Effects of Controlled Mycorrhization on Production of *Jacaranda mimosifolia* D.Don

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**ABSTRACT**

Growth and mineral absorption of ornamental woody plant such as *Jacaranda mimosifolia* D. Don., in gnotoxenic conditions out of a mycorrhization by a commercial inoculum containing *Glomus irregulare* were studied. Used *Jacaranda* plants are one- year old, they were first grown, in 10/12cm caliber pots then in 16/18cm caliber pots for 6 months in a sterile substrate consisting of 2/3 commercial peat and 1/3 sand. Four treatments, achieved three times, were processed. Apart from the control, two other treatments consisted of the addition of commercial inoculum containing *Glomus irregulare* to the culture substrate, at a rate of 62.5 mg / plant for the first and 100 mg / plant for the second. These quantities respectively correspond to 100 propagules / plant and 160 propagules / plant. The fourth treatment is illustrated by the incorporation of 4g/plant of OSMOCOT EXACT standard-Scotts fertilizer; 15 + 9 + 12 (+2.5), into the substrate. Mycorrhization rate of inoculated plants by 100mg/plant dose reached 43.21% while it only reached 21.48% for those that received 62.5mg/plant. Colonization remains at the beginning of installation presenting essentially mycorrhizal hyphae structures. Mycorrhization significantly improves nitrogen, phosphorus and potassium nutrition of *Jacaranda*. This result in a stimulation of plant growth Level of mycorrhization by 21.48%, and achieve gains of aerial dry biomass of about 45.5% and root volume of about 46.29%. When the level of mycorrhization is equal to 43.21%, these gains correspond to, respectively, 59.1% and 67.28%. Fertilization provides for gains of 29.58% in aerial dry biomass and 9.69% in plants height. It has no effect on roots volume.
1. INTRODUCTION

Benefits offered by mycorrhizae to host plants, have been widely described, especially for the water uptake (Nouaim and Chaussood, 1996; Marulanda et al., 2006; Aroca et al., 2007) and mineral nutrition (Clark and Zeto, 2000; Graham, 2000; Mäder et al., 2000; Hodge et al., 2001; Aliasgharzad et al., 2009). The vesicular-arbuscular mycorrhizae enable the plant to access a larger volume of soil and a greater surface absorption through fungal hyphae (Bucher, 2007; Smith and Read, 2008)

Since Glomus is the most ubiquitous vesicular-arbuscular mycorrhizal fungi, it is one of the most studied genus. There has been a big interest in mycorrhization by Glomus for the growth of woody plants, such as olive tree (Cilinnesi et al., 1998), Kiwi (Schubert et al., 1992), date palm (Oihabi, 1991), argan tree (Bousselname et al., 2002) and apple tree (Morin et al., 1994), as well as for ornamental woody plants such as Juniperus sabina 'Blue Danube', Cornus stolonifera var coloradensis and Prunus x cisten (Trépanier, 1998). The inoculations are performed in vitro axenic conditions or in gnotoxenic conditions (nursery and on a sterile substrate) (Bousselname et al., 2002).

Jacaranda mimosifolia D. Don is an ornamental woody plant originating from north-western Argentina and southern Brazil (Miyajima et al., 2005). It is used in Tunisia as ornamental plants along avenues and parks. Its mycorrhization may result in better nutrition and growth, as well as smoother adaptation to climatic and urban conditions of Tunisia.

The aim of this study is to evaluate the potential of commercial mycorrhizal inoculum to foster the growth and production of Jacaranda mimosifolia D. Don plants in nurseries.

2. MATERIALS AND METHODS

2.1. Description of the used inoculum

The endomycorrhizal inoculum used is a commercial product. It belongs to the brand MYKE PRO which is produced by the company Premier Tech Ltée de Rivière-du-Loup. This product contains an active ingredient; Glomus irregulare which is an arbuscular mycorrhizal fungi, with a proportion of 1600 propagules / g, a density of 500 g / l and a granulometry of 0.5 mm. The inoculum bag was stored at 4 ° C until use.

2.2. Treatments applied

Four treatments were applied (Table 1). Apart from non fertilized control substrate without inoculum (T), a treatment is conceived through a substrate mixed with diffusion NPK fertilizer containing magnesium and trace elements 15 + 9 + 12 (+2.5): Osmocote EXACT standard-Scotts (F). The detailed composition of this fertilizer is displayed in Table 2. The used fertilizer dose is equal to 4g / plant. The proportions of nitrogen (N), phosphorus (P2O5) and potassium (K2O) sources in accordance with the fertilizer dose are presented in Table 3. The other two treatments comprise two doses of inoculum; 62.5 mg inoculum / plant (100 propagules / seed) and 100 mg of inoculum / plant (160 propagules / plant) (M1 and M2). Each treatment was performed three times, that is each experimental unit included three pots containing only one plant to be assessed. Since all the assessments were carried within a greenhouse, it was assumed that all experimental units were homogenous. An experimental design completely randomized was used according to the following model:

\[ y_{ijk} = \mu + I_i + F_j + \epsilon_{ijk} \]

where \( y_{ijk} \) = is the value of the response measured for the ith inoculum dose (I), jth fertilizer dose (F) in the kth pot

\( \mu \) : overall mean response

\( I_i \) : Effect of the ith inoculum dose (I)

\( F_j \) : effect of the jth fertilizer dose (F)

\( \epsilon_{ijk} \): experimental error.

2.3. Plant Material and Cultivation

The study is carried in an experimental greenhouse, under homogeneous conditions where the temperature was maintained constant at 25°C. The greenhouse is located at the horticultural science laboratory of the “Institut National Agronomique de Tunisie” (INAT). The experiment started in May 2010. The culture duration is 6 months. One- year old and 40cm high, young Jacaranda mimosifolia D. Don plants bare rooted are planted in pots of 10/12cm caliber (Figure 1). To ensure that the treatments are not pre-mycorrhized, roots are collected randomly at various levels of the underground part of each plant. Microscopic observations of colored root fragments revealed that these plants are not mycorrhized since no mycorrhizal structure was detected. Thus, the plants are considered free of any mycorrhizal infection. A slight thinning out of the roots is carried before potting. The growth substrate consists of 2/3 commercial peat and 1/3 sand. Before planting, pots are washed with tap water and then placed in immersion in chlorine bleach (Chlore 12 °) for 48 hours. Peat and sand are disinfected twice at 120 ° C within an autoclave for 15 minutes for two consecutive days in order to eliminate all germs and pathogens that may harm mycorrhizal colonies and avoid introduction of mycorrhizal propagules in non-inoculated cultures (control or fertilized plants).

Jacaranda plants are placed in the greenhouse and are watered to field capacity every two days. After two months of cultures, these plants are transplanted into clod in 16/18 dimension pots (Figure 1). The substrate aimed at repotting also comprises 2/3 peat and 1/3 sand. Pots and culture substrate are disinfected by the same method used in the cultivation.
2.4. Observed parameters

Jacaranda plant growth is evaluated through measurements of their dry biomass, height (Measurement of longest stem from the neck to the top), leaf area and root volume. Leaf area is measured by a planimeter for bench top. The root volume is assessed according to the method of Musick et al. (1965) consisting of comparing the levels of water before and after immersion of the roots in a given volume. The dry biomasses of root and aerial parts are weighed after drying at 60 °C until reaching a constant weight (Heitholt, 1989). The effects of mycorrhization on major elements nutrition of Jacaranda are evaluated by measuring nitrogen, phosphorus and potassium levels in root and aerial parts of the plants. Samples of dry plant material are ground into powder using a grinder. Nitrogen is dosed using the Kjeldahl method (1883). For phosphorus and potassium contents dosing, samples of dried plant powder were calcined in a muffle furnace at a temperature of 450 °C and they were attacked by hydrochloric acid and filtered. Obtained extract is used for the dosage of phosphorus by the monovanadate method through an atomic absorption spectrophotometer (Olsen et al., 1954). For potassium, it was done using flame photometer.

2.5. Evaluation of mycorrhizal colonization

An estimation of root colonization rates is performed not only to determine the levels of mycorrhization of Jacaranda plants inoculated with Glomus irregularare but also to confirm that there was no endomychorrhizal infection of control or fertilized plants. Root fragments are randomly selected at various levels of underground parts of different plants. Intracellular constituents are eliminated from root tissue by thinning out using KOH (10%) and oxidation of organic matter. Fungal structures are preserved. The roots are then stained with fushine acid (0.05% in lactoglycerol) and stored in lactoglycerol (25% lactic acid, 25% glycerol, and 50% water) to dilute the unfixed colorant and prevent drying. Fushine acid is a colorant which fixes chitin of all fungal structures. According to Tellal et al. (2008), root is considered as endomychorrhized when it presents endomychorrhizal structures (mycelium, vesicule and arbuscule). The rate of mycorrhization of a root by endomychorrhizal fungus is obtained through the method of McGonigle and Fitter (1990). For the quantification of endomychorrhizae, microscopic examination (40 to 200x) of 80 roots one-centimeter-length fragments is performed for each plant. The root fragments were randomly selected and put in parallel by groups of 10 between slide and cover. Three readings per fragment were conducted to determine the type of fungus structure.

3. RESULTS

Jacaranda mycorrhization rate recorded 6 months after Glomus irregularare intake is illustrated in Figure 2. Despite intra treatment variability for M2 dose inoculated plants, they display in average, a higher mycorrhization level than those that received M1 dose. The root infection is more significant when there are larger quantities of inoculum. Increasing the inoculum dose from 62.5 mg / plant to 100mg/plant improves the Jacaranda mycorrhization rate up to 50.29%. Thus, this rate increases from 21.48% to 43.21%. Microscopic observations show small colonies dispersed over mycorrhized roots instead of mycorrhizal fungal structures extending over their entire lengths. Colonization is essentially observed under the shape of hyphae, with very few vesicules and arbuscules (Figure 3).

Mycorrhization of Jacaranda at the rate of 43.21% (M2 treatment) improves the plant’s growth, especially the aerial biomass, height and root volume (p = 0.01) (Table 4). In this case, the gains fulfilled respectively correspond to 59.10%, 24.94% and 67.28%.

The mycorrhization values of dry root biomass and leaf area of fertilized and control plants are not significantly different (Table 4). However, the highest values are observed through a mycorrhization of 43.21% (Table 4). Even the effect is not clear; improvements of root biomass and leaf area of the mycorrhized plants are probably related to the symbiotic association. In fact, according to the results shown in Table 6, these two growth parameters are positively and directly correlated with the Jacaranda mycorrhization rate. This mycorrhization increases output by 73.62% of root dry biomass and allow for gaining 56.35% in leaf area compared to control samples. Figure 4 shows the mean dry biomass of Jacaranda treatments, assessed after six months of cultivation. Mycorrhized plants, including the M2 treatment (mycorrhization rate 43.21%) have the greatest dry biomass.

Compared with controls, mycorrhization improves nitrogen and phosphorus levy balance sheet in aerial and root parts of Jacaranda plants (table 5). A potassium yield improvement of 79.34% is also observed at the root parts of M2 Jacaranda treatments (table 5).

For the aerial parts of M1 and M2 treatments, improvements in yields are respectively 55.08% and 57.57% for nitrogen nutrition, and 66.24% and 65.65% for phosphate nutrition. For the root parts of M1 and M2 treatments, improvements in yields are respectively 41.64% and 78.98% for nitrogen nutrition, and 40% and 70.5% for phosphate nutrition.

Fertilization with 4g / plant of Osmocote EXACT standard-Scotts (treatment F) slightly accelerates the growth of Jacaranda compared to controls (Table 4). This improvement is reflected by increases in yields of aerial dry biomass and heights of fertilized plants respectively equal to 29.58% and 9.69%. Fertilization with 4g/plant shows nutritional yield close to that of controls (table 5). Only an improvement of 48.36% of root nitrogen balance is observed.

4. DISCUSSION
In nursery conditions, inoculation of Jacaranda with two doses of Glomus irregulare shows, after 6 months, average levels of mycorrhization. Increasing the inoculum dose provides a greater root colonization. In fact, for equivalent culture substrate quantities, two treatments of inoculum dose were tested: 62.5mg inoculum/ plant and 100mg inoculum/ plant, which correspond to 100 propagules/ seedlings and 160 propagules/ plant. Plants grown in pots containing the highest number of propagules are more mycorrhized because their roots are more likely to be displayed to an endomycorrhizal propagules and be infected by it.

According to Bousselmane et al. (2002), the kinetics of mycorrhization is characterized by three phases: the installation of a mycorrhizal fungus, the rapid increase of the infection and finally the stabilization of the latter, which is represented by a tray (Moss et al., 1981). Based on the observed mycorrhizal structures which are essentially hyphae, root colonization of Jacaranda focuses on the two first phases. The low rate of the exchange and reserve structures reflects a beginning of installation of a symbiotic association between the host plant and Glomus irregulare. The large mycorrhization variability rates of M2 treatments dismiss the fact that the infection may have occurred in the stabilization phase. Mycorrhization rate and duration of symbiotic association establishment depend on the host, the infectious potential of mycorrhizal fungi and the substrate cultivation used (Plenchette and Fardeau, 1988). Bousselmane et al. (2002) obtained mycorrhized argan plants using two strains of Glomus genus, at a rate of 70% after 4 months of their inoculation. The argan tree cultivation was conducted on a similar substrate used for Jacaranda. This delay in the infection of the latter by Glomus irregulare can be attributed to the late inoculation of one- year- old- young plants while, for the argan tree Bousselmane et al. (2002) inoculated seeds. The mycorrhizal potential of an inoculum is greater when it is brought at an early stage, especially in seedlings (Echaire et al., 2008).

Mycorrhization by Glomus irregulare of young Jacaranda plants has improved nutrition especially that of nitrogen and phosphorus, but also that of potassium with less apparent effect. This improvement is much greater than that obtained by fertilization. These results corroborate those of Leye et al. (2009), where mycorrhization with Glomus irregulare, Glomus fasciculatum, Glomus aggregatum and Glomus mosseae on different provenances of Jatropha curcas L. showed, after four months of cultivation in greenhouses, a significant improvement in the phosphorus, nitrogen and potassium plants concentrations. Dione et al. (2004) observed an improvement in the nitrogen absorption of Allocaucarina verticillata (Lam.) L. Johnson and Casuarina equisetifolia (L.) after a mycorrhization with Glomus fasciculatum and Glomus mosseae. These same authors found that mycorrhization with Glomus aggregatum significantly improves phosphorus and potassium absorption of Allocaucarina verticillata and had no effect on that of Casuarina equisetifolia.

Improved potassium nutrition has also been observed in Allocaucarina verticillata after its mycorrhization with Glomus fasciculatum. Bousselmane et al. (2002), observed a positive effect on phosphorus and potassium nutritions of mycorrhized argan tree by two strains of Glomus. No significant effect was found concerning the nitrogen nutrition.

Jacaranda greatly benefits from the action of Glomus irregulare, including its ability to access and take nitrogen and phosphorus from the substrate, which promotes the growth of seedlings. mycorrhization increases the absorption surface of the host plant by fungal hyphae that prolong somehow its root system, allowing access to a greater volume of mineral elements, especially those that are less mobile (N, P, K) (Bolan, 1991).

Mycorrhization of young Jacaranda plants by Glomus irregulare stimulates their mineral nutrition. This phenomenon resulted in a higher production of dry biomass at the aerial part level and a stimulation of the establishment of a highly ramified root system involving much larger root volumes than those of the control samples. The improvement of the growth is clearer for the more colonized plants, i.e. those whose mycorrhization rate is 43.21%. These have the highest stems. However, the results show that the "stem height" parameter is not correlated with the rate of mycorrhization. Several studies have focused on the stimulatory effects of mycorrhizae on biomass production of their host plants, such as Calliandra calothyrsus (Ingleby et al., 2001). Mycorrhization of onion variety "Sivan" by Glomus aggregatum, Glomus fasciculatum and Glomus mosseae allows a significant increase in the average weight of bulbs compared to non-inoculated or fertilized plants (Sow et al., 2008). Laminou Manzo et al. (2009) found a significantly positive effect on the total biomass production of dunes fixing species mycorrhized by Glomus irregulare: Acacia raddiana Savi; Acacia nilotica (L.) Wild Ex Del. var. adansonii; Acacia senegal (L.) Willd; Prosopis chilensis Stunz and Bauhinia rufescens Lam. According to Strullu and Plenchette (1991), the extramatriciels hyphae of arbuscular endomycorrhizal fungus are responsible for this positive effect of mycorrhization on growth and nutrition of host plants, since they allow exploring of a greater volume of substrate.

Based on the results, it appears that the leaf area of young Jacaranda plants is not influenced by Glomus irregulare intake. However, an increase of this parameter is observed for mycorrhized plants at a rate of 43.21%. The results also indicate that it is positively correlated with the rate of mycorrhization (p = 0.05). This effect could be highlighted if the colonization would have reached a more advanced stage and if the exchange structures between the symbiotic and the woody plant had been installed. A positive effect was observed by Schubert et al. (1992) on the leaf surface of mycorrhized kiwi vitroplants (Actinidaria deliciosa) by a strain of Glomus.

5. CONCLUSION
Mycorrhization by *Glomus irregulare* significantly stimulates nutrition and growth of young *Jacaranda* plants cultivated under nursery conditions. The results of this experiment can confirm the advantages of mycorrhizal inoculation for the nursery culture of *Jacaranda* since the growth of inoculated plants is faster than control plants growth. In comparison with yields of fertilized culture, mycorrhization made similar gains, even greater. This promotes the use of mycorrhizae as substitutes for pollutant chemical products. These results are also encouraging to conduct further studies aimed at selecting indigenous strains of mycorrhizae, which are more efficient and better adapted to soil environmental conditions. However, the earlier addition (at seeding) of commercial *inoculum* could have allowed to getting more mycorrhized plants in order to obtain a faster growth.

### Table 1: Treatments applied

| Treatment               | T  | F | Mycorrhized 1 (M1) | Mycorrhized 2 (M2) |
|-------------------------|----|---|--------------------|--------------------|
| Inoculum dose (mg)      | 0  | 0 | 62.5               | 100                |
| Fertilizer dose (g)     | 0  | 4 | 0                  | 0                  |
| Repetitions number      | 3  | 3 | 3                  | 3                  |

### Table 2: Composition of fertilizer Osmocote EXACT standard-Scotts

| Element                  | Concentration (%) |
|--------------------------|-------------------|
| Nitrogen (N)             | 15                |
| Phosphoric anhydride (P2O5) | 9 soluble in neutral ammonium citrate and in water; 6.8 soluble in water |
| Potassium oxide (K2O)    | 12 soluble in water |
| Magnesium oxide (MgO)    | 2.5 and 1.3 soluble in water |
| Bore (B)                 | 0.02 soluble in water |
| Copper (Cu)              | 0.068 and 0.051 soluble in water |
| Total iron (Fe)          | 0.45 and 0.08% chelated by EDTA |
| Manganese (Mn)           | 0.06 et 0.03% soluble in water |
| Molybdenum (Mo)          | 0.025 soluble in water |
| Zinc (Zn)                | 0.02 and 0.013 soluble in water |

### Table 3: Proportions in nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) sources corresponding to the dose of fertilizer applied

| Dose of fertilizer Osmocote Exact (g/plant) | 4 |
|--------------------------------------------|---|
| Quantity brought of Nitrogen source (N:mg/plant) | 600 |
| Quantity brought of phosphorus source (P_2O_5:mg/plant) | 360 |
| Quantity brought of potassium source (K_2O:mg/plant) | 480 |
Figure 1: Young Jacaranda plants cultivated in pots, under greenhouse in a sterile substrate. A: plants cultivated in 10/12 cm caliber pots; B: Plant grown in a 16/18 cm caliber pot.

Figure 2: Mycorrhization rate of jacaranda plants by Glomus irregulare.
M1 = 62.5 mg inoculum/plant
M2 = 100 mg inoculum/plant
T: control
F: 4 g Osmocote/plant

Figure 3: Observed structures on a root fragment of jacaranda mimosifolia D. Don, 6 months after inoculation by a commercial inoculum containing Glomus irregulare. A: Hyphae forming arbuscule (100 times larger picture); B: hyphae (400 times larger picture).
Table 4: Effects of inoculation with *Glomus irregulare* on aerial and root dry biomass, height, foliar surface and root volume of *Jacaranda* plants

| treatment | Aerial dry biomass (g) | Root dry biomass (g) | Stem height (cm) | foliar surface (cm²) | root volume (ml) |
|-----------|------------------------|----------------------|------------------|----------------------|-----------------|
| M1        | 7.01 b                 | 2.21 b               | 49.54 b          | 233.04 b             | 22.34 b         |
| M2        | 9.34 a                 | 6.14 a               | 66.17 a          | 355.22 a             | 36.67 a         |
| F         | 4.95 bc                | 2.13 b               | 55.00 ab         | 280.64 b             | 14 c            |
| T         | 3.82 c                 | 1.62 b               | 49.67 b          | 155.06 c             | 12 c            |

F trait: ** ns ** ns ** ns

R²: 0.93 0.58 0.94 0.50 0.98

Coefficient of variation: 6.85 14.92 5.79 25.54 6.24

** : Significant difference between treatments (p= 0.01)
ns : No significant difference between treatments
(Values followed by the same letter are not significantly different)

Table 5: Effect of inoculation by *Glomus irregulare* on nitrogen, phosphorus and potassium balance sheets taking of the aerial and root parts of *Jacaranda* plants

| Treatment | Aerial part | Root part |
|-----------|-------------|-----------|
|           | N (10⁻² g) | P (10⁻² g) | K (10⁻² g) | N (10⁻² g) | P (10⁻² g) | K (10⁻² g) |
| M1        | 8.37 a      | 4.65 a     | 18.23 ba   | 2.45 b      | 1.20 b      | 1.29 b      |
| M2        | 8.86 a      | 4.57 a     | 38.17 a    | 6.80 a      | 2.44 a      | 5.42 a      |
| F         | 6.14 ba     | 2.10 b     | 10.20 ba   | 2.77 b      | 1.03 bc     | 1.62 b      |
| T         | 3.76 b      | 1.57 b     | 4.91 b     | 1.43 c      | 0.72 c      | 1.12 b      |

F trait: * ns ** ns **

R²: 0.82 0.95 0.56 0.98 0.96 0.99

Coefficient of variation: 20.28 14.99 88.28 12.67 15.63 13.28

** : Significant difference between treatments (p= 0.01)
* : Significant difference between treatments (p= 0.05)
ns : No significant difference between treatments
(Values followed by the same letter are not significantly different)

Figure 4: Mean dry biomass of *Jacaranda* treatments evaluated after six months of culture.

M1 = 62.5 mg inoculum/plant
M2 = 100 mg inoculum/plant
F = 4 g Osmocote/plant
T = control
Table 6: Correlation between the mycorrhization rate and growth parameters of *Jacaranda*, after 6 months from the inoculation with *Glomus irregulare* (Pearson correlation coefficient, $N = 12$)

| Mycorrhization rate | Aerial biomass dry | Root biomass dry | Root volume | Stem height | Surface foliaire |
|---------------------|--------------------|------------------|-------------|-------------|------------------|
|                     | 0.96 **            | 0.83 **          | 0.98 **     | 0.54 ns     | 0.62 *           |

** The correlation is significant at the level of 0.01
* The correlation is significant at the level of 0.05
ns No correlation

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