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Water status and stomatal behaviour of cowpea, *Vigna unguiculata* (L.) Walp, plants inoculated with two *Glomus* species at low soil moisture levels

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Abstract – The effects of arbuscular mycorrhizal (AM) fungi on water status and stomatal behaviour of cowpea, *Vigna unguiculata* (L.) Walp. cv. B89-504, under water-stressed conditions in the greenhouse were studied. The 3 × 2 experimental design included two levels of mycorrhizal colonisation (Glomus *mosseae, Glomus versiforme*) and non-mycorrhizal control treatment and two soil moisture levels (well-watered pots and pots allowed to dry). Relative water content and leaf water potential values were higher in well-watered mycorrhizal and non-mycorrhizal plants than in water-stressed mycorrhizal and non-mycorrhizal plants. AM species had no significant effect on leaf osmotic potential, stomatal conductance and leaf transpiration in both well watered and water-stressed plants. The values of stomatal conductance and leaf transpiration were high during the vegetative stage and low during the flowering stage. These responses which can be related to the age of the plant suggest that mycorrhizal colonisation did not affect stomatal closure of cowpea plants during water stress. The decrease in plant growth and dry matter production in both mycorrhizal and non-mycorrhizal plants shows that drought resistance in cowpea was unaffected by mycorrhiza in the vegetative phase. © 2001 Editions scientifiques et médicales Elsevier SAS

Glomus / stomatal response / *Vigna unguiculata* / water status / water stress

1. INTRODUCTION

Most crop plants form symbiotic associations with arbuscular mycorrhizal (AM) fungi [29] which have been shown to improve productivity in soils of low fertility [37]. This response is usually attributed to enhanced uptake of immobile nutrients such as P, Zn, and Cu [23, 36, 40, 42, 43, 45]. Some authors have suggested that mycorrhizae may be even more beneficial to plant growth under dry conditions than when soil moisture is plentiful [1, 42, 50, 51].

Increased drought resistance of crops by AM colonisation can occur through several mechanisms : an intensive absorption of water and mineral nutrients by external hyphae [6, 14, 49], the regulation of stomatal conductance in response to hormonal signals [18, 19, 27], the reduction of leaf osmotic potential for turgor maintenance [3, 15] and a modification of photosynthetic and metabolic activities [28, 51, 58]. In addition, other factors associated with AM colonisation may influence drought resistance. These include changes in leaf elasticity [4], increased leaf water potentials and maintenance of transpiration [5], increased rooting length, depth and development of external hyphae [14, 22, 39] and enhanced grain yield [59]. However, other reports indicate that drought resistance is unaffected or decreased by mycorrhiza [2, 12, 32-34, 56].

Cowpea, *Vigna unguiculata* (L.) Walp, a food crop grown mainly in semi-arid regions is usually considered as a drought-avoiding plant with stomata that are extremely sensitive to declining soil water [53], and usually shows relatively small changes in leaf water potential. Very little is known about the effects of AM *Glomus* species on water-stressed cowpea plants. Previous studies have focused on the stomatal response of the plant and hydraulic and hormonal factors implicated in dry conditions [6, 19–21]. The aim of this study is to establish the impact of two *Glomus* species on the plant water status of a variety of cowpea (cv. B89-504) and also to examine the potential strategy of this species in resisting soil moisture stress during the vegetative stage.

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2. MATERIALS AND METHODS

2.1. Soil and biological material

The sandy soil used for the experiment was collected from Bambey (Senegal) with a pH of 5.1 and a conductivity of 18.13 µS. This soil contained 0.17 ‰ of available P (P_2O_5-extractable), 0.1 ‰ of total N, *0.84* ‰ of total C, 94.25 % sand, 1 % clay, 1 % fine silt and 1 % coarse silt. Before use, the soil was sieved (2 mm) and autoclaved at 120 °C for 1 h for 3 consecutive da ,s. Each pot was filled to 4/5 of its volume (18.10^{-3} m^3) with the sterilised soil. All mycorrhizal inocula consisted of soil, spores, mycelium and infected root fragments obtained from open pot culture of *Zea mays* L. The two AM species used, *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe and Glomus *versiforme* Karsten and Berch, were obtained from the collection of the *'Laboratoire de microbiologie'* des sols (ORSTOM, Dakar, Sénégal).

2.2. Plant material and growth conditions

The experiment was conducted between July and September 1997 in CERAAS' greenhouse at the 'Centre national de recherches agronomiques' (CNRA), Bambey, Senegal (14°42'N, 16°28'W). Seeds of *V. unguiculata* (L.) Walp. cv. B89-504 were treated with a fungicide (deltamethrine, 12 g L^{-1}), surface sterilised by immersion in 0.1 % mercuric chloride for 10 min, rinsed in sterile distilled water and germinated on wet filter paper in Petri dishes under sterile conditions. After 3 days, the seedlings were transplanted to pots in the greenhouse containing sterilised sandy soil, and inoculated with 40 g of inoculum per pot separately with G. *mosseae* or G. *versiforme*. Controls received sterilised inoculum. Seedlings were thinned 10 days after sowing (das) to one seedling per pot. The plants were watered with sterile distilled water. A compound fertiliser (NPK, 8:18:28) was incorporated into the soil at a rate equivalent to 150 $kg^{-1}ha^{-}$ at 20 days.

2.3. Experimental design

Treatments consisted of two soil moisture regimes (watered plants and water-stressed plants) and three mycorrhizae inoculations *(Glomus mosseae, G. versiforme* or non-mycorrhizal) and arranged in a completely randomised block design (one plant per pot) with five replicates of each treatment to give a total of 30 pots. From germination up to 29 days of growth, the plants were watered daily to field capacity. From 29 days onwards, one half was maintained close to field capacity and the other half was subjected to water stress and then re-watered at 39 das.

2.4. Micro-climate parameters

Dry and wet air temperatures in the greenhouse were measured each day at 8 h, 15 h and 18 h with a ventilated psychrometer in order to calculate relative humidity (RH) and vapour pressure deficits (*Vpd*).

2.5. Soil moisture measurements

Soil samples were extracted at 5 cm intervals to a depth of 20 cm. Volumetric soil water content (*Hv*) was determined by weighing samples before and after drying at 105°C for 24 h. In each pot, the soil water content was the average of the four values obtained to a depth of 20 cm. In each treatment, *Hv %* values were the means of five replications.

2.6. Plant water status

Relative water content (*RWC*) was measured every week (one or two measurements) from 11.00 h to 13.00 h using leaf discs (1 cm diameter). Leaf discs were immediately placed in pre-weighed vials, sealed and reweighed to derive their fresh weight (*FW*). They were rehydrated by floating on distilled water for 4 h to obtain their turgid weight (*TW*). Their dry weight (*DW*) was obtained after oven-drying at 85 °C for 24 h. *RWC* was calculated according to Turner [62] as:

$$RWC = [(FW - DW)/(TW - DW) \times 100]$$

Between 11.00 h and 13.00 h, water potential (Φ_L) and osmotic potential ($\Phi\pi$) were determined at days 3 1, 36 and 39 after sowing for the youngest fully expanded trifoliate leaves from apex of five plants selected at random from each treatment. Water potential was measured using a pressure chamber (3005 PWSC, PMS Instrument Company, Corvallis, Oregon, USA) [52] whereas osmotic potential of expressed cell sap (or juice) was determined using a vapour pressure osmometer (5500, Wescor Inc., Logan, Utah, USA), calibrated daily with a graded series of NaCl solutions.

2.7. Gas exchange measurements

Stomatal conductance (g_s) and transpiration (*T*) of unshaded leaves were measured twice per week with a diffusion porometer (LI-1600, Licor Inc., Lincoln, Nebraska, USA) [11]. On each sampling day, measurements were made between 11.00 h and 13.00 h on both surfaces of the third fully expanded leaves from the shoot apex of five plants in each treatment.

2.8. Growth and root colonisation

The length of each plant was measured with a ruler twice per week. Plants were harvested at 53 das. For dry weight determinations, shoots, roots and pods of each plant were separated and dried at 85°C for 48 h. A small sample of roots (1 g) was randomly removed in each treatment from the root system before drying. Colonisation by various mycorrhizal structures 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

Table I. Evolution in soil volumetric moisture (%) under well-watered (HO) and water stress conditions (HI) and after rewatering.

Treatments	Withholding water		After rewatering	
	29 days	31 days***	46 days	50 days
HO	8.33a	9.51a	11.65a	11.09a
H1	8.36a	7.78b	11.74a	11.04a

Values are the means of five replications; within each column, means followed by the same letter are not significantly different ($P < 0.05$) as determined by Newman-Keuls test; *** : $P < 0,001$.

Hayman [46]. The percentage of colonised roots by an AM fungus was quantified as described by Furlan and Fortin [25].

2.9. Statistical analysis

Two-way analysis of variance was performed by using the following parameters as sources of variation : block, fungus, soil moisture, and fungus-soil moisture interaction. Differences between means were evaluated for significance by a Newman-Keuls test at a probability of $P < 0.05$.

3. RESULTS

Average values of soil volumetric moisture at 20 cm depth were similar in welljwatered pots of non-mycorrhizal and mycorrhizal plants of cowpea (table I). After three days of withholding water (day 31 after sowing), *Hv* declined significantly ($P < 0.001$) from 8.36 to 7.78%. There was no fungal main effect or water x mycorrhizae interactions during water stress. After rewatering, *Hv* values became similar to those of pots maintained at field capacity.

The range of minimum and maximum air temperatures were 25-29°C and 32–36°C, respectively. The minimum and maximum values of relative humidity varied between 39 to 52% and between 60 to 90%, respectively. The minimum and maximum vapour pressure deficits were 3-15 mbar and 23-35 mbar, respectively (*figure 1*).

Relative water content was similar in non-mycorrhizal and mycorrhizal plants maintained close to field capacity and ranged frqm 80 to 96% (figure 2). Mycorrhizal colonisation did not affect *RWC* in fully watered conditions. On the last day of measurement (39 days), mycorrhizal and non-mycorrhizal stressed plants showed low *RWC* values ranging between 85 to 88%, whereas well-watered plants maintained high *RWC* values ranging betweed 90 to 92% (*figure* 2). This difference between well-watered and water-stressed treatments was maintained at 43 days after rewatering. After this date, rewatered plants showed high values of *RWC* which were similar to those shown by plants maintained in wet conditions.

Leaf water potentials (Φ_L) measured at midday were high in non-mycorrhizal and mycorrhizal plants under well-watered conditions and ranged from -0.57 to -0.44 MPa (*figure* 3). Concurrently, values of leaf osmotic potential ($\Phi\pi$) showed little variation throughout the experimental period, in spite of mycorrhizal colonisation (*figure* 4). On the other hand, after 10 days of withholding irrigation (39 days), Φ_L values decreased significantly in both non-mycorrhizal and mycorrhizal plants compared to those of well-watered plants (*figure* 3). These lower values of Φ_L ($P < 0.05$) were induced by only soil moisture level, and no significant effect of the *Glomus* species was observed. At 43 days, $\Phi\pi$ of re-watered plants was significantly reduced in comparison to that of well-watered plants (*figures 4).*

Under well-watered conditions, inoculated and non-inoculated plants showed high values of stomatal conductance and transpiration (*figures 5, 6*). The values of these physiological parameters decreased significantly in non-mycorrhizal and mycorrhizal plants during the vegetative stage, and remained unchanged during the flowering stage (from 39 to 50 days). Values of g_s and *T* were high during soil drying in AM and non-AM plants and similar to those obtained under well-watered conditions (*figures* 5, *6*). After rewatering, g_s and *T* decreased but no significant difference was found in the case of AM infection among the rewatered treatments. However, at day 50 after sowing, g_s and *T* were significantly higher in non-mycorrhizal and G. *versiforme* colonised plants than in plants colonised by G. *mosseae.*

Under well-watered conditions, plant growth was significantly higher in plants colonised by AM fungi than in non-mycorrhizal plants between 29 and 43 das during the vegetative stage and similar in all well-watered plants after 43 days (*table II*). *The* beneficial effect of the *Glomus* species on shoot length was also observed during water stress (*table II*). In contrast, the growth of non-mycorrhizal plants was reduced. These plants maintained reduced growth after rehydratioh whereas mycorrhlzal plants showed higher values of shoot length. G. *versiforme* showed the most effective effect on shoot growth.

Roots of *Vigna unguiculata* were well-infected by each *Glomus* species as shown by the presence of intraradical hyphae, vesicles and arbuscules (*table III*). In general, the *Glomus* species assayed did not differ In their ability to colonised plant root under water stress (*table* ZZZ). The percentages of total root colonisation by the species were similar and between 29% a& 50%. No mycorrhizal structure was observed in plants of non-mycorrhizal treatments.

Shoot biomass was not significantly affected by the two *Glomus* speçies used in this experiment under well-watered conditions (*table* ZZZ). Water stress significantly decreascd shoot dry matter by 32% in plants colonised by G. *mosseae* and 63% in plants inoculated witb G. *versiforme*. A greater effect of water stress conditions on shoot dry matter was observed in non-inoculated plants (72%). Water stress did not significantly affect root dry weights (table ZZZ). Nevertheless,

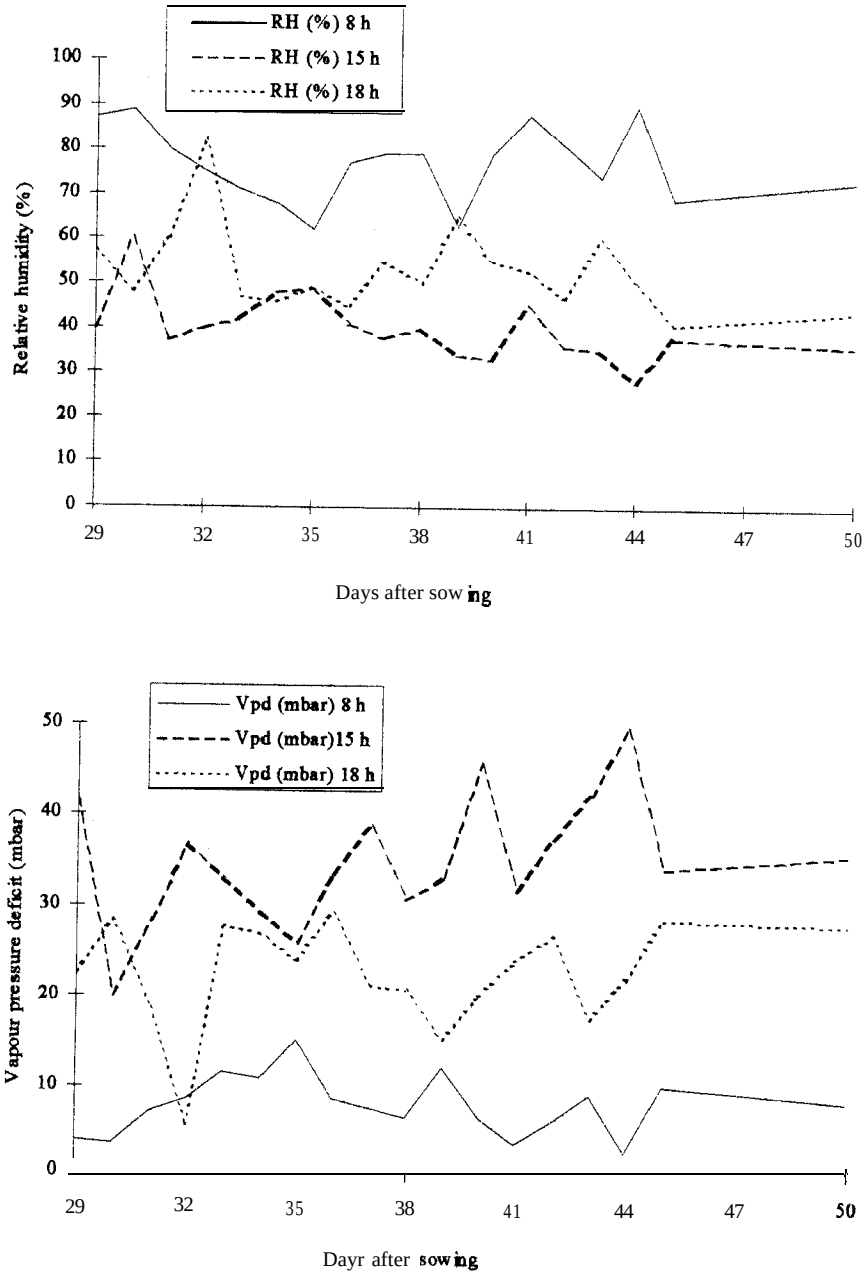

Figure 1. Evolution in micro-climate parameters, relative humidity (%) and vapour pressure deficit (mbar) in the greenhouse during the experiment.

compared to well-watered plants, root dry matter of stressed plants decreased and the relative percentages of reduction were 43% in non-mycorrhizal plants and 41% and 48% in plants colonised respectively by G. *versiforme* and G. *mosseae*. The ratio of rootlshoot dry weights was higher in non-inoculated plants (61%) and plants infected by G. *versiforme* (107%) and decreased in plants colonised by G. *mosseae (table ZZZ)*. The relative pod dry matter of water-stressed cowpea was significantly reduced by the water deficit regime in both mycorrhizal and non-mycorrhizal plants *(table ZZZ)*.

4. DISCUSSION

Water stress is generally characterised by decreases in relative water content (*RWC*) and turgor, resulting in wilting, stomatal closure and reduced growth [41]. In this study, the leaf relative water content of cowpea did not drop significantly (85 to 88%) when plants were subjected to water deficits. Previous work [44] indicated the same results in three genotypes of cowpea, including the genotype B89-504. In addition, similar values of *RWC* in both non-mycorrhizal and mycorrhizal plants throughout the experimental period

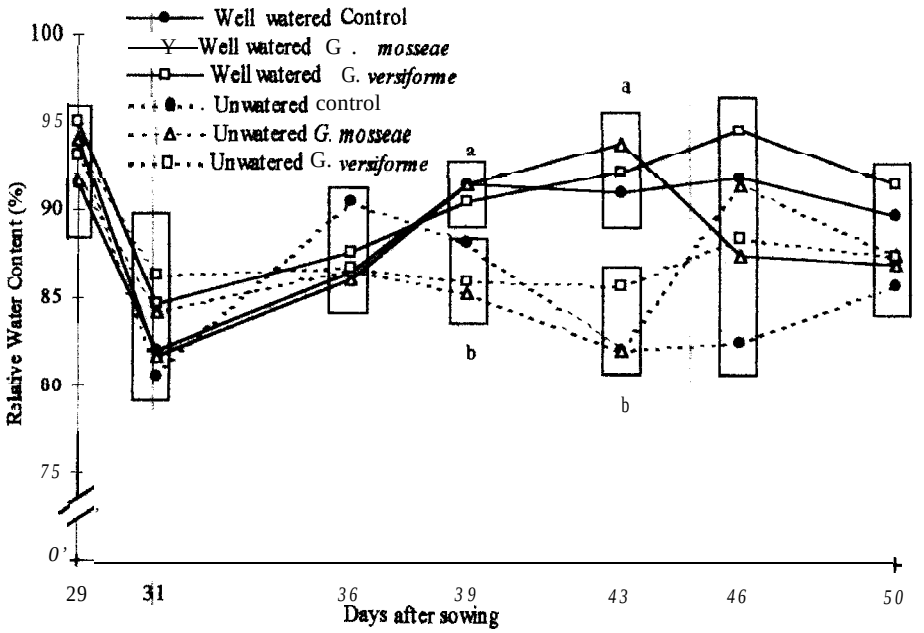

Figure 2. Leaf relative water content (%) in cowpea plants colonised by two *Glomus* or control under two soil moisture regimes and after rewatering. Significantly different means are presented with different letters and in distinct rectangles.

showed that leaf ***RWC*** of cowpea was not affected in any consistent way by mycorrhizal inoculation. These results were observed by Augé et al. [8] in sorghum, *Sorghum bicolor* (L.) Moench 'G1990A, plants inoculated separately witb *Glomus* ***intraradices*** Schenck and Smith and G. *etunicatum* Becker and Gerd.

At high soil water content, leaf water potentials were similar in non-mycorrhizal and mycorrhizal plants. However, after 3 days of withholding water, only non-mycorrhizal plants showed a significant reduction in leaf water potentials. In comparison with non-mycorrhizal plants, lower values of Φ_L were observed in mycorrhizal plants 10 days after withholding water. The midday Φ_L were generally lower than -1.5 MPa for field-grown cowpeas that had npt been inoculated and did not receive water for up to

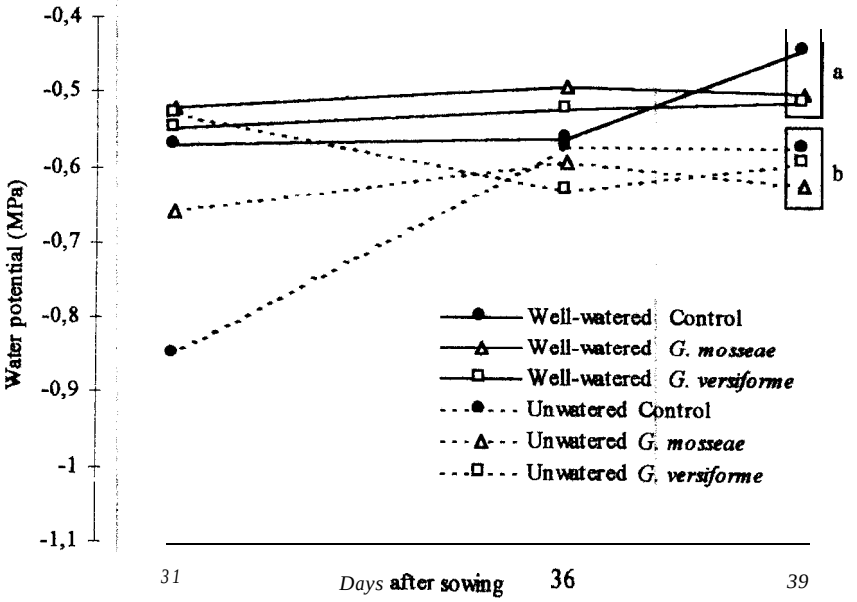

Figure 3. Leaf water potentials (MPa) in cowpea plants colonised by two *Glomus* species or control under two soil rnoisture regimes. See *figure* 2 for legends.

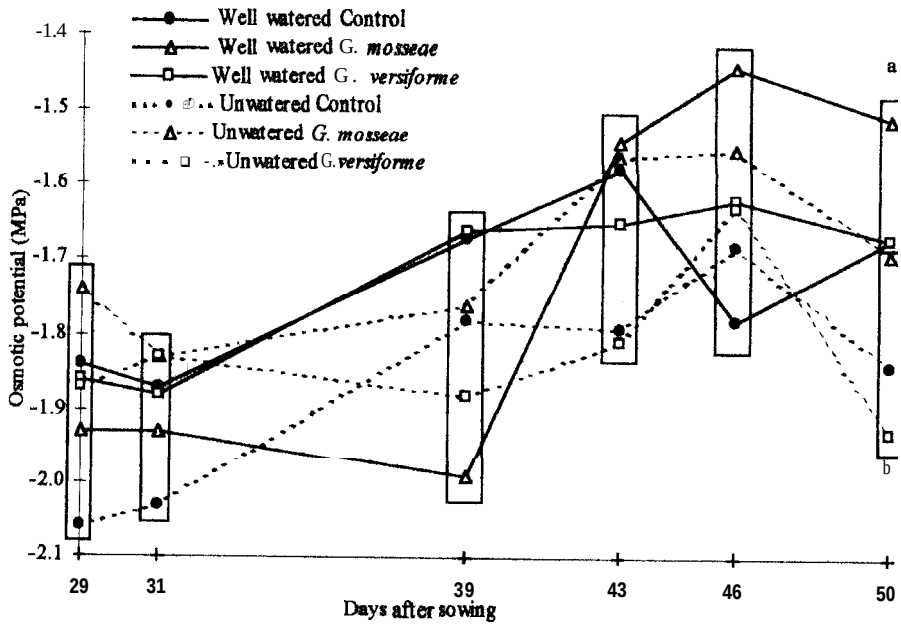

Figure 4. Leaf osmotic potentials (MPa) in non-mycorrhizal and mycorrhizal cowpea plants under two soil moisture regimes and after rewatering. See *figure* 2 for legends.

61 days [9, 10, 53, 54, 55, 61]. In contrast, values of midday Φ_L in non-mycorrhizal pearl millet [*Pennisetum americanum* (L.) Leeke] were as low as -2.9 MPa for field-grown plants subjected to soil drying [30, 31, 57]. In our study, colonisation of roots by *Glomus* allowed Φ_L to be maintained at lower soil water content than in uncolorrised plants. This little variation in Φ_L might explain the maintenance of turgor in cowpea plants. As described for leaf water potential, $\Phi\pi$ was maintained in non-mycorrhizal and mycorrhizal plants in both fully watered and drying conditions. Similar results were observed by Augé et al. [8] in mycorrhizal sorghum that did not exhibit a reduction in $\Phi\pi$ measured before midday. According

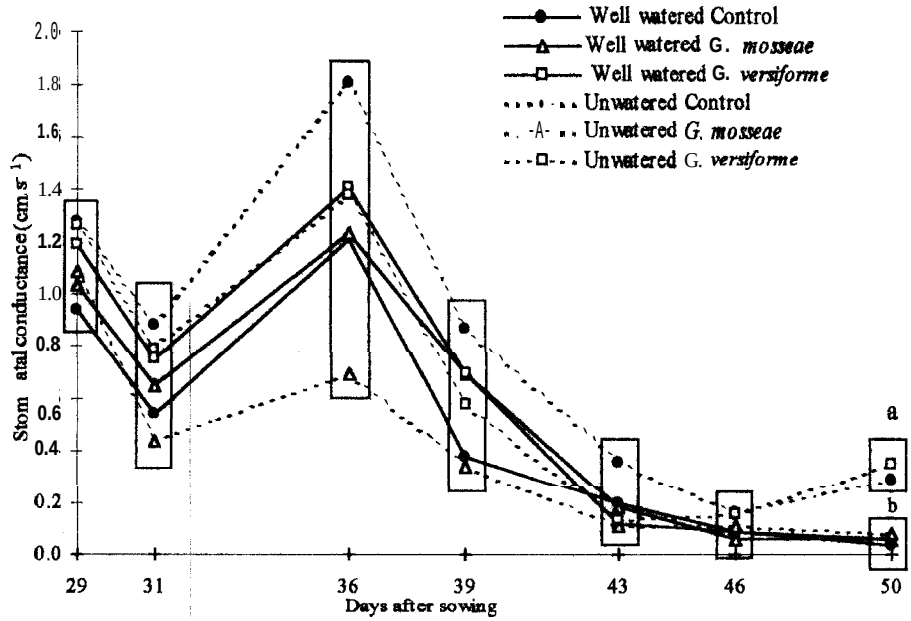

Figure 5. Evolution in leaf stomatal conductance (cm s^{-1}) under two soil moisture regimes and after rewatering in non-mycorrhizal and mycorrhizal cowpea plants. See *figure* 2 for legends.

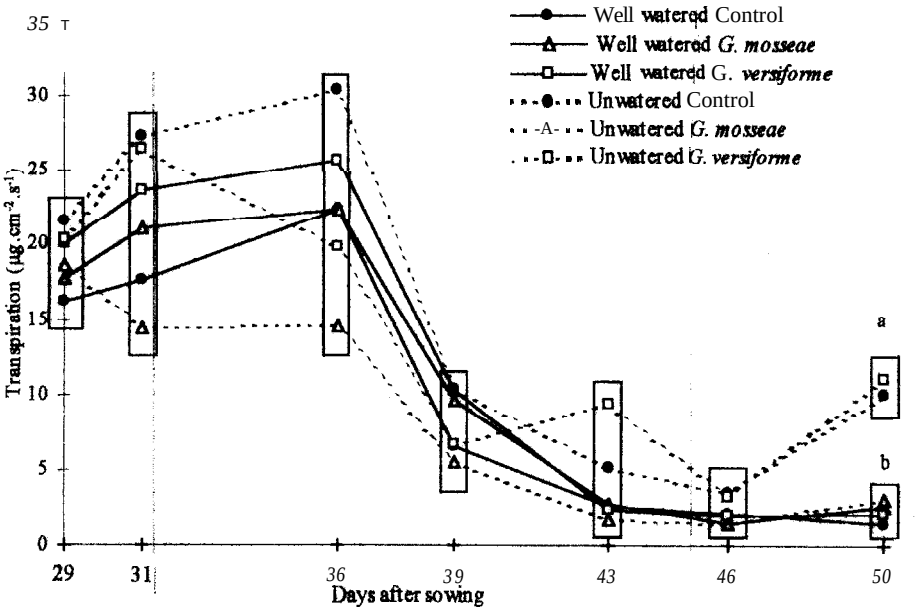

Figure 6. Evolution in leaf transpiration ($\mu g\ cm^{-2}\ s^{-1}$) under two soil moisture regimes and after rewatering in non-mycorrhizal and mycorrhizal cowpea plants. See *figure* 2 for legends.

to these authors, it is possible that measurements of leaf $\Phi\pi$ may not always indicate changes in leaf water status as sensitively as measurements of total leaf Φ_L, if small reduction in water content result in larger Φ_L reductions than $\Phi\pi$ reductions. However, leaf $\Phi\pi$ can be sensitive as or more sensitive than leaf Φ_L as an indicator of decline in soil and plant water status [26, 38]. This response confirms that cowpea is a drought avoider and did not develop osmotic adjustment as a mechanism to resist drought. The maintenance 06 relatively high *RWC* values despite the development of low leaf water potentials by cowpea appears to be a

Table II. Effect of AM fungi on shoot length (cm) of well-watered and water-stressed cowpea plants

Plants	Treatments	Withholding water				After rewatering		
		29 days**	*31* days**	*36* days**	*39* days**	*43* days**	*46* days*	*50* days*
Well-watered	Control	18.1b	15.2b	53.4b	76.9b	100.0b	107.5a	111.6a
	G. *mosseae*	19.4a	24.5a	65.6a	85.5a	112.2a	114.1a	114.7a
	G. *versiforme*	24.0a	28.9a	78.6a	94.1a	102.7a	105.4a	106.1a
Water-stressed	Control	15.2b	16.6b	37.4b	38.9b	41.7b	43.2b	43.5b
	G. mosseae	25.5a	31.7a	61.2a	66.2a	67.2a	67.6ab	68.0ab
	G. versiforme	24.3a	31.6a	84.4a	98.0a	98.1a	98.3a	102.1a

Otherwise as for *table I*; *: $P < 0.05$; **: $P < 0.01$.

Table III. Percentage of root colonisation (%), shoot dry matter (g), root dry matter (g), root/shoot ratios (g/g) and pod dry matter (g/plant) of mycorrhizal and non-mycorrhizal cowpea plants under well-watered (ww) and water-stressed conditions (ws) after harvesting.

Treatments	% infected		shoot dry matter		root dry matter		root/shoot ratios		pod dry matter	
	ww	*ws*	*ww*	*ws*	*ww*	*ws*	*ww*	*ws*	*ww*	*ws*
Control	–	–	3.77a	1.06b	1.25a	0.72a	0.33b	0.53a	3.02a	1.38b
G. mosseae	46.2a	29.3a	1.86b	1.28b	0.93a	0.49a	0.50a	0.38b	1.92b	1.52b
G. versiforme	40.6a	50.6a	3.20a	1.21b	0.94a	0.56a	0.29b	0.60a	3.22a	1.50b

Otherwise as for *table I*.

common trait in drought-resistant species [13]. The significant reduction in $\Phi\pi$ of rehydrated plants at the end of the experiment can be related to the age of the plant.

Stomatal conductance of cowpea was not affected by AM colonisation in well watered conditions as Ebel et al. [20] observed in Vigna *unguiculata cv.* White Acre. However, Ebel et al. [21] reported in a following work that at high soil water contents, mycorrhizal plants had higher stomatal conductance than did non-mycorrhizal plants. The prompt response through stomata1 closure during the flowering stage in unstressed and stressed cowpea confirms the relative sensitivity of this stage. The lower stomatal conductance of G. *mosseae*-colonised plants can be related to quicker soil drying in mycorrhizal pots [20]. Hyphae of the mycorrhizal fungus G. *mosseae* can apparently make significant contributions to water uptake of cowpea roots [24]. Results from this study suggest that mycorrhizal fungi cannot affect the point of stomatal closure during a soil drying episode. In contrast, the maintenance of stomatal opening and transpiration has been observed in mycorrhizal plants of *Rosa hybrida* [3], maize [7], sorghum [8], lettuce [47, 48], and in *Glomus intraradices* colonised *Vigna unguiculata* [19]. In addition, Davies et al. [16] suggested that *gs* might be regulated by xylem [ABA] or ABA flux to leaves. However, the experiments with both attached leaves and detached leaves of cowpea did not suggest the possibility that mycorrhizal symbiosis influenced host gs by altering stomatal sensitivity to ABA [19]. It appears that cowpea, a drought avoiding species such as sorghum, S. *bicolor* (L.) Moench, avoids water deficits by maximising water uptake and minimising water stress.

These physiological mechanisms evolved to withstand drought stress can affect plant growth. Our results show a beneficial effect of AM colonisation on shoot length of cowpea under well-watered regime during the plant cycle. The restrictive effect of water deficit, indicated by the decrease in growth, was observed in non-mycorrhizal and *Glomus* colonised plants. Water deficit significantly reduced shoot dry matter. These results as regards the reduced plant growth during the vegetative stage are not in agreement with those previously reported by Turk et al. [60]. However, the growth of the vegetative organs was among the most sensitive processes during water deficit [35]. In spite of the indeterminate growth habit of this legume, the decrease of the vegetative dry matter was not adjusted after the restoration of watering. The decreased dry matter can be related to the decrease of CO, assimilation. Rapid stomatal closure may have interfered with CO, assimilation, especially during reproductive growth in cpwpea. Stomatal control and the adjustment of transpiration (decreased growth, paraheliotropy) allow the maintenance of plant water status and physiological activity as plant water deficit persist. However, except their reversible characteristic, these morpho-physiological adjustments are analysed like reversed-productive mechanisms because of their direct interaction with carbon assimilation [17]. AM infection was not associated with root dry matter increase in cowpea. Nevertheless, the root dry matter of mycorrhizal plants may even have been underestimated, because part of the external mycelium was presumably lost when soil was washed from the roots.

In conclusion, colonisation by *Glomus* species did not alter significantly the physiological response and plant water status when cowpea plants were subjected to water deficit during the vegetative stage. The common strategy used by cowpea in response to drought remained the prompt stomatal closure.

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