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

Ali M. KHALVATI, Yuncai HU and Urs SCHMIDHALTER

Institute of Plant Science, Chair of Plant Nutrition, Technical University Munich, Am Hochanger 2, D-85354 Freising

Abstract

Experiments were carried out to determine the effects of vesicular-arbuscular mycorrhizal fungi (VAM) on water uptake and on drought acclimation of the host plant and to quantify water uptake by hyphae in barley (*Hordeum vulgare* L. cv. Scarlet). Four treatments (drought and well-watered with VAM and without VAM plants) (*Glomus intraradices*) were induced in this experiment. The initial gravimetric soil water content in the plant and hyphae compartments was 23 %. Plants grew for 94 days. The results from this experiment are as follows:

The gravimetric soil water content in hyphae compartments was on average 2-4 % lower than in the same compartments in non-VAM plants (control) in comparison to the initial soil water content. Differences in the gravimetric soil water content of the hyphae compartment in the VAM plants as compared to the non-VAM plants might be due to water uptake of extra radical hyphae from hyphae compartment and may have improved plant drought tolerance. The root colonization with mycorrhizal fungus improved water relation parameters in VAM plants at least 3 weeks after sowing.

Introduction

Vesicular-arbuscular mycorrhizal fungus (VAM) may increase drought resistance of host plants due to several mechanisms, including increased water uptake by hyphal extraction of soil water (HARDIE 1985), decreased stomatal sensitivity to leaf-air vapour pressure deficit (HUANG et al. 1985), regulated stomatal conductance in response to hormonal signals (ALLEN 1982). Despite the importance of external hyphae for water and nutrient uptake, few researchers attempted to quantify VAM hyphae in soil. Techniques that have been developed to quantify VAM hyphae include direct measurement of hyphae on root surfaces. This project tries to answer the question how much water is taken up by hyphae and whether hyphal extraction contributes to water uptake by plants? The main question is: Is improved plant growth under drought due to the contribution of mycorrhizal fungi to water uptake? The main objectives are to quantify water uptake in split-root hyphae systems and to test whether smaller-sized plants better tolerate drought conditions.

Materials and methods

Construction of split-root-hyphae system chamber

Split-root-hyphae system were made of plexiglas and consisted of two soil compartments separated with nylon net (with 30 µm pore size) and air gap (Figure 1).

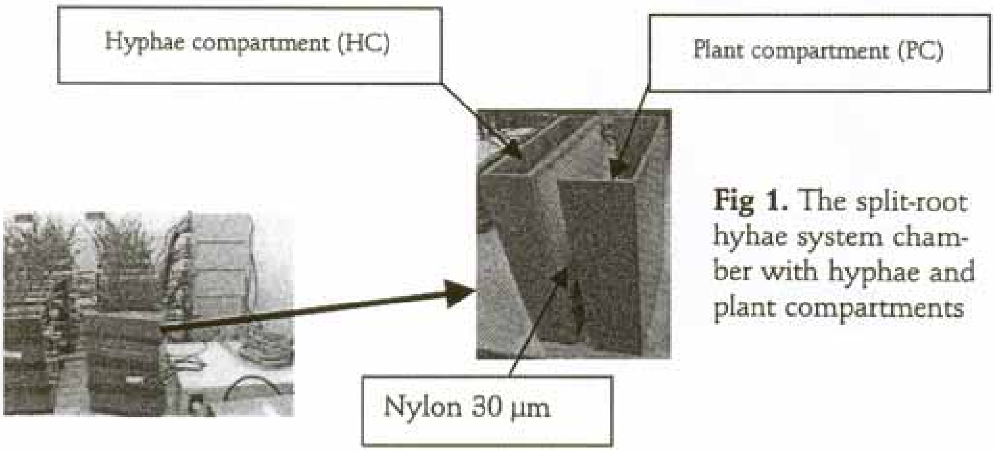

Fig 1. The split-root hyhae system chamber with hyphae and plant compartments

The air gap with 5 mm is efficient to prevent water diffusion and mass flow from the plant compartment.

Root colonization studies

The VAM-colonized roots were stained with hot staining (KROMANIK and MCGRAW 1982). One-hundred 1 cm root segments per treatment were examined for hyphae. Root mycorrhization was determined at the end of the experiments.

Physiological responses

Leaf water (LWP) and osmotic potential (LOP) were measured at the end of each drying cycle using a Scholander bomb (SCHOLANDER et al. 1964) with N_2 gas. To determine leaf osmolality, plant sap was analyzed by using a osmometer (VAPROtm Model 5520, Wescor Inc, Germany).

At the end of each drying cycle, leaf relative water content (RWC) was determined by using the formula RWC = (FW-DW / TW-DW) X 100, where FW is fresh weight, DW is leaf dry weight and TW is leaf turgid weight.

Leaf net photosynthesis rate was measured by using a porometer (Lci Console ADC Bioscientific Limited, England).

Results

Gravimetric soil water content in plant/hyphae compartment

The gravimetric soil water content status of the plant compartment in seven drying cycles is shown in Figure 2. Gravimetric soil water content in hyphae compartments is shown in Figure 3. The gravimetric soil water content values show that the initial gravimetric soil water content is reduced by about 2-4 % in the hyphae compartment (HC).

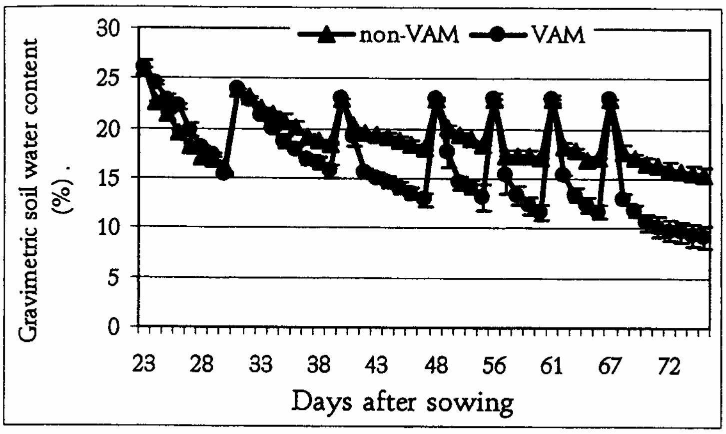

Fig 2. Gravimetric soil water content in plant compartment under drought conditions (error bars indicate standard deviations)

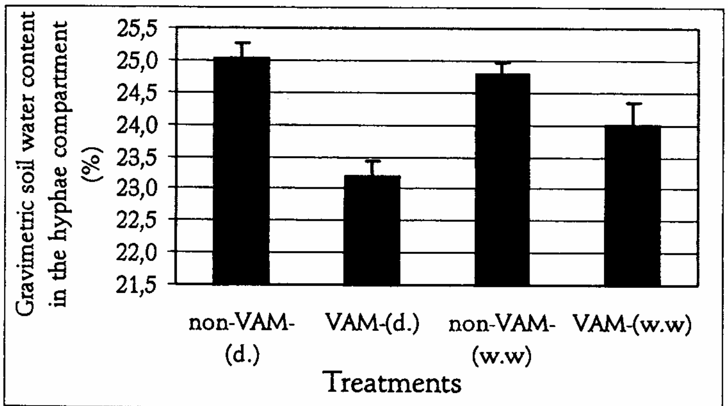

Fig 3. Hyphae compartment gravimetric soil water content of VAM changed during 90 days compared to non-VAM chambers under well-watered (w.w) and drought (d.) conditions (error bars indicate standard deviation)

Leaf water relations

The leaf relative water content (RWC) in VAM and non-VAM plants under drought and well-watered conditions is shown in Figure 4. Leaf relative water content in the VAM plants was higher than in non-VAM plants throughout the drying cycles in both treatments.

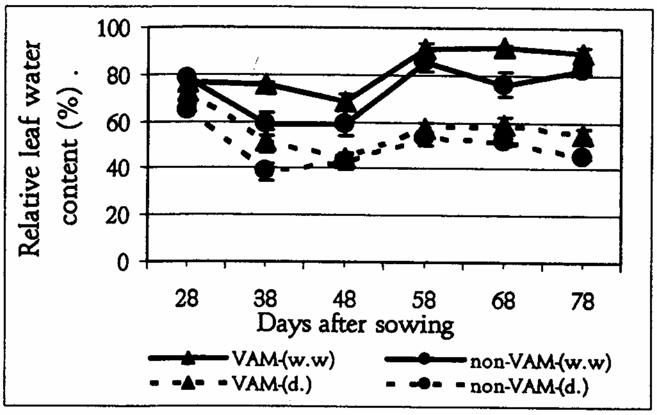

Fig 4. Interactive effect of VAM on relative leaf water content in VAM and non-VAM plants under well-watered (w.w) and drought (d.) conditions (error bars indicate standard deviations

Leaf water potential in VAM and non-VAM plants under well-watered and drought conditions is shown in Figure 5. Leaf water potentials were different between VAM and non-VAM plants. The results show that actually VAM and non-VAM plants under well-watered condition had higher water potentials (less negative) than as plants under drought conditions. Under drought conditions, VAM plants had lower (more negative) water potentials than non-VAM plants during the whole drying cycles. Interactive effect of VAM on leaf stomatal conductance $g_{(s)}$, leaf respiration (Re) and net photosynthesis rate (A) under well watered and drought conditions were measured during the last 20 days to assess the progressive drought effects on VAM and non-VAM plants.

The leaf net photosynthesis rate results are shown in Figure 6. During the drying

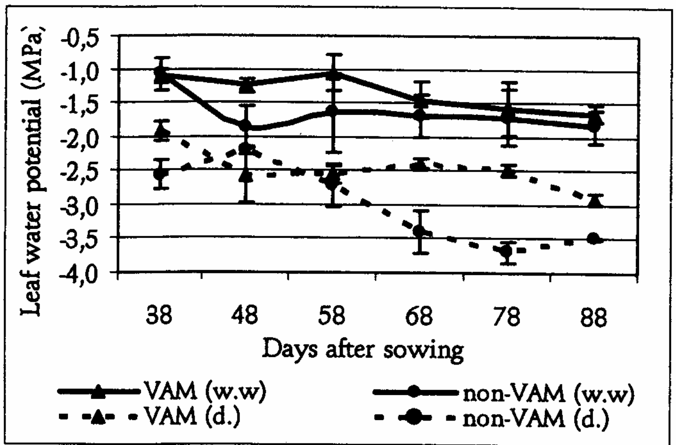

Fig 5. Interactive effect of VAM on relative water potential VAM and non-VAM plants under well-watered (w.w) and drought (d.) conditions (error bars indicate standard deviations

conditions, VAM-plants often maintain higher stomatal conductance $g_{(s)}$ and actually leaf respiration became higher than in similarly sized non-VAM plants. In the same time net photosynthesis rate (A) in VAM plants was relatively higher than in non-VAM plants. At reduced soil water contents mycorrhizal plants maintained higher g(s), transpiration and leaf water potential than non-VAM plants.

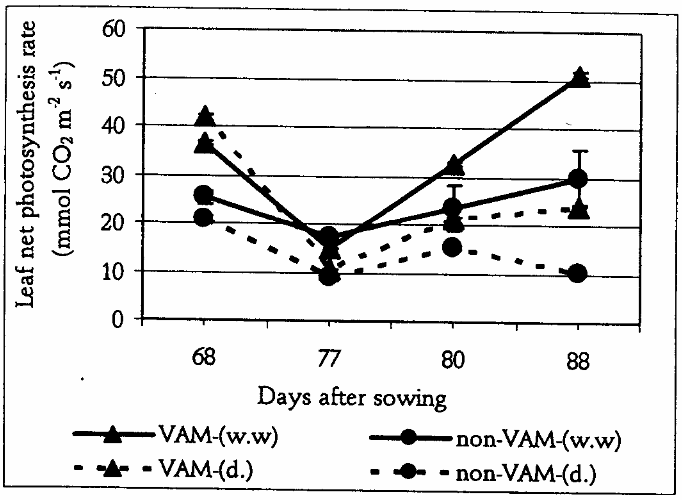

Fig 6. Interactive effect of VAM on leaf photosynthesis rate in VAM and non-VAM plants under well-watered (w.w) and drought (d.) conditions (error bars indicate standard deviations)

Biomass

The results of the interactive effects of VAM on fresh weight of shoots in non-VAM plants and VAM plants are shown in Figure 7. Shoot fresh weight values were markedly different. The yield component in VAM plants was much higher in VAM-plants under both well watered and drought conditions.

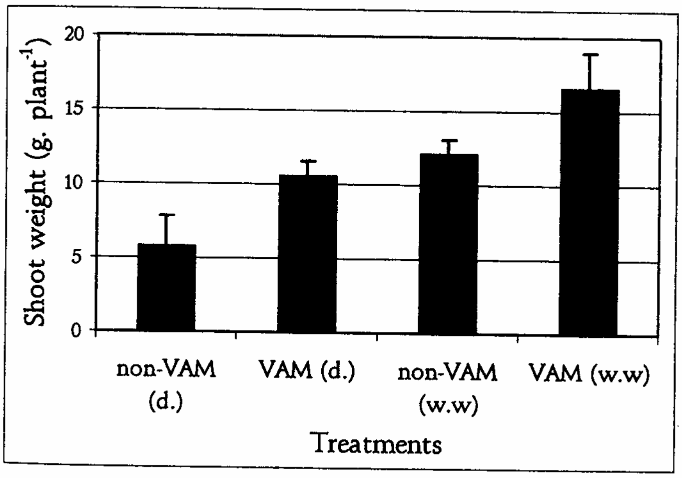

Fig 7. Interactive effect of VAM on shoot fresh weight in non-VAM and VAM plants under well-watered (w.w) and drought (d.) conditions (error bars indicate standard deviations)

Discussion

Seven drying cycles totally were induced in plant compartments during the 90 days period of this experiment. The gravimetric soil water content in the hyphae compartment in VAM plants was 2-5 % lower than in non-VAM plants. The reason might be due to a more efficient water uptake by VAM plants.

It seems that extraradical hyphae contribute to plant water uptake from this compartment particularly under drought conditions. There is excellent evidence to demonstrate that external hyphae of mycorrhizal fungi support water uptake for plant (MOSSE and HAYMAN 1971, BUSSE and ELLIS 1985, HUANG et al. 1985). Root colonization with *G. intraradices* in barley had a beneficial effect on host-plant drought tolerance by maintaining higher (less negative) LWP and LOP, higher RWC, higher net photosynthesis rate that cause higher production of dry mass, higher P content during drought. Our data agree with the finding of other studies (FITTER 1988, SYLVIA 1986), which showed that colonization by VAM fungi improved water relation and plant drought tolerance under drought conditions.

Mycorrhizal colonization does affect the water relations of plants, eventually by increased photosynthesis, or elevated cytokinin levels, which stimulate stomatal openings. Stomatal conductance and transpiration were higher in VAM plants than in non-VAM plants in both well watered and drought conditions. This result is also reported by other scientists (ALLEN 1982, READ and BOYD 1986, NELSEN 1987, GUPTA 1991, KOIDE 1993, SANCHEZ- DIAZ and HONRUBIA 1994, AUGE 2000). In about 80% of mycorrhizal studies reporting plant growth during drought, VAM plants were larger than non-VAM plants, which seem to suggest an important role for VAM fungi in promoting the drought resistance of their hosts.

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