

Study on biotic and abiotic factors explaining Acacia saligna growth heterogeneity in revegetated Terga pit in western coast of Algeria

Nouria Derkaoui¹, Zoulikha Bouchiba¹, Zineb Faiza Boukhatem¹, Nouredine Yahia², Robin Duponnois³, Ezékiel Baudoin³, Abdelkader Bekki¹

1. Laboratoire de Biotechnologie des Rhizobiums et Amélioration des Plantes, Département de Biotechnologie. Université d'Oran 1 Ahmed Ben Bella, Es Senia, Algeria.
2. Laboratoire Génétique et Amélioration des Plantes Département. De Biologie. Faculté des Sciences Naturelles et de la Vie. Université d'Oran 1 Ahmed Ben Bella. B.P. 16 Es-senia, Oran, Algérie.
3. IRD, UMR 113, laboratoire des symbioses tropicales et méditerranéennes, campus Cirad de Baillarguet, TA-A 82/J, 34398 Montpellier cedex 5, France.

Corresponding author email: nouria64@live.fr

ABSTRACT: Excessive sand quarrying and extraction generally caused vegetation cover disappearance and erosion processes amplification. To fight against this phenomenon, the rehabilitation of career seems necessary. A revegetation trial in Terga sand pit (Ain Témouchent; Algeria) was conducted in 2010 by the introduction of Acacia saligna inoculated with selected Bacteria Nodulating Legumes (BNL) and endomycorrhizal fungi. However, after two years, significant heterogeneity between introduced Acacias biomass was observed on the replanted site. The objective of our work was to study whether biotic or abiotic factors were responsible for this heterogeneity. Seedlings of A. saligna, inoculated with legumes and grasses roots taken from the studied site, were planted on barren soil and non-sterile soil taken from three zones where heterogeneity in biomass was observed. Soil analyses of the three selected zones were quite close. After four months of cultivation in greenhouse, the effects of different inoculants on plant growth were evaluated by measuring plant height, aerial biomass and the frequency and intensity of mycorrhizal infection. The frequency and the average intensity of endomycorrhizal colonization of plant roots grown on sterile soil were higher than non sterile one. These results indicated that this heterogeneity is due to the adaptation and competitiveness of introduced inoculum and not to abiotic factors represented by soil physic-chemical parameters.

Key words: Exploitation, Acacia saligna, revégétation, endomycorrhizal fungi, biotic factor, abiotic factor.

INTRODUCTION

Natural ecosystems are under great stress when subjected to overgrazing, accidental fires, and industrial activities such as the opening of quarries, mines or new communication roads (Brunel et al., 2007). This anthropization could lead to soil weakening and increasing of erosion impact in the surface layer (Pieri, 1991) resulting in vegetation degradation. In addition to soil physical and chemical characteristics, telluric microorganisms, including bacteria and fungi which are essential for plant life, are also affected (Requina et al., 2001). Mycorrhizal fungi are part of microbial components sensitive to such damage (Duponnois et al., 2001). The use of exotic trees has been recommended as a sustainable management option for improving the productivity and biodiversity of disturbed ecosystems (Cossalter, 1987) by rehabilitating the physical, chemical and biological properties of degraded soils (Duponnois et al., 2005a). Exotic, fast growing species have generally been successfully used for revegetation (Dumancic and Houérou, 1980; Gasques and Garcia-Fayos, 2004; Jeddi et al., 2009; Tiedeman and Johnson, 1992).

Acacia saligna (Labill.) Wendl. (A. cyanophylla Lindley) is originated from South Western Australia and was introduced in Algeria since 1870. It is considered as one of the most used exotic plants for land rehabilitation (El-Lakany, 1987; Tiedeman and Johnson, 2004). This species is a fast growing plant that thrives in areas with low rainfall (250 mm) and in saline and alkaline soils (Vercoe, 1987; Fox, 1995; Thomson, 1987; Amrani et al., 2010). Despite its resistance to limestone and sea spray, this plant is still facing environmental constraints such as low levels of phosphorus and nitrogen in the soil (Brockwell et al., 2005). Mycorrhizal

symbiosis is a relationship involving the exchange of metabolic resources between plants and fungi. The symbiosis is one of the most common and extensively studied biological associations between plants and microorganisms. In this relationship, the fungi improve water and mineral nutrition for the plant, and in return, the fungi receive photosynthesis products (Marx, 1969; Smith and Read, 1997). Studies have shown that 80% of terrestrial plant species, 90% of vascular plant species and more than 95% of all plant families are mycorrhized (Harley, 1989; Allen, 1991; Smith and Read, 1997). In addition, rhizobia, aerobic soil bacteria, have the ability to form root nodules in symbiosis with plants of the family Fabaceae. The host plant provides the BNL (Bacteria Nodulating Legumes) with carbon substrates produced by photosynthesis, in return, the bacteria will fix and reduce atmospheric nitrogen into ammonium, for a direct assimilation by the host plants. These fungal and bacterial symbionts are among the key elements for sustainable agroforestry, which is able to improve plant growth even when they grow in adverse soil conditions; AMF (Arbuscular Mycorrhizal Fungi) and rhizobia allow better growth of host plants through improved mineral nutrition, especially for phosphate (AMF) and nitrogen (BNL).

A. saligna is a leguminous tree which has the advantage of establishing symbiosis with AMF and BNL. A rehabilitation trial was conducted to revegetate the Terga quarry in the region of Ain Témouchent (Western coast) in Algeria by the introduction of *A. saligna* seedlings inoculated with rhizobia and selected mycorrhizal fungi. After two years, however, significant heterogeneity in introduced Acacias' biomass was observed on site in some plots. It is in this context that we focused on the trial rehabilitation, with the aim to study the biotic or abiotic factors responsible for this heterogeneity observed in situ.

MATERIALS AND METHODS

Site of study and sampling protocol

The revegetated site of Terga sandpit presented heterogeneity in established trees' growth. We selected three zones of sampling on the basis of *A. saligna* density. The first zone (zone 1) was characterized by strong growth of Acacias associated with spontaneous accompanying legumes (*Ononis natrix* and *lotus creticus*). The second zone (zone 2) was characterized by weak growth of trees and total absence of other vegetation. The third zone (zone 3) was characterized by weak growth of Acacias associated with herbaceous species and grasses.

Soil sampling was performed for each zone, according to the grid method described by (Pauwels et al., 1992). For each zone, thirty five soil sub-samples (about 700 g) were collected around each plant (it exists an interval of 3 m between trees) at 20 cm depth; representative soil homogenates of each zone were prepared by mixing the various individual sub-samples.

Seeds' disinfection and scarification

Seeds of *A. saligna* were collected the year preceding soil sampling from revegetated sandpit from the collection of laboratory of Rhizobia Biotechnology and Plant Amelioration (LBRAP). *Acacia saligna* seeds were scarified with sulfuric acid (95%) for 90 minutes and then rinsed thoroughly with sterile distilled water. The seeds were then germinated in Petri dishes containing 0.8% water agar. The prepared dishes were placed in the dark at 28°C for four days.

Fungal material

The accompanying legumes' roots of *Lotus creticus* and *Ononis natrix* (R1) present at zone 1, as well as grasses and herbaceous' roots in zone 3 (R3) were picked randomly for mycorrhizal inoculation.

Plant culture substrate and experimental inoculation

Culture substrate was a soil homogenate representative of each zone. Physico-chemical properties of the three zones' soils were characterized: nitrogen, assimilable phosphorus, organic matter and ph were assessed. Assays were conducted on sterilized soil of the three zones by autoclaving at 120°C for one hour at 24 hours intervals, 3 times and on natural soil from zone 2.

Well germinated *A. saligna* seedlings were transferred aseptically into PVC tubes filled with 700 g of soil, taken from bulk soil for each zone. Three treatments were carried out for autoclaved soils of zone1, zone 2 and zone 3, and natural soil of zone 2: inoculation with spontaneous legumes mixed roots (R1: *Lotus creticus* and *Ononis natrix*), inoculation with roots of herbaceous species and grasses (R3) and a non-inoculated treatment was included as control. Eight replicates were conducted for each treatment. A total of 96 plants were followed up for four months.

Inoculation took place at seedlings transfer time. It consisted in pushing in the middle of each pot containing the growth substrate, 1 g of root inoculum at 2-3 cm depth.

The experimental design was randomized at glasshouse. The plants were watered with nutrient solution (Dilworth and Broughton, 1971) devoid of nitrogen, alternating with sterile distilled water.

Estimation of endomycorrhizal infection rates

The demonstration of endomycorrhizal infection was achieved by staining the roots of the selected plants according to the method of (Phillips and Hayman, 1970). Thirty root fragments, taken from the root system of four plants per treatment were examined. The roots were first washed thoroughly with tap water and then placed in a solution of potassium hydroxide (KOH) 10% for 45 minutes at 90°C in water bath. The roots were then rinsed with tap water and cleared in a solution of lactic acid for 20 minutes at room temperature.

Thereafter they were stained in a solution of trypan blue 0.1% for 20 minutes. The treated roots were cut into fragments of about 1 cm length. Thirty randomly chosen fragments are mounted between slide and coverslip, with 10 fragments per slide. The fragments were observed by light microscopy (40x). To assess the degree of endomycorrhizal infection of roots, we used the method described by (Trouvelot et al., 1986). The degree of endomycorrhizal colonization of each fragment was estimated using a scale of six classes rated from zero (0) to five (5) set by (Trouvelot et al., 1986) using the following formulas:

Frequency of mycorrhization (F%)

$$F\% = 100 \times (N0 - n0) / N$$

With, N: number of observed fragments and n0: number of non-mycorrhizal fragments. Intensity of mycorrhization (M%)

$$M\% = (95 n5 + 70 n4 + 30 n3 + 5 n2 + n1) / N$$

With, n = number of fragments assigned with the index 0, 1, 2, 3, 4 or 5.

0: no, 1: trace, 2: less than 10%, 3: 11 to 50%, 4: 51 to 90%, 5: more than 91%.

The measured parameters

After 4 months of culture, plant height was measured and the shoot biomass was evaluated after plant drying in oven at 60°C for 3 days. Mycorrhizal parameters were also measured on samples of fine colored roots as mentioned. The frequency and intensity of mycorrhiza were recorded. Root nodules were observed and recorded.

Statistical analysis

All collected data for the various parameters were subjected to an analysis of variance (ANOVA) with XLSTAT-Pro 7.5 software and the statistical variable means were compared by using Newman Keuls test at (P = 5%).

RESULTS

Soil characterization

Soil physico-chemical analysis of the three studied zones in Terga sand pit (Table 1) shown that all were alkaline and showed low available phosphorus and organic matter levels. The soil of zone 2 was poorer in nitrogen than soils of zones 1 and 3.

Plant height

After 4 months of growth in greenhouse, plant height varied depending on soil and inocula type (Figure 1). Plants growth on sterilized soil of Zone 1 and 2 showed a relatively better vegetal development compared to those grown on barren soil of Zone 3. Generally, four replicates on eight of inoculated plants with (R1) presented root nodules in the three sterilized soils and native Zone 2 soil. Otherwise; nodulated plants shown a better growth compared with non nodulated ones.

For plants grown on sterile Zone 1 soil, height of plants inoculated with legume roots of Zone 1 (R1) was significantly greater than that of non-inoculated controls. No significant difference was observed between the plants inoculated with the roots of herbs (R3) and uninoculated plants. Similarly no significant difference was noted between the plants inoculated with the roots of legumes (R1) and plants inoculated by the roots of herbs (R3).

For the sterile soil of Zone 2, significant differences between inoculated and non-inoculated plants were observed as well as between inoculated plants with legume roots (R1) and those inoculated with herbs' roots (R3). The highest values were obtained with inoculum (R1).

Statistical analysis showed no significant difference between inoculated plants and control for non-sterile soil in zone 2.

Concerning Zone 3 sterile soil, there were no significant differences between inoculated plants with legume roots (R1) and controls. In this case, (R3) inoculation had afforded the highest values.

Biomass

Shoot dry matter of plants of different treatments, showed variability between different treatments

(Figure 2). Statistical analysis showed highly significant differences between plants depending on soil type and inoculum used. Indeed, the aboveground biomass of all the inoculated plants was significantly higher than control plants, except for the treatment of plants grown on non-sterile soil in zone 2.

Endomycorrhizal infection parameters of *Acacia saligna* plants

Statistical analysis showed a significant difference between both treatments (R1 and R3) and controls (table 2).

For Zone 1 soil, the frequency of mycorrhization for inoculated plants with (R1) and (R3) were 96% and 92% respectively, which were not significantly different, the same observations were obtained for mycorrhizal intensity.

In Zone 3 soil, better frequency and intensity of root mycorrhization were obtained with (R3) (78% and 45.12% respectively) than (R1) treatment (51% and 24.14% respectively).

The highest values of mycorrhizal frequency and intensity were reported in plants grown on Zone 2 sterilized soil with a frequency and intensity of 98% and 63.10% respectively.

On another hand, inoculated plants grown on Zone 2 non-sterilized soil presented the lowest values of mycorrhizal frequency and intensity. Even for control plants which grown in this natural soil, they presented endomycorrhizal structures but at a weak rate (30% of frequency and 1% of intensity). The mycorrhizal intensity appeared correlated with frequency in all treatments.

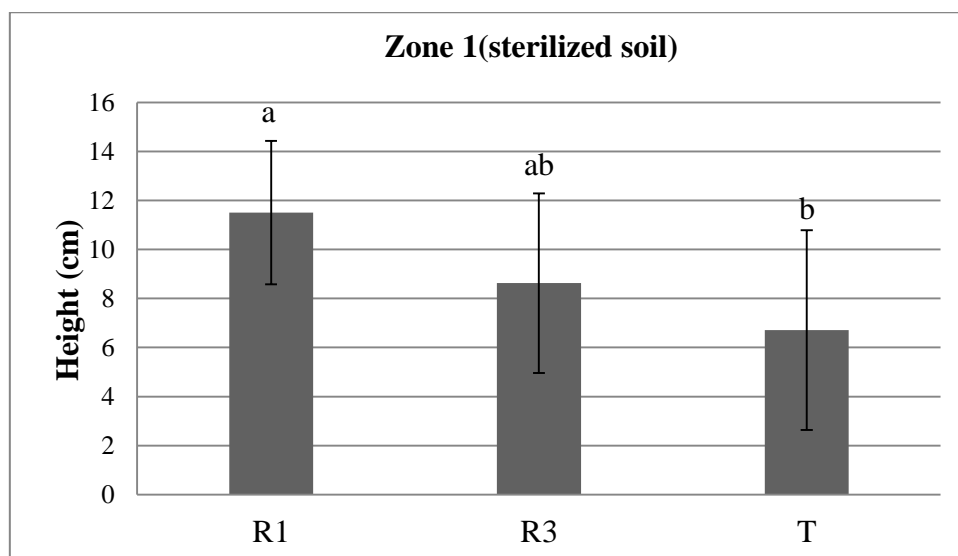
Table 1. Physicochemical characteristics of the three soils

Soil	pH	Total nitrogen (%)	Assimilable phosphorus (ppm)	Organic matter (%)
Zone 1	8	0.7	23	1.9
Zone 2	8.2	0.4	20	2.3
Zone 3	8.2	0.9	19	2.6

Table 2. Frequency and intensity of mycorrhization of *Acacia saligna* plants inoculated and non-inoculated with mycorrhizal inocula after four months of growing

Zones	inoculum	Mycorrhizal frequency %	Mycorrhizal intensity %
Zone 1(sterilized soil)	R1	94,250 a	57,203 a
	R3	92,000 a	46,943 a
	T	0,000 b	0,000 b
Zone 2 (sterilized soil)	R1	98,250 a	63,105 a
	R3	94,750 a	61,875 a
	T	0,000 b	0,000 b
Zone 3 (sterilized soil)	R1	77,750 a	45,123 a
	R3	51,000 b	24,140 b
	T	0,000 c	0,000 c
Zone 2 (natural soil)	R1	66,663 a	21,465 a
	R3	63,330 a	25,728 a
	T	30,413 b	1,080 b

T: uninoculated, R1: inoculated by legumes' roots, R3: inoculated by the herbaceous roots). The values that share a common letter are not significantly different according to the Newman-Keuls test at level 5%.



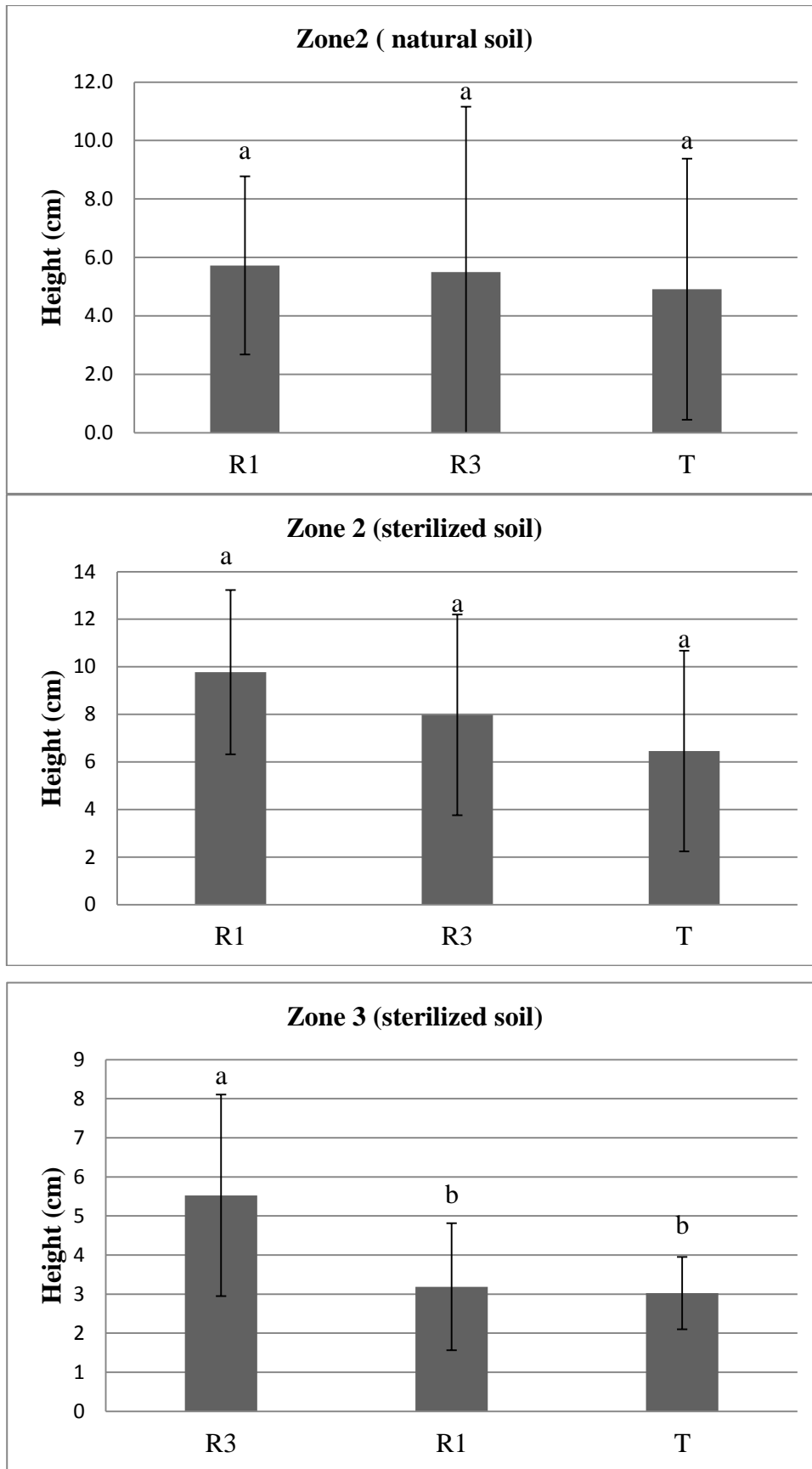
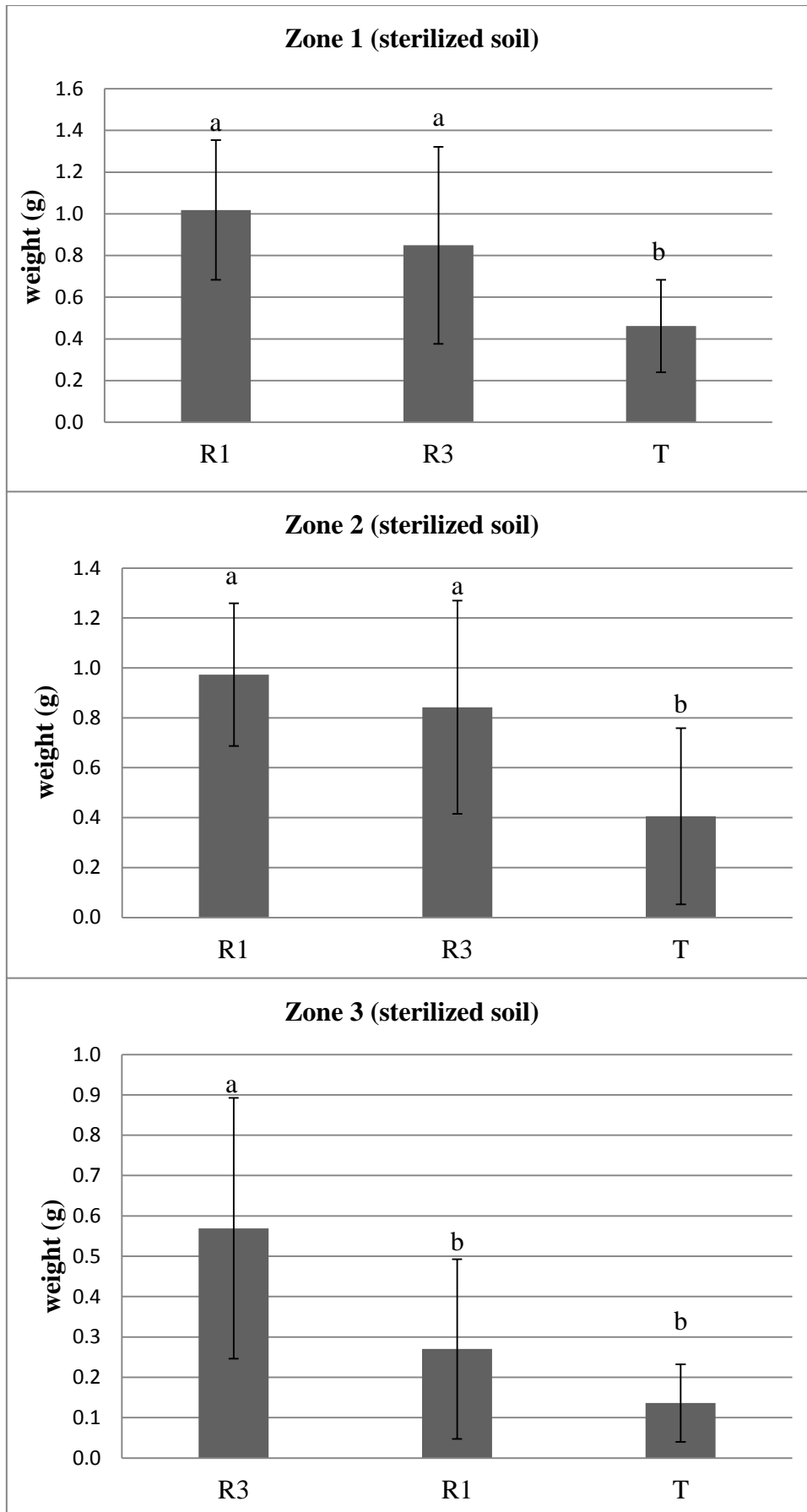


Figure1. High of *Acacia saligna* plants inoculated and non-inoculated with mycorrhizal inocula after four months of growing (T: uninoculated, R1: inoculated by legumes' roots, R3: inoculated by the herbaceous roots). The values that share a common letter are not significantly different according to the Newman-Keuls test at level 5%.



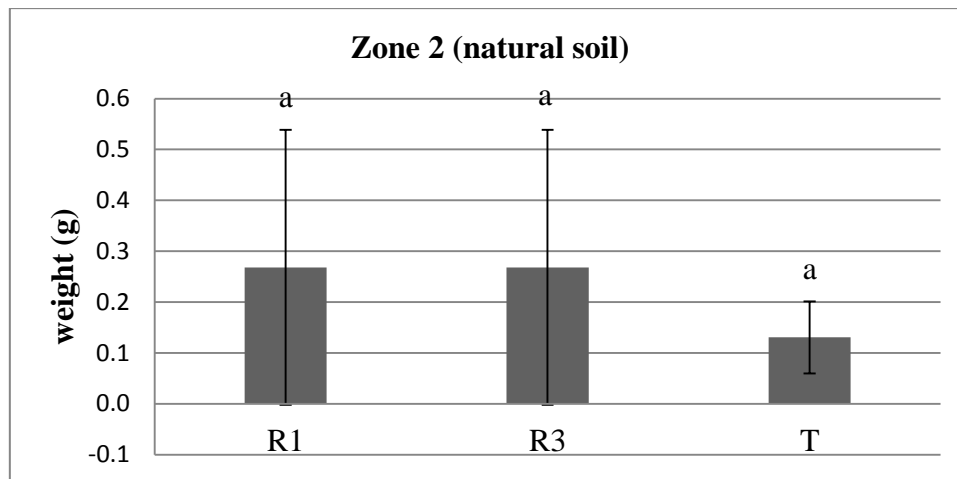


Figure 2. Aerial Biomass of *Acacia saligna* plants inoculated and non-inoculated with mycorrhizal inocula after four months of growing (T: uninoculated, R1: inoculated by legumes' roots, R3: inoculated by the herbaceous roots). The values that share a common letter are not significantly different according to the Newman-Keuls test at level 5%.

DISCUSSION

This work evaluated and compared the response of *A. saligna* to mycorrhizal inoculation, grown on sterilized soil of three zones (Zone 1, 2 and 3) and non-sterilized soil of zone 2. These revegetated zones had shown heterogeneity in growth in the rehabilitated Terga sandpit.

The obtained results clearly shown that soil and mycorrhizal inoculation had an effect on the weight and height of *A. saligna* plants in greenhouse. Even soil physico-chemical characters were quite close for the three studied soils; this parameter shown a significant effect on plant growth. We could assume that the observed differences in the measured growth parameters are mainly due to the effect of inoculation. Mycorrhizal fungi can promote plant growth by improving their mineral nutrition of phosphate in particular (Bolan, 1991; Plenchette and Fardeau, 1988; Sanders and Tinker, 1971; Duponnois et al., 2001) and water uptake (George et al., 1992; Sieverding, 1991), But if the nutrient is available, the plant will have no recourse to form this symbiosis (Ojala et al., 1983), this was confirmed by several authors (Dommergues and Mangenot 1970; Gianinazzi Pearson and Gianinazzi, 1986; Plenchette, 1982; Strull, 1991) who argue that this symbiosis will be established and will grow gradually with the depletion of nutrients readily available to plant roots in the soil. In our case, the three studied soils shown low assimilable phosphorus content which permitted the symbiosis establishment.

Results on biomass showed that inoculation significantly improved dry matter production. These results are consistent with those obtained in the case of *Acacia holosericea* inoculated with *Glomus intraradices* (Duponnois et al., 2005b) and *Acacia Senegal* inoculated with mixed spores of arbuscular mycorrhizal fungi (AMF) from different genera of *Glomus*, *Gigaspora* and *Acaulospora* (Ndiaye et al., 2011). It has been shown that controlled mycorrhizal symbiosis can significantly improve the growth of Australian *Acacias* in degraded soils (Cornet and Diem, 1982; Duponnois and Plenchette, 2003; Duponnois et al., 2007).

The results of the greenhouse experiment showed a significant reduction in growth when the plants were grown in non-sterile soil. In addition, this growth decline was accompanied by significant reductions in root colonization by AM fungi. The adaptive capacity of inoculum to soil environment, its extraradical development and its competitiveness with indigenous microflora were important parameters (Caravaca et al., 2003). Microbial interactions and native AM fungi may decrease the promoting effect of the introduced AM fungal isolate on the plant growth (Duponnois et al., 2005a). Indeed, competition between endomycorrhizal strains could negatively affect the nutritional relationship with the plant species (van der Heijden, 2006). Ba et al (1996) reported that the inoculation is beneficial only if the used strains were more competitive than the existing strains in soil. (Coperman et al., 1996) stated that mycorrhizal fungi behavior and their effectiveness would be related to their background and precisely to environmental factors of their habitats. This suggests adaptation of this inoculum to natural environment conditions (abiotic and biotic conditions defined by the ground).

CONCLUSION

Although the level of heterogeneity of *Acacia* plants' development is well expressed, the physicochemical analyzes of 3 zones soil exhibited almost similar characters; this allows us to deduce that the main factor responsible for this discrepancies in the outcome of rehabilitation is not related to soil characteristic

(abiotic factors). Inoculation induced better plant growth in autoclaved soils than in non-sterilized soils. Also inoculation with legumes' roots (R1) induced a better growth than inoculation with grasses and herbaceous' roots (R3). This can be explained by the compatibility between the inoculum issued from legumes and *A. saligna* species. It appears that the controlled mycorrhization of *A. saligna* on sterilized soil was a beneficial tool in improving the survival and productivity of *Acacia* species; however inoculum behaviour in natural soil depended drastically on its competitiveness with native microflora. This is why inoculation on natural soil is an important step before conducting trials on the field and consequently for its use in degraded sites rehabilitation.

REFERENCES

- Allen MF. 1991. Ecology of Mycorrhizae. 184.
- Amrani A, Noureddine NE, Bhatnagar T, Argandona M, Nieto JJ, Vargas C. 2010. Phenotypic and genotypic characterization of rhizobia associated with *Acacia saligna* (Labill.) Wendl. In nurseries from Algeria. *Syst Appl Microbiol* 33: 44 – 51.
- Broughton WJ, Dilworth MJ. 1971. Control of leghaemoglobin synthesis in snake beans. *Biochem J* 125: 1075– 1080.
- Bolan NS. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plant. *Plant Soil* 134, 189–207.
- Bâ AM, Dalpé Y, Guissou T. 1996. Les Glomales d'*Acacia holosericea* et d'*Acacia mangium*. *Bois et Forêts des Tropiques*, **250**: 5-18.
- Brockwell J, Searle SD, Jeavons AC, Waayers M. 2005. Nitrogen Fixation in Acacias: An Untapped Resource for Sustainable Plantations, Farm Forestry and Land Reclamation, ACIAR, Canberra.
- Brunel B, Domergue O, Maure L, Brahic P, Galiana A, Josa R, De Lajudie P, Attallah T, Risk H, El-Hajj S. 2007. Potentialité des associations symbiotiques plantes–micro-organismes pour réhabiliter des sites fortement dégradés en milieu méditerranéen. *CAHIERS AGRICULTURES*16(4): 324-329.
- Cornet F, Diem HG. 1982. Etude comparative de l'efficacité des souches de *Rhizobium* d'*Acacia* isolées de sols du Sénégal et effet de la double symbiose *Rhizobium*—*Glomus mosseae* sur la croissance de *Acacia holosericea* et *A. raddiana*. *Bois Forêts Trop.*198, 3– 15.
- Cossalter C. 1987. Introduction of Australian acacias into dry, tropical West Africa. *For. Ecol. Manage.* 16, 367–389.
- Coperman RH, Martin CA, Stutz JC. 1996. Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soils. *Hortic. Sci.*, 31: 341–344.
- Caravaca, F., Figuerola, D., Barea, J.M., Azcon-Aguilar, C., Plenzuela, J., Roldan, A. 2003. The role of relict vegetation in maintaining physical, chemical and biological properties in an abandoned *Stipa*-grass agroecosystem. *Arid Land Res. Manage.*17, 103–111.
- Dommergues YR, Mangenot F. 1970. *Ecologie Microbienne du Sol*. Edition Masson: Paris; 796 p.
- Dumancic D, Le Houérou H.N. 1980. *Acacia cyanophylla* Lindl. as supplementary feed for small stock in Lybia. In: Le Houérou, H.N. (Ed.), *Browse in Africa. The Current State of Knowledge*. Internat. Livestock Center for Africa, Addis Ababa.
- Duponnois R, Plenchette C, Thioulouse J, Cadet P. 2001. The mycorrhizal soil infectivity and arbuscular mycorrhizal fungal spore communities in soils of different aged fallows in Senegal. *Appl Soil Ecol* 17: 239–251.
- Duponnois R, Plenchette C. 2003. A Mycorrhiza helper Bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species. *Mycorrhiza* 13, 85–91.
- Duponnois R, Paugy M, Thioulouse J, Masse D, Lepage M. 2005a. Functional diversity of soil microbial community, rock phosphate dissolution and growth of *Acacia seyal* as influenced by grass-, litter- and soil-feeding termite nest structure amendments. *Geoderma* 124(3-4) :349–361.
- Duponnois R, Colombet A, Hien V, Thioulouse J. 2005b. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. *Soil Biol Biochem* 37:1460–1468.
- Duponnois R, Plenchette C, Prin Y, Ducousso M, Kisa M, Ba AM, Galiana A. 2007. Use of mycorrhizal inoculation to improve reforestation process with Australian *Acacia* in Sahelian ecozones. *Ecol Eng* 29: 105– 112.
- El-Lakany HH. 1987. Protective and productive tree plantations for desert development. Proc. of the 2nd International Conference on Desert Development, 25–31 January, 1987, Cairo, Egypt.
- Fox JED. 1995. A review of the ecological characteristics of *Acacia saligna* (Labill.) H. Wendl, *Mulga Research Centre Journal* 12: 39–55.
- Gianinazzi-Pearson V, Gianinazzi S. 1986. The physiology of improved phosphate nutrition in mycorrhizal plants. In *Les Mycorrhizes, Physiologie et Génétique*. INRA: Paris; 101-109.
- George E, Haussler K, Vetterlein G, Gorgus E, Marschner H. 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Can. J. Bot.* 70, 2130–2137.
- Gasque M, Garcia-Fayos, P. 2004. Interaction between *Stipa tenacissima* and *Pinus halepensis*: consequences for reforestation and the dynamics of grass steppes in semi-
- Harley JL. 1989. The significance of mycorrhiza. *Mycol Res* 92(2): 129-139.
- Jeddi K, Cortina J, Chaieb M. 2009. *Acacia salicina*, *Pinus halepensis* and *Eucalyptus occidentalis* improve soil surface conditions in arid southern Tunisia. *J. Arid Environ.* 73, 1005–1013.
- Marx DH. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. II. Production, identification, and biological activity of antibiotics produced by *Leucopaxillus cerealis* var. *piceina*. *Phytopathology* 59(4): 411-417.
- Ndiaye M, Cavalli E, Manga AGB, Diop TA. 2011. Improved *Acacia* Senegal growth after inoculation with arbuscular mycorrhizal fungi under water deficiency conditions. *Int. J. Agric. Biol.*, 13: 271–274.
- Ojala JC, Jarrel WM, Menge JA, Johnson ELV. 1983. Influence of mycorrhizal fungi on the mineral nutrition and yield of onion in saline soil. *Agronomy* 75, 225-259.
- Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 55: 158–161.
- Plenchette C. 1982. Recherches sur les endomycorhizes à vésicules et arbuscules. Influence de la plante hôte, du champignon et du phosphore sur l'expression de la symbiose endomycorhizienne. Thèse de PhD, Université Laval, Québec, Canada, p. 182.
- Plenchette C, Fardeau JC. 1988. Prélèvement du phosphore par les racines et les mycorhizes. *C.R. Acad. Sci.* 4, 117–123.
- Piéri C. 1991. Les bases agronomiques de l'amélioration et du maintien de la fertilité des terres des savanes au sud Sahara. In: *Savanes d'Afrique, terre fertile? Actes des rencontres internationales*. Montpellier, France, pp 43–74 (10–14 Décembre 1990).
- Pauwels Jm, Van Ranst E, Verloo M, Mvondo ZA. 1992. Méthodes d'analyses d'éléments majeurs dans la plante. Manuel de laboratoire de pédologie : Méthodes d'analyses des sols et des plantes. Equipement, gestion des stocks de verrerie et produits chimiques. Publications agricoles. AGCD, Bruxelles

- Requena N, Perez-Solis E, Azcon-Aguilar C, Jeffries P, Barea JM. 2001. Management of indigenous plant–microbe symbioses aids restoration of desertified ecosystems. *Appl Environ Microbiol* 67: 495–498.
- Sanders FE, Tinker PB. 1971. Mechanism of absorption of phosphate from soil by endogone mycorrhizas. *Nature* 233, 278–279.
- Sieverding E. 1991. Vesicular–Arbuscular Mycorrhiza Management in Tropical Agrosystems. *Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ)*. Eschborn, Germany, p. 371.
- Strullu DG (1991). *Les Mycorrhizes des Arbres et des Plantes Cultivées*. Edition Lavoisier: Paris, France; p. 250.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis: second edition*, Academic Press. 600.
- Trouvelot A, Kough JL, Gianinazzi-Pearson V, Gianinazzi S. 1986. Mesure du taux de mycorhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. *Mycorrhizae : physiology and genetics*: 217-221.
- Thomson LAJ. 1987. Australian acacias for saline, alkaline soils in the hot, dry Subtropics and Tropics, in: J.W. Turnbull (Ed.), *Australian Acacias in Developing Countries*, ACIAR, Canberra, pp. 66–69
- Tiedeman JA, Johnson DE. 1992. *Acacia cyanophylla* for forage and fuelwood in North Africa. *Agroforestry Systems* 17:169-180.
- Tiedeman JA, Johnson DE (2004). *Acacia cyanophylla* for forage and fuel wood in North Africa *Agroforestry Systems*, 17,169-180.
- Van der Heijden, M.G.A, Streitwolf-Engel,R, Riedl, R., Siegrist,S.,Neudecker,A, Ineichen,K, Boller,T.,Wiemken,A, Sanders, I.R. 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure In experimental grassland. *New Phytologist*, 172:739-752.
- Vercoe TK. 1987. Fodder potential of selected Australian tree species, in: J.W. Turnbull (Ed.), *Australian Acacias in Developing Countries*, ACIAR, Canberra, pp. 95–100.