elongation rate and total leaf length of barley. Association of VAM with barley increased the uptake of water and translocation of K to the plants, especially under water-deficit conditions. Our data suggest a positive relationship between hyphal contribution to water and nutrient uptake and leaf water relations and leaf growth (length), even if the improved water uptake was only 2-4%. It is conceivable the VAM assisted the plants to withstand drought conditions, by for example facilitating direct water uptake and transport through their hyphae to the roots (Hardie, 1985; Faber et al., 1991).

The plasticity of the extraradices mycelium might be an important strategy for adaptation and survival in a diverse range of ecosystems. Although the experimental system used is somewhat artificial, our results seem to reflect accurately the morphogenetic processes known to occur in VAM fungi when growing in soil. The results of well-watered plants showed somehow parasitism activities of mycorrhizal fungus for host plants. In fact, a small growth depression in terms of total dry weight root was recorded in VAM barely probably due to competition for photosynthesis between host and fungus (Abbott and Robson, 1985).

3.7 References

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4 Dynamics of water uptake by hyphae and roots of mycorrhizal barley under drought conditions as measured with capacitance sensors

4.1 Abstract

Effect of VAM on the transport of water to barley roots from a physically separate soil compartment (hyphae compartments) was measured by means of capacitance sensors. Our objective in this study was to investigate the dynamics of water uptake by extraradical hyphae in hyphae compartment and by roots of mycorrhizal barley under drought conditions. With our approach, the sensors were positioned within each of plant and hyphae compartments. Drought stress was applied with totally seven drying cycles in this experiment. Soil water content was instantaneously recording over 10 minutes. Results indicated a decrease in soil water content in the hyphae compartments of plants colonized by vesicular arbuscular mycorrhize (VAM) when compared to the corresponding values in the compartment of control treatment (non-VAM plants). This was taken as evidence for the uptake of water by the extraradical hyphae and its transfer to the host plants in the adjacent, but physically unattached, plant compartment (PC). Measurements showed that under drought condition, VAM hyphae transported 5-7% of the soil water from the hyphae compartment to the plant compartment as compared with non-VAM plants. The data also showed indications for a reverse mass transport of water from the plant compartment (when plants were periodically watered) to the hyphae compartment by the hyphae strands connecting the two compartments.

4.2 Introduction

Soil water content is a key factor in plant growth and production agriculture. Soil water content influences the fates of several nutrients applied to soils and impacts crop growth directly. Accurate estimation of soil water content is therefore very important and has been extensively studied. Basically there are three methods available to measure soil water content under field conditions, i. e. 1) gravimetric techniques, 2) nuclear technique (e.g., neutron scattering), and 3) electromagnetic techniques. Of these, electromagnetic techniques have become popular because they allow a rapid, safe, non-destructive, and easily automated estimation of soil water content.

Soil water content can be evaluated by measuring the dielectric ε_s of soil because of the large difference between the dielectric constant of water ($\varepsilon_w \approx 80$) and that of dry soil ($\varepsilon_D \approx$ 2-5) (Thomas, 1966). Two measuring principles were introduced for the ε_s determination. Among the electromagnetic techniques; time domain reflectometry (TDR) is the most common method (Fellner-Felldeg, 1969; Topp et al., 1980; Baker and Allmaras, 1990; Heimovaara, 1994; Noborio, 2001). However, the emergence of high quality, low-cost high frequency oscillators has led to increased interest in capacitance sensors techniques (e.g., Dean et al., 1987; Evett and Steiner, 1995). Time domain reflectometry

(TDR) is based on velocity measurements of pulses on a transmission line (Fellner-Feldegg, 1969; Wobscall, 1978; Topp et al., 1982), and it has found widespread application. Increasing å of the material between and around the electrodes reduces the pulse velocity and modifies the reflection at the end of the line (Dasberg and Dalton, 1985). The tip of coaxial structure contacts the soil surface and the amplitude and phase of the reflected signal provide information about the soil moisture (Brisco et al., 1992). Another method utilizes a serpentine-like TDR probe placed on the soil surface (Selker et al., 1993). Comparative measurements with the gravimetrically determined volumetric water content as control show typical RMS (root mean square) errors between 0.01 and 0.05 for the TDR method (Topp et al., 1982; Amato and Ritchie, 1995; Kelly et al., 1995). High frequencies oscillators, however, have the advantage of increasing the accuracy of the soil water measurements and minimizing the influence of the soil type on the signal (Wobschall, 1978). Gradner (1991) mentioned the high initial cost of a TDR device as a disadvantage. The smallest practical TDR probe has a length of 2.1 cm and a rod separation of 1.4 cm (Amato and Ritchie, 1995). Difficulties arise from the short travel times in the pulses and the electronics. Therefore, the accuracy decreases with a smaller length (Kelly et al., 1995) and measurements on dry soil become less accurate because the propagation velocity is maximum in this case (Amato and Ritchie, 1995). Time-domain reflectometry probes are often pushed vertically into the soil (Topp et al., 1982; Dalton et al., 1984; Zegelin et al., 1989). This generates an artificial connection between the different layers of the soil and it facilitates the water flow into deeper layers. In this way, the natural conditions are destroyed. Capacitance probes are relatively inexpensive and easy to operate. Furthermore, the sensor geometry is very adaptable, facilitating the development of a variety of configurations (Robinson et al., 1993). However, capacitance probes are influenced by soil type and require calibration. Also, there is concern about the influence of soil salinity and soil temperature on capacitance sensors. The dependence of the dielectric constant $å_s$ of the soil on the volumetric water content è can be described empirically by third-order polynomials as a fit function that can be applied to all soils (Topp et al., 1980). The objective of this study was to investigate the dynamics of water uptake by hyphae in hyphae compartment and by roots of mycorrhizal barley under drought conditions using capacitance sensor technique.

4.3 Materials and Methods

4.3.1 Capacitance sensors

Figure 23 shows a capacitance sensor with a pair of isolated wires as electrodes. As illustrated by the top view in Fig. 23a, the wires are stretched in a frame with the overall dimensions of 7 by 7 cm and an opening of 5 by 5 cm. It is advantageous to produce the frame from a perforated circuit board of water-resistance material (glass fibre epoxy). As all holes have a precise separation of 2.5 mm, the wires are parallel and have this constant separation. The wires are crossed in such a way that each wires section of Electrode 1 is situated between two wires sections of Electrode 2 and vice versa. In this way, the parallel wires in the frame opening have alternate charge. This increases the capacitance slightly compared with a single pair of wires with equal length. A further advantage is the stability of the capacitance due to variations of the wires position. If one considers only two adjacent sections of the wires in the frames, the partial capacitance C_p can be calculated by:

$$C_p = \Pi \epsilon_z \epsilon_o l / \ln (d/r)$$

With the length l of the considered wires section (opening of the frame), the wire radius r, the separation d, and the relative dielectric constant ϵ_z and ϵ_i of the intermediate material and Π the number of Pi (Π=3.14). A displacement of one wire section within the wire plane increases the capacitance if the separation d becomes smaller. However, if one regards the wire of the other side of the displaced wire, the same displacement increases the separation and the capacitance becomes lower. This is a first-order compensation of capacitance variation due to wire displacement. The copper wire has a diameter of $2r$ = 0.54 mm and a total diameter of 1.1 mm, including the polyvinyl chloride (PVC) insulation, with a dielectric constant ϵ_i. Welding the PVC ends of the wire completes the insolation of the wires against water. In this way, the insulation significantly reduces the influence of the conductivity. The measuring method also operates with wires coated by varnish. The frame is slightly bent, as illustrated in an exaggerated manner by the first lateral view in Fig. 23b. This produces a certain mechanical stress on the wires in order to stabilize their position when the sensor is brought into the soil. In addition, it reduces the time fluctuations of the capacitance.

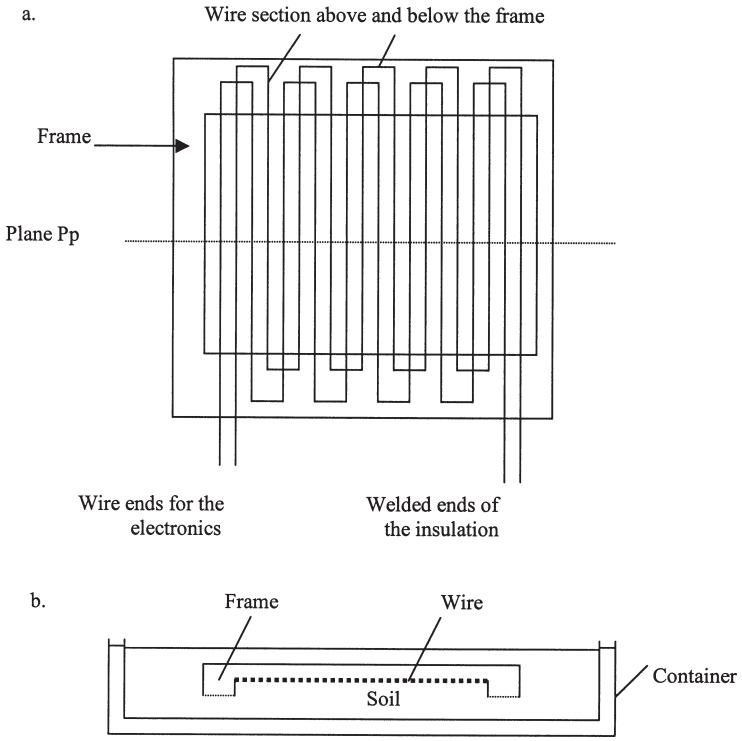

Figure 23. Sensor with a pair of insulated wires as electrodes. (a) wires in the frame are crossed in such a way that each section of Electrode 1 is situated between two sections of Electrode 2 and vice versa. (b) Sensor in the container with soil (Ruth, 1999).

4.3.2 Construction of split-root-hyphae system chamber

Split-root-hyphae system was made with plexiglass and consisted of two compartments: the hyphae compartment (H x L x W: 30 x 19 x 3 cm) and the plant compartment (30 x 19 x 5 cm). Two layers of nylon net with a pore size 30 ìm and an air gap of about 5 mm between the two nylon nets separated root and hyphae compartments. The air gap of 5 mm is believed to be sufficient to prevent water diffusion and mass flow between the plant and hyphae compartments (see Figure 8, chapter 3). In order to avoid water loss by evaporation from the hyphae compartment, the soil surface of the hyphae compartment was covered with a perforated plastic film during the entire experiment.

4.3.3 Plant growth conditions

Soil from horizon (0-15 cm) of a loamy-silt soil belonging to a field of the Research Station-Dürnast, of the Chair of Plant Nutrition, Technical University Munich, was used in this study (see Table 1). The soil was ground, and passed through a 5-mm mesh

screen. Before the soil was filled into both compartments, it was sterilized by autoclaving at 120 °C and 1.3 bar pressure. The initial gravimetric soil water content of the soil (23% on dry soil basis) was achieved by adding distilled water and thoroughly mixing. The soil bulk density was 1.4 g cm^{-3}.

Seeds of a commercial variety of barley (*Hordeum vulgaris* L. Scarlet) were surface sterilized using a 0.5 % NaClO solution for 15 min, then washed three times in sterile water, and germinated in petri dishes. Inoculums were banded 2-3 cm below the surface in plant chambers containers of 4 kg sterilized soil. This amount of inoculums was selected in preliminary tests as the optimum to produce a good colonization level for a total amount of soil in the chamber. The same numbers of chambers were provided without inoculum as control (non-VAM) plants. The number of plants per chamber was reduced to six at seven days after sowing.

Plants were grown in a controlled growth chamber at 14 h photoperiod, PPFD (Photosynthetic Photon Flux Density) of 450-ìmol m^{-2} s^{-1}. The air temperature was 20 / 18 °C (day/night) with 65 % humidity during the germination and later regulated on 15 / 15 °C with 70% humidity for the whole period of plant growth. Drought treatment consisted of withholding irrigation to plants starting the 21st day after sowing. The water was withheld for one week (one drying cycle), which reduced the gravimetric soil water content in the plant compartment to around 8-10% at the end of each drying cycle. After each drying cycle plants were watered once to bring the gravimetric soil water to 23%.

The sensors were positioned within each compartment as follows (Figure 24). Soil was placed into each compartment to 1-cm thickness, a capacitance sensor was placed horizontally on the soil surface and secured for good contact with the soil, and the compartment was filled with more soil to give the bulk density of 1.4 g cm^{-3}. In the four split-root-hyphae chambers, we used a total of eight capacitance sensors connected to a computer to record the signal at 10-minute intervals. Measuring procedure is shown in the following diagram:

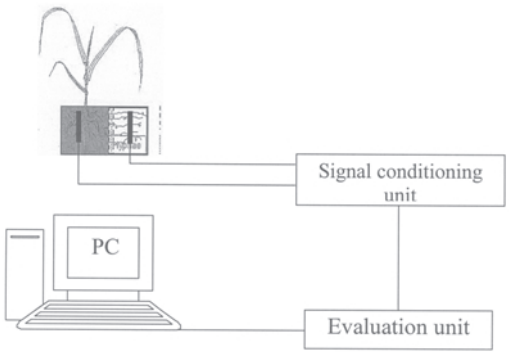

Figure 24. Diagram of the signal processing. Main components of the measuring device.

Because the sensor plane was positioned at exactly mid point between the bottom of the container and the soil surface, and because the soil drying occurs very slowly, the mean water content was used as the actual water content at the location of measurement. This faster way of calibration neglects the nonlinear dependence of water content and depth. Therefore, it must be regarded as a preliminary calibration. The horizontal position of wires and frame in the soil has the advantage that it does not produce an artificial connection (passage way) between soil layers, a situation that is the case if an object is vertically pushed into the soil. We assume that horizontally positioned sensors would not induce a preferential water flow in vertical direction. In addition, the natural vertical flow is only inhibited to a minor extent because the separation of 2.5 mm between the wires with diameter of 1.1 mm leaves enough space for the flow. The measured capacitance at given water content divided by the capacitance of the sensor in air ($\varepsilon = 1$) yields the measured dielectric constant ε_M (Ruth, 1999).

Data conversion

Soil dielectric constant *f* is measured in frequency (MHz), which is converted to capacitance by the following Equations:

$$f\,[\mathrm{MHz}] = \mathrm{f} \times 256/1000000$$

$$C\,[\mathrm{pF}] = [\mathrm{A}/\mathrm{f}\,[\mathrm{MHz}\,] - \mathrm{T}\,] - \mathrm{N}$$

In the above formula *C* is the capacitance and A, T and N are parameters describing the sensor geometry and dielectric constant. Since low or high frequency value gives similar results, one can use each one for the determination of the water content. Both low and high frequencies were used because it may happen that one frequency does not work because of electronic difficulties. Applying the above equations and the parameters allowed us to calculate the soil water content by the program "Theta set 36 file No 58. txt" (Ruth, personal communication).

4.4 Results

4.4.1 Change in the water content in the plant compartments

Measurement of soil water content by gravimetric method in the root compartments of plants whose roots were or were not colonized by vesicular arbuscular mycorrhizae are presented in Figure 25. It was noted that the water content in the drought stressed VAM and non-VAM plants were only slightly different particularly during the last few weeks of plant growth during which water content in compartments with vesicular arbuscular mycorrhizae decreased gradually presumably because plant roots colonized by VAM had a much greater contact surface with the soil. Our data shows that, at the initial and final days of drying cycles, measurements of soil water by the capacitance method could fully describe the soil water status as measured by the gravimetric method (Figures 25 and 26).

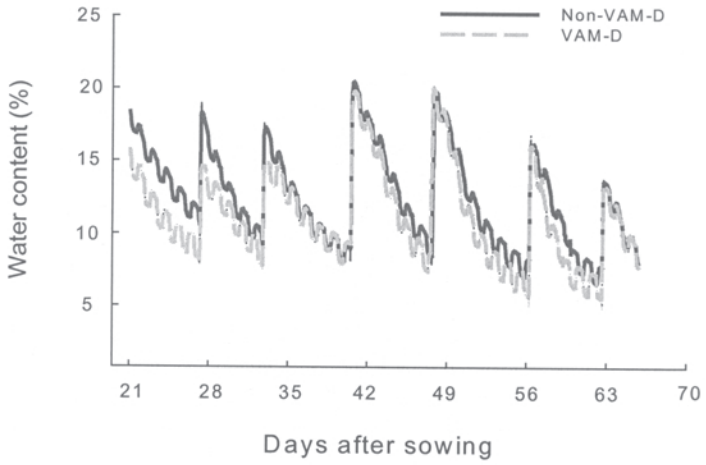

Figure 25. Change in the water content in plant compartments (PC) of drought stressed (D) VAM and non-VAM plants measured by capacitance sensors.

4.4.2 Change in the water content in the hyphae compartments

Gravimetric soil water content in the hyphae compartments of plants with or without vesicular arbuscular mycorrhizae is shown in Figures 26 and 27. A clear difference was observed between the soil water content in hyphae compartments of drought stressed VAM plants as compared with that in non-VAM plants. This difference was noticeable from the time plants were subjected to drought (28 days after sowing). The water content of drought stressed VAM plants decreased throughout the experiment up to the time when plants were harvested. No change, however, was noted in the water content of the corresponding compartment in control

(non-VAM) plants neither on well-watered nor on drought conditions. Slight increases of soil water content in the hyphae compartment of drought stressed VAM and non-VAM plants corresponded to increasing water content in the each initial chambers rewatering at the end of each drying cycle.

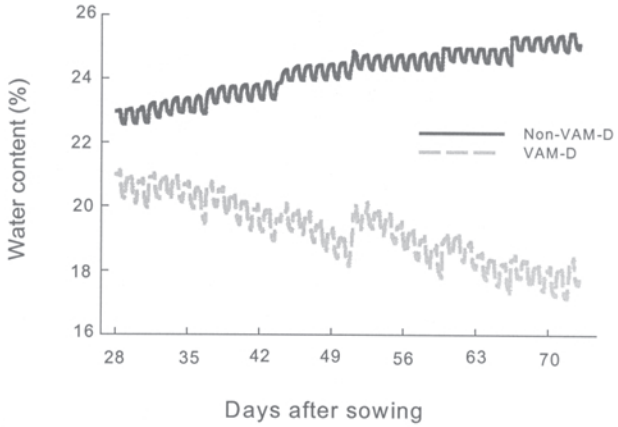

Figure 26. Change in the water content in hyphae compartments (HC) of drought stressed (D) VAM or non-VAM plants measured by capacitance sensors.

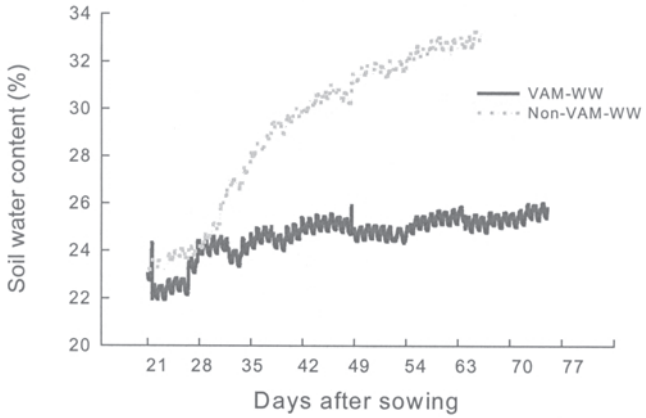

Figure 27. Change in the water content in hyphae compartments (HC) of well-watered (W) VAM or non-VAM plants measured by capacitance sensors.

Gravimetric measurement of soil water content was performed by sampling the soil at different depths in the hyphae compartments (Figure 28). The results were very similar to those obtained from the same compartments using the capacitance sensors. On the average, water content in hyphae compartments of drought stressed VAM plants was 5-7% lower than that in the drought stressed non-VAM compartments. This difference could only be attributed to extraction and transfer of water by the VAM hyphae of from the hyphae compartment to the plant compartment of drought stressed VAM plants.

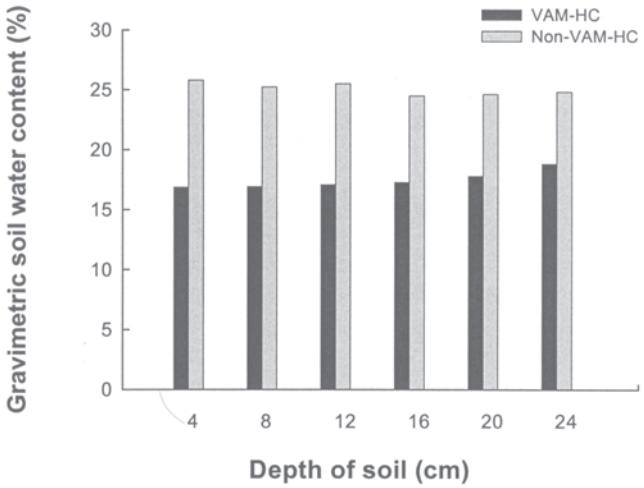

Figure 28. Gravimetric soil water content in the hyphae compartments of drought stressed plants with or without vesicular arbuscular mycorrhizal fungi (VAM; Non-VAM) at 90 days after sowing.

4.5 Discussion

Barley symbiosis with vesicular arbuscular mycorrhizae are illustrated to takeup soil water more thoroughly than non-mycorrhizal barley. One reason for this is the fact the extraradical hyphae of vesicular arbuscular mycorrhizal fungi contribute to water uptake for host plants under drought condition (Auge 2001). With our approach, in this experiment kinetics of water transport from hyphae compartment to plant compartment via extraradical hyphae was monitored using capacitance sensors during drought. Eight capacitance sensors were installed into four plant hyphae compartments of split-root-hyphae system. The capacitance sensors technique is supposed to monitor kinetics of water transferred via hyphae to host plants. The observation showed that a good agreement between the values of water content from the capacitance sensors and values of soil sampling was observed in this evaluation (Figures 26 and 27). Vesicular arbuscular mycorrhizal fungi promoted soil water status at both plant and hyphae compartments when drought stressed VAM plants demand more water in the strict period of water availability. However, with regard to instantaneous measurements of water content with capacitance sensors there is a clear connection between plant behaviours and water status in hyphae compartment. This connection may be due to assemblies of hyphae in both compartments to uptake water as root-like for plant under drought conditions. In our findings, improvement of the water status and leaf net photosynthesis rate of drought stressed VAM plants (see pp 40) as compared with non-VAM plants might be an evidence

for the fact of water uptake by hyphae to contribute to plant growth under water limitation periods. Indeed, our findings in this experiment showed only 5-7% water transferred that most probably can contribute sufficiently to mycorrhizal plants. Consequently, suggesting that this contribution might appear with combination of other aspects of symbiosis impact. The sensors showed difference of water content but negligible during days and night. However, logical and instantaneous recording of values over 10 minutes enables us to following soil water content with about 0.01% during days and nights.

In conclusion, the results of capacitance sensors are in agreement with values of gravimetric soil water content of drought stressed VAM and non-VAM. This amount of water corresponding to water uptake by extraradical mycelium from hyphae compartment contributes to plant under drought conditions. However, other mechanisms must be involved in improving plant growth by hyphae under drought conditions because only small amounts of water were transferred by hyphae.

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5 General discussion

5.1 Differential effects of two vesicular arbuscular mycorrhizal fungi on growth of well-watered and drought stressed barley

In the first comparative and relative basic greenhouse experiment we have investigated effects of two species of VAM on well-watered and drought stressed barley. Similarly, root/ shoot ratio was not affected seriously by root mycorrhization under drought condition. Consequently, no significantly different influence on biomass (shoot and root dry weight) regardless of the species of VAM under drought conditions indicates that the cost of colonization may be greater than beneficial effects in the host plant. However, rather late affection of root mycorrhization by *Glomus intraradices* on leaf water potential of drought stressed barley as compared to root mycorrhized by *Glomus moseae* or non-root mycorrhizated barley has convinced us to consider some unknown effects of VAM. Although, positive correlation between improvement of water potential and root mycorrhization rate in host plant indicates improving of water relations of drought stressed VAM barley.

The results of these studies indicate the potential importance of mycorrhizal infection in the assessment of P efficiency in barley under drought conditions.

High phosphorus uptake strongly reduced the extent of infection of barley roots by the mycorrhizal fungus, *Glomus mosseae* and *Glomus intraradices* in this study. In some studies the significant interactions between cultivars and P addition, and between mycorrhiza and P addition were observed for shoot dry weight but not for root dry weight (Baon et al., 1993).

However, efficiency in utilization of P by barley was negatively correlated with the infection. The colonization of cereals by the indigenous mycorrhizal fungi decreased with the addition of P to the soils.

5.2 Quantification of water uptake by extraradical hyphae of VAM

Experimental determination of water transport by hyphae is difficult because of water and nutrient transfer by other processes in the soil such as solution diffusion and mass flow (Johansen et al., 1992; Frey and Schüepp, 1993). Split-root-hyphae system was constructed to determine the contribution of mycorrhizal hyphae to water uptake and transport to drought stressed plants. Nylon nets with 30-ìm pores was suggested to use for water uptake in these mycorrhizal research studies because this type of nylon is permeable to mycorrhizal hyphae but not to roots. With our approach, two different methods have been applied to determine water uptake by mycorrhizal fungi from hyphae compartment to plant compartments via extraradical hyphae. In the first experiment, using soil sample from several depth of soil in the hyphae compartments indicates water uptake by external mycelium. The results illustrated about 2-4% difference in soil water content of drought stressed VAM plants as compared to that of non-VAM plants, indicate

that extraradical hyphae transferred water from the hyphae compartment to the plant compartment and that hyphae connections grew from plant compartment to hyphae compartment, forming hyphal bridges, which can be simple or may branch as a root. Therefore, existence of external mycelium on both compartments in fact achieved a mycelium-way network for mycorrhizal fungi to deliver water from hyphae compartment to plant compartment in order to contribute to plants under drought conditions. Runner hyphae forming external loops along the surface of the root also initiate secondary colonization (Cox and Sanders, 1974; Brundertt et al., 1985; Friese and Allen, 1991; Wilson and Tommerup, 1992).

In the second experiment to use capacitance sensor technique, the kinetics of water transfer either in plant compartments to monitor root water uptake or the kinetics of water transfer via hyphae from hyphae compartment to plant compartment was investigated. This method enables us to determine soil water content of both compartments. Fungi contributed to drought stressed VAM plants about by 5-7% of the total water supply, which was simlilar to that detected in the first experiment.

Using capacitance sensors in the split-root-hyphae system allowed us to investigate and follow the kinetics of water movement from hyphae compartment to plant compartment via extraradical hyphae. Meanwhile, crossings and vapor transport of water from plant compartment to hyphae compartment resulted in a rather difficult situation to assemble and investigate soil water content data in both hyphae compartments of drought stressed VAM and non-VAM. Regardless of increasing the soil water content on both drought stressed VAM and non-VAM due to rewatering the plant compartments to obtain initial soil water content, the results of capacitance sensors are in agreement with soil sampling results according to the gravimetric soil water contents in the drought stressed VAM and non-VAM barley. However, promoting of water transfer by mycorrhizae network during 90 days plant growth indicates a significant water uptake by extraradical hyphae under drought conditions. This water transfer appeared mostly to have occurred during the intermediate and late periods of drying cycles.

It seems that such a small amount of water in this study might benefit to permit survival under drought conditions. Nevertheless, improvement of water relations and in particular photosynthesis of drought stressed VAM might be convincible to deal with the contribution of mycorrhizal fungi to drought stressed VAM. However, other unknown mechanisms could be involved to contribute to the water relations of drought stressed VAM plants.

5.3 Effects of VAM on barley growth and the significance in plant water relations and photosynthesis with split-root-hyphae system under drought conditions

VAM effects on plant water relations during drought conditions have been associated with morphological effects. Drought stressed vesicular arbuscular mycorrhizal barley had a different morphological feature such as number of tillers, shoot height and leaf

area. Effects of VAM on morphological properties and biomass of VAM plants have demonstrated a higher number of tillers, shoot height and leaf area in drought stressed VAM plants as compared to non-VAM. Biomass production was the other parameter, which took an important place through measurements. Drought stressed VAM barley had higher biomass. Although, the effects of VAM on drought stressed VAM and non-VAM barley was not different in the greenhouse experiment. Consequently, even in VAM and non-VAM plants having similar shoot dry weight and leaf areas, this finding indicates that a relatively larger, more finely separated or more efficient root system improves soil water availability and increases leaf water content. Water uptake by split-root-hyphae system is determined by the amount of roots, the distribution of roots, and the rate of absorption per unit root. When total biomass is even similar in VAM and non-VAM root systems, differences in root distribution rates or specific water uptake rate may result in differing rates of water absorption VAM colonization can change root length, root architecture and root/ shoot ratio (e. g. Berta et al., 1993; Espeleta et al., 1999).

Drying cycles of plant compartments exhibited similar patterns in all experiments (Figures 1, 9 and 25). The results showed that VAM plants root systems could dry the soil faster than non-VAM root systems. This might be because the shoots of the VAM plants were larger (more evapotranspirational leaf surface area) or the root systems of VAM plants were larger or more finely separated. These findings are in agreement with similar researches (Allen et al., 1981; Busse and Ellis, 1985; Ellis et al., 1985; Huang et al., 1985; Sharma and Srivastava, 1991; Osonubi et al., 1992; Osonubi, 1994; Okon et al., 1996).

Because soil and plant water relations are interdependent, it may be difficult to isolate and compare single parameters in VAM and non-VAM plants during drying cycles at the experimental pot study. Therefore, water relations of the greenhouse study did not show a significant difference between drought stressed VAM and non-VAM plants. This can have links to nutrient levels of the experimental soil or probably to not appearing any drought effects due to the late development of drought on both VAM and non-VAM plants. However, in split-root-hyphae system experiments, where leaf and soil water loss have been easily uncoupled, soils still dried faster with VAM than with similar-sized non-VAM root systems. This is an interesting experimental condition: one root compartment is watered while others remain unwatered, allowing measurement of soil drying rates in unwatered compartments of plants whose transpiration does not decrease with soil drying (as inevitably happens when entire root systems are allowed to dry). In this circumstance, one in which the plant does not rely on water supply from the drying compartment or pot because the watered compartment satisfies shoot water requirements, VAM root systems dried the soil more faster than non-VAM root systems of the same size, irrespective of whether about one-quarter, or one-half of the root system remained unwatered. Those results are similar to that of Ebel et al., (1994; 1996).

VAM water relations in most drying cycles were different as compared to non-VAM plants in this study. On the one hand, this might occur by enhanced leaf solute accumulation (lower osmotic potentials) in leaves of VAM plants, resulting in higher bulk leaf turgors at a particular total leaf potential.

If higher photosynthetic rates sometimes associated with VAM symbiosis result in higher concentrations of soluble sugar and other photosynthetic productions in the leaf, this might cause higher osmolality in VAM than in non-VAM plants. Adjustments in leaf osmotic potential and stomatal conductance on drought stressed VAM are related and VAM-induced alteration of leaf osmotic potential may explain VAM-induced promotion of stomatal conductance in this study. However, neither leaf osmotic potential nor leaf turgor potential has generally differed in well-watered VAM and non-VAM plants. These findings are in agreement with Henderson and Davies (1990), Faber et al., (1991), Auge et al., (1992a; 1995), Davies et al., (1993), Ebel et al., (1996), Bryla and Duniway (1997c), Davies (1992), Bryla and Duniway (1997a, c).

We were already interested in characterizing drought hardiness in terms of growth, yield and survival in this study. VAM symbiosis appears to affect these mostly through drought avoidance, often associated with improved nutrients supply. In about 80% of mycorrhizal studies reporting plant growth during drought, VAM plants were larger than non-VAM plants (Auge, 2001), which seem to suggest an important role for VAM fungi in promoting the drought resistance of their hosts. However, in our study, VAM plant growth and yields in dry conditions were higher that in non-VAM plants.

Larger leaf area rate in drought stressed VAM plants of experimental pots study indicates a larger transpiration surface, high stomatal conductance and consequently high leaf net photosynthesis rate as compared to that in non-VAM plants. In a comparative study, drought stressed VAM plant photosynthesis has been increased during the last drying cycles since root mycorrhization supposed to develop in symbiosis with VAM plants, suggests that increased photosynthesis in barley colonized by *G. intraradices* was related to sizeable reductions in both gas phase and liquid-phase resistance to CO_2 transport in leaves. Another suggestion might be that VAM symbiosis may have increased the number of photosynthetic units. Photosynthetic storage and export rates have been increased in VAM plants in terms of high production of biomass and yield in symbiosis plants. However, it is well known that photosynthesis per units has a relationship to plant P nutrition. Our findings indicate that no significant difference between well-watered VAM and non-VAM plants somewhat related to this case in terms of high-level P experimental soil in this study. These results are in agreement with several other researches (Sanchez-Diaz et al., 1990; Davies et al., 1993; Koide, 1993).

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6 Summary

To investigate the quantification of water uptake by extraradical hyphae and effect of root mycorrhization on water relations of host plants several experiments were carried out in this study. We evaluated the effects of colonization of barley (*Hordeum vulgaris* L. var. Scarlett) roots with two VAM fungi (*Glomus intraradices* and *Glomus mosseae*) on growth, yield components, leaf water relations and acquisition of nutrients under simulated drought or well-watered conditions in a greenhouse. We observed mild effects of the two mycorrhizal fungi on leaf water relation between VAM and non-VAM drought stressed plants but no specific effects on yield parameters or plant nutrients uptake. However, that drought conditions surprisingly increased root colonization by VAM. Though under drought condition, only *G. intraradices* changed the leaf area of plants slightly but statistically significant. We also noted some differences between *G. intraradices* and *G. mosseae* in their effect on nutrient uptake by barley under the same growth conditions.

In the split-root-hyphae system experiments we subjected barley plants to simulated drought and studied the effects of symbiosis with vesicular arbuscular mycorrhizal fungi (*Glomus intraradices*) on water uptake and elongation of plant leaves. Ninety days after sowing, the initial gravimetric soil water content was reduced by about 2-4 % in the hyphae compartments of drought stressed plants with vesicular arbuscular mycorrhizal fungi (VAM plants) as compared to that in the plants without vesicular arbuscular mycorrhizal fungi (non-VAM plants). In this study, leaf water potential was lower in VAM plants, relative leaf water content and leaf turgor pressure were all higher in VAM plants than in the non-VAM plants, but at the end of the drying cycles leaf 5 on the mainstem of drought stressed VAM plants was 33% longer than in the non-VAM plants. We suggest, however, that the improved leaf water relations, longer leaf, and faster leaf elongation rate in the drought stressed VAM plants compared with the non-VAM plants, might have been due to the impact of VAM on plants which were independent on the higher contribution of VAM hyphae to water uptake by drought stressed VAM plants.

Effect of VAM on the transport of water to barley roots from a physically separate soil compartment (hyphae compartments) was measured by means of capacitance sensors in the last experiment. Results indicated a decrease in soil water content in the hyphae compartments of plants colonized by vesicular arbuscular mycorrhize (VAM) when compared to the corresponding values in the compartment of control treatment (non-VAM plants). This was taken as evidence for the uptake of water by the extraradical hyphae and its transfer to the host plants in the adjacent, but physically unattached, plant compartment (PC). Measurements showed that under drought condition, VAM hyphae transported 5-7% of the soil water from the hyphae compartments to the plant compartment as compared with non-VAM plants. The data also showed indications for a reverse mass transport of water from the plant compartment (when plants were periodically watered) to the hyphae compartment by the hyphae strands connecting the two compartments.

Howevre, thus the connection possible between both compartments was only due to consisting root-like way of extraradical mycelium for uptake water contributing to droughted stress barley.

7 Zusammenfassung

Die Untersuchung der der Wasseraufnahme durch Extraradikale Hyphen und die Auswirkung der Wurzelmykorrhizierung auf die Beziehung zwischen Wasser und den Wirtspflanzen wurde in mehreren Experimenten untersucht. Wir werteten die Effekte der Besiedelung von Gerste (*Hordeum vulgaris* L. var. Scarlett) mit zwei Mykorrhiza-Pilzen (*Glomus intraradices* und *Glomus mosseae*) in Hinsicht auf Pflanzenwachstum, Erntebestandteile, Blatt-Wasser-Beziehungen und Nährstoffaufnahme unter simulierter Trockenheit im Gewächshaus aus. Wir beobachten eine geringe Wirkung von zwei Mykorrhiza-Pilzen auf die Blatt-Wasser-Beziehung in VAM- Pflanzen und Pflanzen ohne VAM. Wir fanden jedoch nie eine spezifische Wirkung auf die Ertragsparameter der Nährstoffaufnahme der Pflanzen. Überraschender Weise wurde durch die Trockenheit die Wurzelbesiedelung durch VAM erhöht. Bei Trockenheit hat sich nur bei *G. intraradices* die Blattumgebung der Pflanzen leicht, dafür aber signifikant verändert. Wir beobachteten auch einige Unterschiede zwischen *G. intraradices* und *G. mosseae* in ihren Auswirkungen auf die Nährstoffaufnahme von Gerste bei sonst identischen Wachstumsbedingungen.

In dem split-root-hyphae System Versuch haben wir Gerste unter simulierte Trockenheit gestellt und untersuchten die Effekte der Symbiose mit vesikulären arbuskulären Mykorrhiza Pilzen (*Glomus intraradices*) auf die Wasseraufnahme und die Ausdehnung der Blätter. Neunzig Tage nach der Aussat war der anfängliche gravimetrische Bodenwassergehalt um etwa 2 –4 % in den Hyphenkammern der trockengestressten Pflanzen mit vesikulären arbuskulären Mykorrhiza Pilzen (VAM Pflanzen) im Vergleich zu den Pflanzen ohne vesikuläre arbuskuläre Mykorrhiza Pilze (Pflanzen ohne VAM) gesunken. In dieser Untersuchung, war das Blattwasserpotential in den VAM Pflanzen geringer als in den Pflanzen ohne VAM, der realtive Blattwassergehalt und der Turgordruck im Blatt waren in den VAM Pflanzen höher als in den Pflanzen ohne VAM. Am Ende des Trockenzyklus war Blatt 5 des Haupttriebes der trockengestressten Pflanzen um 33 % länger als in den Pflanzen ohne VAM. Wir vermuten jedoch, dass die verbesserten Blatt – Wasser - Beziehungen, die längeren Blätter und die schnellere Blattausdehnungsrate bei trocken gestressten Pflanzen im Vergleich mit Pflanzen ohne VAM, während des Befalls der Pflanzen mit VAM – unabhängig davon ist, dass die Pflanzen unter Trockenstress bei der Wasseraufnahme durch die VAM Hyphen unterstützt wurden. Die Wirkung der VAM auf den Wassertransport von einer physikalisch getrennten, mit Wasser versorgten, Kammer (Hyphenkammer) zu den Gerstenwurzeln in der Pflanzenkammer wurde im letzten Versuch mit capacitance Sensoren gemessen. Die Ergebnisse

weisen auf eine Verringerung des Bodenwassergehalts in den Hyphenkammern die von versikulärer arbuskulärer Mykorrhiza (VAM) erreicht wurden, verglichen mit den Kammern der entsprechenden Kontrollbehandlung (Pflanzen ohnen VAM). Dies wurde als Beweis für die Wassseraufnahme durch extraradikale Hyphen und auf einen Wassertransport hin zu den Wirtspflanzen in der physikalisch abgetrennten, nur durch Hyphen verbundenen, Pflanzenkammer (PK) hin. Messungen bei Trockenheit zeigten dass VAM Hyphen 5 – 7 % des Bodenwassers von der Hyphenkammer in die Pflanzenkammer transportiert haben. Die Daten gaben auch Hinweise dass ein entgegengesetzter Massenstrom von Wasser, von den Pflanzenkammern (vorausgesetzt die Pflanzen wurden regelmäßig gewässert) hin zu den Hypenkammern einzig und allein durch die Wirkung der Hyphen, die ausschließlich für eine Verbindung zwischen den beiden Kammern verantwortlich sind, stattgefunden hat und damit nachgewiesen wurde.

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Abbreviations

A: Net leaf photosynthesis rate
ANOVA: Analysis of Variance
Cp: Partial capacitance
D: Drought conditions
DW: Dry weight
EM: Extraradical mycorrhizae
f: Frequency
FW: Fresh weight
G : Glomus
g_s : Stomatal conductance
HC: Hyphae compartment
LER: Leaf elongation rate
LVDT: Linear varaiable differential transformers
PC: Plant compartment
PPFD: Photosynthetic photon flux density
PVC: Polyvinyl chloride
RWC: Relative leaf water content
TDR: Time domain reflectometry
TW: Turgor pressure
VAM : Vesicular arbuscular mycorrhizal fungi
WW: Well-watered