INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–343/AJL/2011/13–2–271–274
http://www.fspublishers.org

Full Length Article

Improved *Acacia senegal* Growth after Inoculation with Arbuscular Mycorrhizal Fungi under Water Deficiency Conditions

MALICK NDIAYE, ERIC CAVALLI[1]†, ANICET GEORGES BRUNO MANGA AND TAHIR ABDOULAYE DIOP
Laboratoire de Biotechnologies des Champignons, Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, BP. 5005 Dakar-Fann, Sénégal
†Service d'Analyse et de Caractérisation, UFR Sciences et techniques, Université de Franche-Comté, 16, route de Gray - 25030 Besançon cedex, France
[1]Corresponding author's e-mails: eric.cavalli@univ-fcomte.fr; lbc@ucad.sn; manicet@yahoo.fr, papmalic@yahoo.fr

ABSTRACT

In a controlled pot experiment, tropical legume tree *Acacia senegal* L. (Wild.) seedlings were inoculated with three different species of arbuscular mycorrhizal fungi (AMF), *Glomus intraradices, G. fasciculatum* and *G. mosseae*. Three water levels (field capacity, moderate water deficiency & severe water deficiency) were applied to the plants after transplantation. Each treatment was replicated 5 times. After twelve weeks of water deficiency, results showed that growth of plants was improved by mycorrhizal inoculation under water deficiency conditions. Inoculation treatments under water deficiency significantly increased biomass, height and shoot water content. In non-inoculated plants, water deficiency reduced most plant growth parameters. Root colonization average varied from 30.4% to 62.5%. Lowest intensity (30.4%) was observed for field capacity associated with *G. intraradices* and the highest root colonization (62.5%) was observed for severe water-deficiency associated with *G. fasciculatum*. AM fungus *G. fasciculatum* can form adequate mycorrhizae with *A. senegal* under water deficiency conditions.

Key Words: Arbuscular mycorrhiza fungi; *Acacia Senegal*; Water deficiency; Sahel

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) form an association with plants, which benefit is enhanced growth (Smith & Read, 2008). This symbiosis enhances plant ability to become established and to cope with stress situations such as nutrient deficiency, drought and soil disturbance (Morte *et al*., 2001; Zhang *et al*., 2006; Bucher, 2007). Thus, mycorrhizal inoculation with suitable fungi has been proposed as a promising tool for improving restoration success in semi-arid degraded areas (Garbaye, 2000). Forest degradation in North Senegal has already had serious effects on well being of rural population. Traditional production of arabic gum from acacia trees is an income-generating activity for most farmers in this area (Wickens *et al*., 1995). However, gum yields have decreased, because of biotic, physical, socioeconomic and institutional reasons (Ballal *et al*., 2005; Sarr *et al*., 2005). There is a need to look at this traditional forest management from a more perspective. Understanding water balance of plant production is of paramount importance for future management and sustainability.

A. senegal is a leguminous agroforestry tree species belonging to the subfamily *Mimosoideae* with a wide natural distribution in Sahelian zone of Africa (Raddad *et al*., 2005). The tree is highly variable with four distinct varieties (Wickens *et al*., 1995). The variety *senegal*, with which we are concerned here, is the main source of arabic gum and a well-established traditional agroforestry tree component (Raddad *et al*., 2005).

Therefore, the aim of this study was to evaluate the effects of mycorrhizal inoculation on the growth and responses of *Acacia senegal* under various water status.

MATERIALS AND METHODS

Planting of materials: Experiment was set in the department of Plant Biology at latitude 14°41'2''N, longitude 17°27'45''W (University Cheikh Anta Diop/Senegal). The soil used in this study was collected from Sangalkam (50 km from Dakar, Senegal). Soil characteristics are given in Table I. Seeds of *A. senegal* were scarified and surface-sterilized with concentrated sulfuric acid for 10 min and washed in sterile distilled water. They were germinated on sterile water agar 0.8% at 30°C in the dark for 48 h. Four germinated seeds were sown into nursery bags, each containing 500 g sandy soil of Sangalkam and thinned to one seedling per pot, 1 week after

To cite this paper: Ndiaye, M., E. Cavalli, A.G.B. Manga and T.A. Diop, 2011. Improved *Acacia senegal* growth after inoculation with arbuscular mycorrhizal fungi under water deficiency conditions. *Int. J. Agric. Biol*., 13: 271–274

emergence. All seedlings were watered daily for 1 week to allow proper establishment.

AMF inoculation: Mycorrhizal inoculums containing indigenous species (*Glomus mosseae*, *G. fasciculatum* or *G. intraradices*) were obtained from the Laboratory of Fungal Biotechnology of the Department of Plant Biology (University Cheikh Anta Diop/Senegal). Mycorrhizal inoculums of each endophyte consisted of mixed soil, spores, mycelium and infected root fragments obtained from pot culture of *Zea mays*. *Glomus* isolate preparations that had similar characteristics (an average of 40 spores per gram & 85% of infected roots) were used as inocula.

Experimental design and growth conditions: Experiment was performed using four treatments: three different AMF, and non inoculated control. Experimental design was a factorial-randomized block with two factors (inoculation & water status level) with five replicates. A 20 g portion of inoculum was added to each bag at the start of growth *A. senegal* seedlings. Plants were raised from January to March in a greenhouse with the following conditions: average day/night temperature 30/25°C and relative humidity between 55% and 65% during the day. Seedlings were subdivided into three sets. In a first set, plants were kept to 100% of field capacity (FC). In a second set (moderate water deficiency), plants were maintained at 50% FC and in the last set (severe water deficiency), plants were maintained at 25% FC. Plants were harvested after 3 months.

Measurements and harvest: Following parameters were first measured: plant height and stem diameters at 2 cm from the base. Roots were then washed free from soil under a stream of tap water and fresh and dry shoots and roots weights were measured. Shoot water content was calculated as ((FW-DW)/FW) X 100, where FW stands for fresh weight and DW for dry weight. Root and shoot dry weights were estimated after oven-drying the samples at 70°C for 48 h. Percentage of mycorrhizal root infection was estimated by microscope 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 (1970). Mycorrhizal colonization was calculated according to gridline intersect method (Giovannetti & Mosse, 1980).

Statistical analysis: Statistical procedures were carried out with the software package R version 2.5. Two factors analysis of variance (ANOVA) was performed to partition the variance into the main effects and the interaction between Inoculation and water status.

RESULTS

Effect of inoculation and water status on growth of *A. Senegal*: Water deficiency conditions (50 & 25% FC) depressed shoot and root dry weights of inoculated plants and control. Compared to control plants, inoculation with any of the three *Glomus* species increased shoot and root dry weight regardless of water regime (Table II). Greatest increases in shoot and root dry biomass of *A. senegal* plants were recorded for *G. fasciculatum* treatment. The other two AMF also increased root and shoot biomass, but to a lesser extent (Table II).

No significant differences on stem diameters were found among the inoculation treatments. Inoculation treatment also improved plants height. Inoculation with *G. fasciculatum* increased this growth values more than with G. *mosseae* or *G. intraradices*. Water deficiency conditions have reduced height, stem diameter and shoot water content of plants. However, a beneficial effect was found for *G. fasciculatum* inoculation, which relatively increased height and shoot water content whatever the water regime applied (Table II). The most effective AMF in increasing growth and drought tolerance was *G. fasciculatum*. The magnitude of the growth response to AMF was more effective in improving root biomass that shoot biomass.

Analysis of variance was conducted on all data recorded from inoculation, water status and interaction between these two factors (Table III). While water status show a significant effect on shoot dry, plant height and stem diameter, statistical results show that inoculation affected shoot dry, root dry, height and shoot water content. Significant effects of interaction inoculation x water status were observed on root dry and plant height. Table III showed that shoot dry, stem diameter and shoot water content were not significantly affected by combination of inoculation and water status.

Mycorrhizal colonization: Roots of control plants were observed after water treatments and indicated the absence of mycorrhizae (Table IV). Results showed that water deficiency increased AM colonization. Root colonization of infected plants ranged from 30.4% to 62.5%. *G. fasciculatum* showed the highest AM colonization compared to other AMF and for all water status. On the other hand, *G. intraradices* showed the lowest AM roots colonization. Results also showed that deficit of water increased root colonization (Table IV).

DISCUSSION

The finding that AMF colonized *A. senegal* plants grew more under water deficient conditions than did the non colonized plants under field capacity conditions is a very important information for Sahelian forestry. These results evidence the high mycorrhizal dependence of *A. senegal* trees to reach optimum development, particularly under water deficiency conditions. AMF infected seedlings showed higher shoot and root dry weight compared to non inoculated seedlings. Present results suggest that symbiosis increase plant tolerance to water deficient conditions. Positive effects of AMF on host-plant growth and development were already observed in low soil fertility conditions (Jeffries, 1987) and also in drought environments (Sylvia *et al.*, 1992; Picone, 2003; Liu *et al.*, 2007).

Table I: Characteristics of the soil used in this study

Components	Contents
Clay	3.6%
Silt	1.6%
Fine silt	2.9%
Fine sand	51%
Coarse sand	40.9%
Organic matter	0.43%
Total C	2.50%
Total N	0.21%
C/N ratio	11.9
Total P (µg g^{-1})	40
Available P (µg g^{-1})	2
pH (sol/water ratio 1:2)	5.7
pH (sol/KCl ratio 1:2)	4.5

Table II: Growth parameters and shoot water content of *A. senegal* in response to mycorrhizal inoculation and water status

Parameters	FC	MWS	SWS
Shoot dry biomass (g.$plant^{-1}$)			
Contol	0.96 a	0.74 b	0.65 b
G. *intraradices*	1.12 a	1.08 a	0.95 a
G. *fasciculatum*	1.19 a	1.25 a	1.26 a
G. *mosseae*	1.1 a	0.94 a	0.78 b
Root dry biomass (g.$plant^{-1}$)			
Contol	0.21 ab	0.19 b	0.15 c
G. *intraradices*	0.22 ab	0.22 b	0.18 bc
G. *fasciculatum*	0.25 a	0.37 a	0.39 a
G. *mosseae*	0.27 a	0.25 b	0.22 b
Height (cm)			
Contol	14.7 a	13.7 b	10 c
G. *intraradices*	15 a	13 b	11 bc
G. *fasciculatum*	16 a	17 a	18 a
G. *mosseae*	16 a	15 a	14 b
Stem diameter (mm)			
Contol	5 a	4.5 a	4.2 a
G. *intraradices*	5 a	4.8 a	4.5 a
G. *fasciculatum*	5.9 a	5.2 a	4.7 a
G. *mosseae*	5.5 a	5.1 a	4.5 a
Shoot water content (%)			
Contol	52.8 a	50.3 b	46.6 c
G. *intraradices*	50.1 ab	47.1 c	45.5 c
G. *fasciculatum*	52.5 a	58.3 a	55.6 a
G. *mosseae*	54.5 a	53.6 b	50.4 b

FC: Field capacity; MWD: Moderate water deficiency; SWD: Severe water deficiency. Values with different letters in a column followed by are significantly different ($p \leq 0.05$)

Table III: Significance level (F-values) of effects of differents factors and factors interaction on variables based on analysis of variance

Variables	Inoculation	Water status	Inoculation: Water status
Shoot dry biomass	13.8***	4.3*	$1.1^{n.s.}$
Root dry biomass	33.7***	1.5^{ns}	5.9***
Height	27.9***	11.7***	5.8***
Stem diameter	2^{ns}	5.4**	0.2^{ns}
Shoot water content	7.8***	2.4^{ns}	1.2^{ns}

*, **, ***, and ns indicate the level of significance at $P \leq 0.05$, 0.01, 0.001 and the absence of significance, respectively
Values with different letters in a column followed by are significantly different ($p \leq 0.05$)

Table IV: Effects of water status on mycorrhizal colonization (%) of *A. Senegal* seedlings. Values in columns followed by different letters are significantly different (p≤0.05)

Treatments	AM colonization (%)		
	FC	MW	SWD
Control	0.0 d	0.0 d	0.0 d
G. intraradices	30.4 c	37.2 c	43.6 c
G. fasciculatum	50.1 a	55.6 a	62.5 a
G. mosseae	40.9 b	50.3 b	53.4 b

FC: Field capacity; MWD: Moderate water deficiency; SWD: Severe water deficiency
Values with different letters in a column followed by are significantly different ($p \leq 0.05$)

G. *fasciculatum* was the most efficient fungus in terms of *A. senegal* plant performance. Several inoculation studies have reported different improvement of host plant growth by different AMF (Slankis, 1973; Dixon *et al*., 1984; Qu *et al*., 2004). In this study, growth enhancement by AM fungi varied widely, confirming different ability of mycorrhizal fungi to enhance seedling growth.

Water deficiency conditions have a postive effect on AM colonization. *G. intraradices* inoculation resulted in poor colonization on *A. senegal* seedlings (Table IV) and thus indicate a probable consequence of inferior plant-symbiont compatibility. AMF can play a relevant role in promoting root growth and symbiotic development, facilitating plant performance in drought environments (Liu *et al*., 2007). In nursery conditions, Patreze and Cordeiro (2004) found different responses to inoculation for three species of a woody tropical legume. These authors also suggested that specific species of AMF should be selected to stimulate plant growth. Positive effects on plant growth observed in seedling by inoculation should be involved with the roots which improve nutrient mineral uptake (Andrade, 2004). It is well known that roots exude supply nutrients to microbial community and that AMF influence soil composition both qualitatively and quantitatively (Andrade *et al*., 1997, 1998). Symbiosis between roots and AMF improved plant growth, especially in semi-arid regions. Positive impacts of inoculation have been demonstrated for forest tree species in Philippines (De La Cruz *et al*., 1988), India (Khan & Uniyal, 1999) and Kenya (Munro *et al*., 1999).

Inoculation effects under water status levels are more obvious by measuring root dry weight, height and symbiotic capacity rather than shoot dry weight, stem diameter and shoot water content. AM fungus *G. fasciculatum* can form adequate mycorrhizae with *A. senegal* under water deficiency conditions.

CONCLUSION

Under water deficiency conditions, AMF enhances plant growth. Symbiotic association between AMF and *A. senegal* trees is therefore, of great interest in arabic gum production. Inoculation with *G. fasciculatum* showed a promise for successful establishment of *A. senegal* seedlings in semi-arid degraded areas.

Acknowledgement: The authors wish to express their gratitude to Asyila Gum Company and Analysis and Characterization Service (SERAC) at University of Franche-Comté in France.

REFERENCES

Andrade, G., 2004. Role of functional groups of microorganisms on the rhizosphere microcosm dynamics. *In*: Varma, A., L. Abbott, D. Werner and R. Hampp (eds.), *Plant Surface Microbiology*, pp: 51–69. Springer-Verlag, Berlin, Germany

Andrade, G., K.L. Mihara, R.G. Linderman and G.J. Bethlenfalvay, 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular mycorrhizal fungi. *Plant Soil*, 192: 71–79

Andrade, G., R.G. Linderman and G.J. Bethlenfalvay, 1998. Bacterial associations with the mycorrhizosphere and hyphosphere of the arbuscular mycorrhizal fungus *Glomus mosseae*. *Plant Soil,* 202: 79–87

Ballal, M.E., E.A. El Siddig, M.A. Elfadl and O. Luukkanen, 2005. Relationship between environmental factors, tapping dates, tapping intensity and gum arabic yield of an *Acacia senegal* plantation in western Sudan. *J. Arid Environ.*, 63: 379–389

Bucher, M., 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytol*., 173: 11–26

De La Cruz, R.E., M.Q. Manalo, N.S. Aggangan and J.D. Tambalo, 1988. Growth of three legume trees inoculated with VA mycorrhizal fungi and Rhizobium. *Plant Soil*, 108: 111–115

Dixon, R.K., H.E. Garrett, G.S. Cox, D.H. Marx and I.L. Sander, 1984. Inoculation of three Quercus species with eleven isolates of ectomycorrhizal fungi. Inoculation success and seedling growth relationships. *For*. *Sci*., 30: 364–372

Garbaye, J., 2000. The role of ectomycorrhizal symbiosis in the resistance of forests to water stress. *Agriculture*, 29: 63–69

Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.*, 84: 489–500

Jeffries, P., 1987. Use of mycorrhizae in agriculture. *Crit*. *Rev*. *Biotechnol*., 5: 319–357

Khan, S.N. and K. Uniyal, 1999. Growth response of two forest tree species to VAM and Rhizobium inoculations. *Indian For*., 125: 1125–1128

Liu, A., C., Plenchette and C. Hamel, 2007. Soil nutrient and water providers: how arbuscular mycorrhizal mycelia support plant performance in a resource limited world. *In*: Hamel, C. and C. Plenchette (eds.), *Mycorrhizae in Crop Production,* pp: 37–66. Haworth Food and Agricultural Products Press, Binghamton, New York

Morte, A., G. Diaz, P. Rodriguez, J.J. Alarcon and M.J. Sanchez-Blanco, 2001. Growth and water relations in mycorrhizal and nonmycorrhizal (*Pinus halepensis*) plants in response to drought. *Biol. Plant*., 44: 263–267

Munro, R.C., J. Wilson, J. Jefwa and K.W. Mbuthia, 1999. A low-cost method of mycorrhizal inoculation improves growth of (*Acacia tortilis*) seedlings in the nursery. *For. Ecol. Manage*., 113: 51–56

Patreze, C.M. and L. Cordeiro, 2004. Nitrogen-fixing and vesicular–arbuscular mycorrhizal symbioses in some tropical legume trees of tribe *Mimoseae*. *For. Ecol. Manag*., 196: 275–285

Philips, J.M. and D.S. Hayman, 1970. Improved procedures for cleaning roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans*. *Brit. Mycol. Soc.*, 55: 93–130

Picone, C., 2003. Managing mycorrhizae for sustainable agriculture in the tropics. *In*: Vandermeer, J.H. (ed.), *Tropical Agroecosystems*, pp. 95–132. CRC Press, Boca

Qu, L.Y., T. Shinano, A.M. Quoreshi, Y. Tamai, M. Osaki and T. Koike, 2004. Allocation of C14 carbon in two species of larch seedlings infected with ectomycorrhizal fungi. *Tree Physiol*., 24: 69–76

Raddad, E.Y., A.A. Salih, M.A. Elfadl, V. Kaarakka and O. Luukkanen, 2005. Symbiotic nitrogen fixation in eight (*Acacia Senegal*) provenances in dryland clays. *Plant Soil*, 275: 261–269

Sarr, A., A. Faye, A. Oihabi, M.A.J.O. Houeïbib, M. Neyra and D. Lesueur, 2005. Inoculation en station et au champ d'*Acacia senegal* avec des souches sélectionnées de Rhizobium. *Bois For. Trop.*, 283: 12–13

Slankis, V., 1973. Hormonal relationships in mycorrhizal development. *In*: Marks, G.C. and T.T. Kozlowski (eds.), *Ectomycorrhiza-their Ecology and Physiology*, pp: 232–298. Academic Press, New York

Smith, S.S. and D.J. Read, 2008. *Mycorrhizal symbiosis*. Cambridge, UK: Academic Press

Sylvia, D.M. and S.E. Williams, 1992. Vesicular arbuscular mycorrhizal and Environmental stress. *In*: Bethlenfalvay, G.J. and R.G. Linderman (eds.), *Mycorrhizae in Sustainable Agriculture*, pp: 101–124. American Society of Agronomy

Wickens, G.E., A.G. Seif El Din, G. Sita and I. Nahal, 1995. *Role of Acacia Species in the Rural Economy of Dry Africa and the Near East*. FAO Conservation Guide n 27. Rome, Italy

Zhang, X.H., A.J. Lin, B.D. Chen, Y.S. Wang, S.E. Smith, and F.A. Smith, 2006. Effects of *Glomus mosseae* on the toxicity of heavy metals to (*Vicia faba*). *J. Environ. Sci.*, 18: 721–726

(Received 01 July 2010; Accepted 17 August 2010)