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Drought responses of arbuscular mycorrhizal grapevines

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Abstract
Recent studies have focused on the effects of drought on arbuscular mycorrhizal (AM) vines, but many of the mechanisms of drought tolerance remain unclear. Gas exchange and nutritional strategies of drought tolerance in AM grapevines were investigated. One-year old grapevines, colonised with an AM fungus, were cultivated under glasshouse conditions, after which a 4 week drought period was induced. Shoot xylem water potentials were lower in all the drought stress plants. Although drought stress resulted in a decline in AM colonisation, the proline levels and biomass of AM plants were higher than the non-AM controls during the drought period. Despite AM plants having lower stomatal conductances and substomatal CO_2 concentrations, similar photosynthetic rates were found between AM and non-AM plants under drought stress. As a result the water use efficiency was higher in the AM plants under drought stress. The photosynthetic response of AM plants during drought was associated with an increase in specific leaf mass and higher Rubisco activities and electron transport rates. These results indicate that drought stressed AM grapevines exhibited enhanced water use efficiencies by increasing proline accumulation and having higher investments of photosynthetic capacities to maintain similar CO_2 fixation rates as non-AM grapevines.

Keywords: Grapevines, drought stress, mycorrhizae, proline, water relations

1. Introduction

Grapevines appear to be reliant on arbuscular mycorrhizal (AM) colonisation for normal growth and development (Menge et al., 1983; Karagiannidis et al., 1995; Biricolti et al., 1997; Linderman and Davis, 2001). The beneficial effect of mycorrhizae is of special importance to plants such as grapevines, which have a coarse and poorly branched root system, as a result coarse rooted species are more reliant on AM colonisation than fine rooted species (Bolan, 1991; Eissenstat, 1992; Motosugi et al., 2002). One of the primary benefits of AM fungi is enhanced host nutrition, AM fungi can supply 80% of P and 25% of N to the host plants (Marschner and Dell, 1994). The enhanced P nutrition of AM plants growing in phosphate limited soils, usually leads to higher plant growth rates than non-AM plants (Sanders and Tinker, 1971; Smith, 1982; Bolan, 1991; Orcutt and Nilsen, 2000). The benefits of nutrient acquisition impose a carbon cost on host reserves and the AM can use between 10% and 23% of the host plant's photosynthetically fixed C (Snellgrove et al., 1982; Koch and Johnson, 1984; Kucey and Paul, 1982; Jakobsen and Rosendahl, 1990).

Under non-irrigated conditions grapevines can experience drought stress and the formation of AM may benefit the host plants during water stressed conditions (Nikolaou et al., 2003a,b). AM colonisation may be more important under drought conditions for the host plant growth than in non-stressed conditions (Nelson and Safir, 1982; Fitter, 1985). There is speculation as to whether the positive effects of AM on the water relations of the host are the result of improved mineral nutrition or due to altered hormonal (Druge and Schonbeck, 1993) or non-hydraulic signals (Ebel et al., 1994), owing to the effects of the AM fungus. A major feature of the physiological impact of AM colonisation on drought-stressed host plants, appears to be the control of water relations via the stomatal conductance (Augé, 2001). This has been demonstrated in AM rose hybrids as a lowering (Augé and Duan, 1991) and an increase (Augé et al., 1986) in stomatal conductance. During water stressed conditions AM plants have been

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shown to maintain higher transpiration rates and improved stomatal conductances, compared to non-AM plants (Hardie and Leyton, 1981; Levy et al., 1983; Davies et al., 1993; Augé, 2001).

The accumulation of proline as an osmoticum in AM hosts appears to be variable under low water supply. Bethlenfalvay et al. (1988) found that the leaves of AM plants had lower levels of proline under water stressed conditions, indicating lower levels of stress. However, other studies (Ramakrishnan et al., 1988; Ruiz-Lozano et al., 1995; Azcon et al., 1996; Goicoechea et al., 1998) found higher proline levels in AM hosts under drought stress, suggesting that the symbiont can affect the host proline concentration.

Recent work by Nikolaou et al. (2003a,b) on drought stress in grapevines indicated AM-related changes in photosynthetic gas exchange, water relations and cytokinin levels of host plants. However, in order to gain further insight into the effects of AM fungi on vines experiencing drought stress, the underlying mechanisms of photosynthetic adjustments and the site of osmotic adjustment via osmolytes was elucidated. The aim of this study was therefore to determine where proline-induced osmotic changes occur and how photosynthetic gas exchange is affected by AM formation during drought stress in grapevines.

2. Methods and Materials

Cultivation of the vines

One-year old vines (*Vitis vinifera* cv. Chenon blanc, 12 months under sterile conditions in cold storage) were obtained from a commercial supplier (KWV, Paarl, South Africa) and four plants were used per treatment. The vines were planted in 10 litre pots filled with sterilised sand. The sand was mixed with 20 g of live mycorrhizal inoculum for the AM treatments, whilst the non-AM control plants received an autoclaved (3 h at 110°C under steam pressure at 200 kPa) dose of the inoculum. The inoculum consisted of *Glomus mosseae* (Nicol. and Gerd.) Gerdeman and Trappe (Agricultural Genetics Co. LTD, UK) chlamydospores and fragments of roots in a clay support medium. The inoculum was placed 5 cm below the grapevine rootstock in each pot. The non-AM grapevine rootstocks were irrigated with 50 ml H_2O extract (5 g inoculum per 1 ml H_2O) of the live inoculum filtered through a 30 µm mesh to introduce non-mycorrhizal microbes which may have been killed during the autoclaving process.

The vines were watered by drip irrigation to field capacity (800 ml H_2O per 12 kg of sand) with Long Ashton nutrient solution (Hewitt, 1966) modified to contain 100 µM P and 4 mM NO_3^- as the N source. Vines were grown in a tunnel greenhouse from September to December with a midday irradiance of between 1,000 and 1,500 µmol m^{-2} s^{-1} and an average day/night temperature of between 28°C/19°C. The vines were grown for eight weeks following transplantation and inoculation, after which a four week period of drought stress was induced. During this drought period, all the grapevine plants received 800 ml of Long Ashton nutrient solution at the beginning of the week and every second day thereafter the control plants were supplied with 800 ml H_2O and the drought stress plants with 100 ml H_2O (12.5% of field capacity). This was done to ensure that the nutrient supply remained the same, and that only the H_2O supply was varied during the drought period.

Plant harvest

The plants were divided into root, leaf and stem components and weighed to determine the fresh weight. The leaf areas of the plants were measured with a leaf area meter (Li-cor, model LI-3000, Lambda Instruments Corporation, USA). Using the leaf areas and leaf weights, the following leaf parameters were calculated: Specific leaf mass (g m^{-2}) = leaf dry weight/leaf area; plant leaf area ratio (m^2 g^{-2}) = leaf area/plant dry weight; leaf weight ratio = leaf dry weight/plant dry weight. The roots were carefully blotted dry, a piece of the root was cut off, weighed and stored in a vial with 50% ethanol (v/v) solution for estimation of mycorrhizal colonization. The components were dried at 80°C for more than 72 h and weighed to determine the dry weight.

Chemical analysis

After the four week drought period, the proline concentration of the leaves and roots was determined with freshly harvested plant material. Five replicates of each treatment were used to determine the proline concentration colourometrically using the ninhydrin method of Bates et al. (1972). For total N analysis, oven-dried (72 h, 80°C) plant material of each treatment was milled in a Wiley mill (A.H. Thomas, Philadelphia, USA) using a 60 mesh screen for leaf, stem and root (0.05 g) material. The plant N was determined by a commercial laboratory (BemLab, De Beers Rd, Somerset West, South Africa), using a LECO-nitrogen analyser with Spectrascan standards (Norway).

Water potential measurements

Xylem water potentials (XWP) were taken at midday, using a pressure chamber (PNS instruments Co. Oregon, USA). A terminal branch bearing the first fully expanded leaf was placed in the pressure bomb, with the leaf inside the chamber and cut surface of the stem protruding from the chamber. The pressure was gradually increased, until the xylem sap evenly covered the cut surface. At this point the pressure was turned off and recorded as the shoot water potential.

Table 1. Biomass and arbuscular mycorrhizal colonisation of 1 year old grapevines grown in sand culture. Plants were supplied with live *Glomus mosseae* inoculum or with an autoclaved inoculum and grown under drought and non-drought conditions. The resulting plant treatments were: non-mycorrhizal without drought (NM-D); mycorrhizal without drought (M-D); non-mycorrhizal with drought (NM+D); mycorrhizal with drought (M+D). Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4)

Biomass parameters	Non-drought		Drought	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Dry weight (g)				
Plant	18.78 b	20.10 b	15.13 a	23.06 b
Roots	3.75 b	3.30 b	1.75 a	2.78 b
Shoots	15.03 a	14.29 a	13.13 a	20.27 b
Root:shoot	0.25 b	0.10 a	0.14 a	0.13 a
Leaves	11.25 a	12.78 ab	10.86 a	14.03 b
Leaf parameters				
Leaf area (m^2)	0.157 a	0.160 a	0.152 a	0.154 a
Specific leaf mass (g m^{-2})	74.45 b	63.08 a	63.43 a	78.31 b
Plant leaf area ratio (m^2 g^{-1})	0.86 a	0.96 a	0.86 a	0.80 a
Leaf weight ratio	0.60 a	0.63 a	0.73 a	0.61 a
Shoot water potential (MPa)	−0.97 a	−0.93 a	−1.52 b	−1.47 b
Arbuscular mycorrhizas				
% colonisation	0 a	68.17 c	0 a	38.57 b

Table 2. Mineral nutrition of 1 year old grapevines grown in sand culture. Plants were supplied with live *Glomus mosseae* inoculum or with an autoclaved inoculum and grown under drought and non-drought conditions. The resulting plant treatments were: non-mycorrhizal without drought (NM-D); mycorrhizal without drought (M-D); non-mycorrhizal with drought (NM+D); mycorrhizal with drought (M+D). Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

Nutrient parameters	Non-drought		Drought	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Leaf proline (mmol g^{-1} fw)	4.10 bc	3.42 ab	2.70 a	4.33 c
Root proline (mmol g^{-1} fw)	5.63 ab	3.49 a	6.22 b	13.53 c
Specific root proline (mmol proline $mmol^{-1}$ N)	0.45 a	0.28 a	0.46 a	1.12 b
Leaf N (mmol g^{-1} dw)	18.41 ab	16.50 a	19.45 b	17.38 ab
Root N (mmol g^{-1} dw)	12.41 a	12.30 a	13.57 a	12.16 a

Table 3. Photosynthetic properties of 1 year old grapevines grown in sand culture. Plants were supplied with live *Glomus mosseae* inoculum or with an autoclaved inoculum and grown under drought and non-drought conditions. The resulting plant treatments were: non-mycorrhizal without drought (NM-D); mycorrhizal without drought (M-D); non-mycorrhizal with drought (NM+D); mycorrhizal with drought (M+D). Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

Photosynthetic parameters	Non-drought		Drought	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
% stomatal limitation of photosynthesis	29.161 ab	37.894 b	31.136 ab	23.703 a
Stomatal conductance (μmol m^{-2} s^{-1})	197.625 c	135.050 b	118.250 b	71.925 a
Internal substomatal CO_2 (μmol CO_2 m^{-2})	230.725 c	205.700 b	188.275 b	147.175 a

Photosynthesis

The youngest fully expanded leaf for each plant was used for the photosynthetic determinations. The photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (E) were determined at midday, using a portable infrared gas analyzer (LiCor, Lambda Instruments Corporation, USA).

Photosynthetic nitrogen-use efficiency (PNUE) was obtained by dividing Pn by the leaf N concentration. Photosynthetic water-use efficiency (PWUE) was calculated from measurements of Pn and transpiration rate. Intercellular CO_2 response curves were determined using the facility on the infrared gas analyser, by manually adjusting the CO_2 concentration in the leaf chamber. The CO_2

response curves were used to calculate carboxylation-limited Rubisco activity (Vc_{max}) and electron transport capacity (J_{max}), using the equations of Von Caemmerer and Farquhar (1981). The percentage stomatal limitation of photosynthesis was obtained from the CO_2 response curve, using the difference in Pn at ambient atmospheric CO_2 concentrations (380 ppm) and Pn at internal substomatal CO_2 concentrations in the equation:
% Stomatal limitation = (Pn at 380 ppm CO_2 – Pn at internal substomatal CO_2 concentrations / Pn at 380 ppm CO_2) * 100.

Mycorrhizal colonisation

During plant harvest, lateral roots were removed and stored in 50% ethanol. Root segments were cleared with 20% KOH for 48 h at room temperature. Afterwards, the KOH was rinsed from the segments and acidified with 1% HCl for 24 h, also at room temperature. The roots were stained with 0.05% Analine blue in 70% acidified glycerol for 48 h at room temperature. Roots were cut into 1 cm pieces and examined at 400× magnification under a light microscope. Infection was determined according to the methods described by Brundrett et al. (1994).

Statistical analysis

The percentage data were arcsine transformed (Zar, 1999). The influence of the two factors (AM inoculation and drought) and their interactions were tested with a two-way analysis of variance (2-way ANOVA) (Statgraphics Version 7, 1993, Statgraphics Corporation, USA). Where the 2-way ANOVA revealed significant effects by the factors, the differences between treatments were separated using a post hoc least-significant difference (LSD), multiple comparison test ($P \leq 0.05$).

3. Results

Xylem pressure potentials indicated that the plants in the drought treatment were experiencing water stress as a result of the limited water supply (Table 1). Drought stress lowered the AM colonisation from 68% to 39% (Table 1). In spite of lower AM colonisation during drought stress, the symbiotic benefits were apparent in host growth (Table 1). Compared to non-AM plants, AM plants under drought stress had higher dry weights of roots, shoots and total plant (Table 1). Although there was no difference between the leaf area of AM and non-AM plants under drought stress, the specific leaf mass (SLM) was higher in AM than non-AM plants during the drought period (Table 1).

There were no differences in the leaf and root N concentrations between AM and non-AM plants under drought stress (Table 2). However, under these conditions AM plants had higher root, leaf and specific root proline levels than non-AM plants (Table 2).

The positive correlation between leaf proline and photosynthetic water-use efficiency (WUE) in both AM and non-AM plants under drought stress (Fig. 1), indicates a role for compatible solutes in affecting leaf gas exchange properties during droughts. The higher WUE in AM plants (Fig. 2) was attained during lower stomatal conductances (Gs) (Table 3). The lower Gs may have led to the lower internal leaf CO_2 concentrations in the AM plants under drought stress (Table 3).

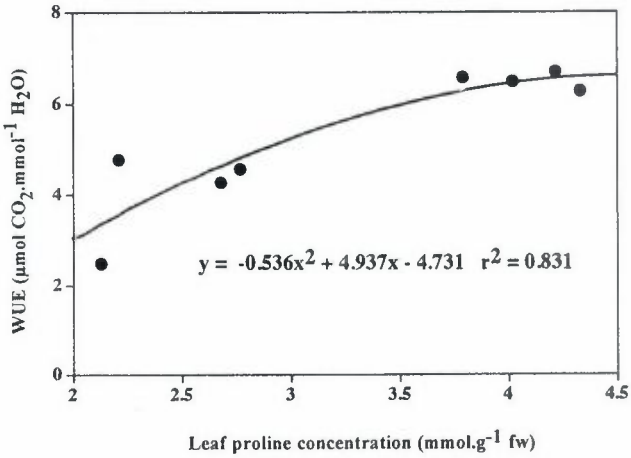

Figure 1. Correlation between leaf proline concentrations and photosynthetic water-use efficiencies (WUE) of 1 year old grapevines grown in sand culture. Plants were supplied with live *Glomus mosseae* inoculum (mycorrhizal), or with an autoclaved inoculum (non-mycorrhizal) and grown under drought and non-drought conditions.

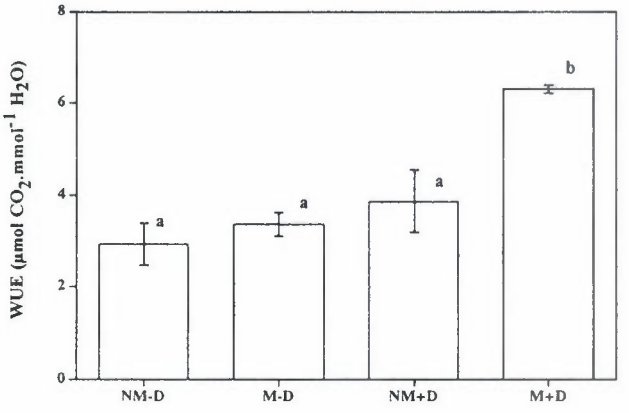

Figure 2. Photosynthetic water-use efficiency (WUE) of 1 year old grapevines grown in sand culture. Plants were supplied with live *Glomus mosseae* inoculum or with an autoclaved inoculum and grown under drought and non-drought conditions. The resulting plant treatments were: non-mycorrhizal without drought (NM-D); mycorrhizal without drought (M-D); non-mycorrhizal with drought (NM+D); mycorrhizal with drought (M+D). Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

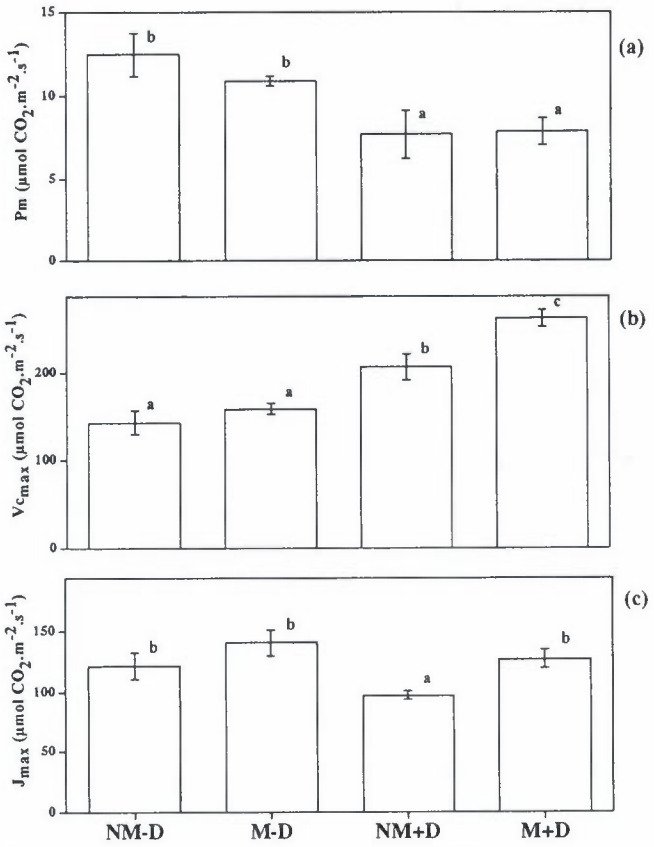

Figure 3. Rubisco activity (Vcmax) (a) Electron transport capacity (Jmax) (b) and Photosynthetic rate (Pn) (c) of 1 year old grapevines grown in sand culture. Plants were supplied with live *Glomus mosseae* inoculum or with an autoclaved inoculum and grown under drought and non-drought conditions. The resulting plant treatments were: non-mycorrhizal without drought (NM-D); mycorrhizal without drought (M-D); non-mycorrhizal with drought (NM+D); mycorrhizal with drought (M+D). Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

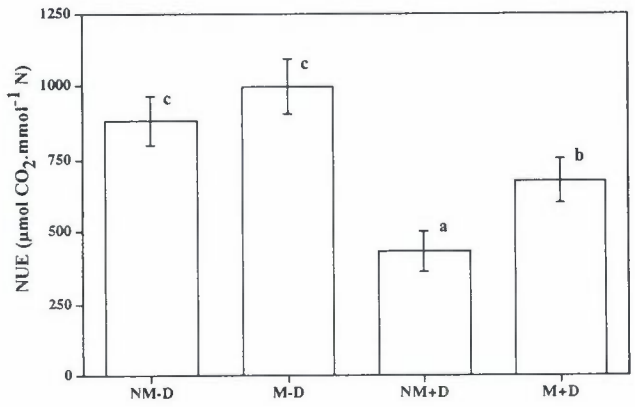

Figure 4. Photosynthetic nitrogen-use efficiency (NUE) of 1 year old grapevines grown in sand culture. Plants were supplied with live *Glomus mosseae* inoculum or with an autoclaved inoculum and grown under drought and non-drought conditions. The resulting plant treatments were: non-mycorrhizal without drought (NM-D); mycorrhizal without drought (M-D); non-mycorrhizal with drought (NM+D); mycorrhizal with drought (M+D). Different letters indicate significant differences between each treatment ($P \leq 0.05$, n=4).

Despite the lower Gs, the percentage stomatal limitation of Pn indicates that under drought, the Pn of AM plants were less limited by stomata than in non-AM plants (Table 3). However, with higher Rubisco activity (Vc_{max}) (Fig. 3b) and electron transport capacity (J_{max}) (Fig. 3b), similar Pn were obtained between AM and non-AM plants (Fig. 3a). The more efficient photosynthetic N-use efficiency (NUE) of AM plants under drought stress (Fig. 4), coincided with the enhanced Vc_{max} (Fig. 3b) and J_{max} (Fig. 3b) activities per unit leaf N in AM plants.

4. Discussion

The decline in AM colonisation with drought stress, concurs with previous studies of AM fungi during low water supply (Ruiz-Lozano and Azcon, 1996; Pande et al., 1999). The lower AM colonisation, under drought conditions may be related to the increased allocation of host C to the symbiont root component not the AM symbiont. In this regard, an increased root investment has been reported in previous AM studies where increased root length, root length density and altered root morphology were reported to aid soil water exploration (Kothari et al., 1990). The decline in AM colonisation in the present study, may be related to the observed increase in root growth and the possible increase in root area of AM plants under drought stress. Despite the lower AM colonisation, AM plants had greater dry weights under drought stress than non-AM plants. In accordance with the work of Fay et al. (1996), these current findings show that in spite of lower colonisation, the AM symbionts still provide benefits to the host.

In the absence of any change in N uptake, the enhanced growth of AM plants under drought stress can be attributed to improved water relations. Previous studies for AM hosts have shown an improvement in water relations, but the underlying causes varied in each investigation (Di and Allen, 1991; Cui and Nobel, 1992; Gemma et al., 1997; Al-Karaki and Clark, 1998; Augé et al., 1987). In the present study, the enhanced water use efficiency (WUE) of AM hosts may have resulted from photosynthetic adjustments or improved proline accumulation. The photosynthetic adjustment was evident in the lower stomatal conductance (Gs), without a significant compromise in the photosynthetic rate (Pn). The control of stomatal conductance by AM plants, has been demonstrated in rose hybrids as a lowering (Augé and Duan, 1991) and an increase (Augé et al., 1986) in stomatal conductance. In the present study, the sub-stomatal CO_2 concentration (Ci) declined as a result of the lower Gs, but the photosynthetic rates were maintained by the increase in the capacities for Rubisco activity and electron transport.

These increases reflect the higher investment in photosynthetic capacity of AM host plants, and correspond with the enhanced specific leaf mass (SLM) and

photosynthetic N-use efficiency (NUE) of the AM plants. The positive relationship between SLM and photosynthetic capacity previously has been reported as a cause for improved Pn since SLM reflects the N investment in photosynthetic capacity (Evans and Seemann, 1989; Abrams et al., 1994; Reich et al., 1999; Niimetes, 1999). This is possibly the underlying cause for the high Rubisco activity and electron transport rate in the AM plants.

Although Augé et al. (1992) found a decrease in total amino acid concentration of mycorrhizal rose hybrids under drought stress, certain amino acids such as proline may have enhanced levels to induce drought tolerance in plants (Paleg and Aspinall, 1981). In this regard, higher proline levels of AM hosts under drought stress, concurs with other findings of AM plants during low water supply (Ramakrishnan et al., 1988; Ruiz-Lozano et al., 1995; Azcon et al., 1996; Goicoechea et al., 1998). The accumulation of proline can aid in the uptake of water by lowering the water potential of the roots (Paleg and Aspinall, 1981). However, the role of proline may not only be as an osmolite, it may also act as a sink for energy to regulate redox potentials under drought stress (Kishor et al., 1995).

5. Conclusion

The AM mediated adjustments to host drought tolerance may be based on proline-induced osmotic adjustments and by greater investment in photosynthetic capacity. The combination of these alterations resulted in maintaining similar Pn of AM as non-AM plants, but with higher WUE.

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