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Research Article

Effect of mycorrhizae on growth and root development of *Casuarina* spp. under greenhouse conditions

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ABSTRACT

Casuarina is one of the ecologically and economically important tropical coastal trees nodulated by nitrogen-fixing actinomycete Frankia, forming symbiotic associations with both ecto- and endomycorrhizal fungi and able to form a unique type of roots called "proteoid roots" or "cluster roots". Following field experiments, this work show that the influence of AMF on seedling height growth was limited and a slight presence of arbuscular mycorrhizal structures in roots of inoculated casuarina trees was observed, Casuarina mycorrhization remains low in frequency and intensity namely respectively (66.67%; 1.87%) and the spores number in the rhizospheric soil of inoculated plants was about 6 spores per 100g of soil belonging to the genera *Glomus* and *Acaulospora* with a dominance of the genus *Glomus*, nevertheless the occurrence of cluster roots in both inoculated and non-inoculated casuarina trees was observed the first month of inoculation, but these, were more abundant in control plants, which probably explains the fact that arbuscular mycorrhizal (AM) fungi didn't play an important role in improving the growth and root development of casuarina tree in their early growth stages, moreover, spontaneous actinorhizal infection was found neither in mycorrhizal plants nor in control ones .

Keywords: *Casuarina* spp., mycorrhizae, actinorhiza and cluster roots.

INTRODUCTION

Casuarinas trees, species belonging to the Casuarinaceae family, which includes 4 genera (Allocasuarina, Casuarina, Ceuthostoma and Gymnostoma) and approximately 96 species. Casuarina trees are native from Australia, Southeast Asia and Pacific archipelagoes. They grow very fast and are resistant to drought and high salinity. Casuarina species have been reported to be monoecious[1] and dioecious[2], Flowering occurs principally from April to June, with numerous minute narrow and terminal male flowers crowded in rings among grayish scales, and rounded and lateral female flowers occurring in light-brown clusters[1],[3]. Female flowers are wind pollinated. The multiple fruit, gray brown, 8 to 15 mm in length, 0.3 to 0.6 in diameter ripen from September through December. Seed bearing usually begins by age 5, and good seed crops occur annually[1], in addition, they can grow up to two to 3m per year, and reach a final height of 20-30m [4].

They grow in a wide range of different environments, from tropical forests to arid woodlands and coastal dunes. They frequently occur as pioneer vegetation at early stages of plant succession following disturbances such as fire, landslides, volcanic eruption and flooding[5,6].

They are particularly well adapted to poor and disturbed soils thanks to their capacity to establish symbiotic associations with mycorrhizal fungi -both arbuscular and ectomycorrhizal- and with the nitrogen-fixing bacteria Frankia[7]. In *Casuarina*, N_2-fixation ranges 15 to 80% with the actinomycete *Frankia*, and mycorrhizal colonization ranges 10 to 70% with arbuscular mycorrhizal (AM) or 10 to 50% with ectomycorrhizal (EM) fungi for better soil nutrient and water acquisition[8]. The efficiency of mycorrhizal and Frankia infection depends on the habitat of the host, the prevailing environmental conditions, and the associated plant species. Ectomycorrhizal, endomycorrhizal and Frankia symbionts can occur in the same plant root[9], during mycorrhizal establishment, when AMF is established first, it has no negative effects on EMF infection, however, in the rare cases when EMF is established first, it could reduce AMF colonisation by forming a mantle which acts as a barrier to AMF infection[10].

In addition to mycorrhizas and the nitrogen-fixing bacteria Frankia, actinorhizal plants are also able to form a unique type of roots called "proteoid roots" or "cluster roots" in response to the detrimental effects of nutrient deficiencies in soil. For several authors[11,12], cluster roots are specialized root systems that plants produce to replace mycorrhizae in the absence of effective mycorrhizal fungi in soil or on failure of mycorrhizal infection, they mobilize mineral P that is bound to metal cations such as Fe, Al, and Ca, extract P from organic layers in soil, obtain Fe and Mn from alkaline soils, and take up organic forms of N[13].

Casuarina are mainly used in forestation programs to rehabilitate degraded or polluted sites, to stabilise sand dunes and to provide fuelwood and charcoal and thus contribute considerably to improving livelihoods and local economies[7], and Casuarinas can be planted as pioneer species in hot-dry river valley, dry sandy soil, rock mountain and around desert. However some species are agressive, especially in fragile ecosystems. Root suckering of some species can become a problem around buildings, sidewalks and adjacent agricultural fields, however, this trait can be advantageous in highly erosive areas and in fuelwood plantations[14].

According to Rose[15] and Gardner[16], the role of Casuarina as a pioneering plant and Tsunami protecting tree may be assisted by the presence of mycorrhiza on the root system.

Having regard its potential benefits for the rehabilitation of degraded land, the present study aims at the effect of an endomycorrhizal inoculation on the growth performance of *casuarina* spp. seedling under controlled conditions, species which can be proposed for road slope revegetation and stabilization in morocco.

MATERIALS AND METHODS

Plant material and inoculation

Experiments were conducted under greenhouse conditions, between February and June of 2015, to assess the effect of mycorrhiza on growth parameters and development of Casuarina plants.

Plants arise from Kenitra (Morocco) Forestry nurseries, distributed into plastic pots at the rate of one plant per pot, then, pots were filled with the mixture of Mamora forest soil sterilized by autoclaving at 120 ° C for 2 hours on two consecutive days and the endomycorrhizal inoculum (soil containing AM fungi) collected from the soil and the root samples of different Moroccan ecosystems at a rate of 50% (V: V) in contact with the root system of the trap plant.

Production of the endomycorrhizal inoculum is performed at the laboratory.

The pots were then placed in ambient temperature at nursery and watered daily with distilled water.

Two treatments were performed with and without mycorrhiza.

Soil physico-chemical analysis

The main physico-chemical characteristics of the soil of Mamora forest were determined by standard analyzes performed in the soil analysis laboratory ORMVAG of Kenitra.

Table 1
Physical and chemical characteristics of the Mamora soil.

Physicochemical parameters	Values
pH	7.53
Organic matter (%)	0.7
Total nitrogen (%)	0.05
Total phosphorus P_2O_5 (%)	0.239
Total potassium K_2O (meq/100 g)	0.15
Magnesium (Mg) (meq/100 g)	0.2
Calcium (Ca) (meq/100 g)	6.30

Evaluation of the agronomic parameters of the inoculated plants

After 5 months, agronomic traits evaluated included the height of the vegetative part, stem diameter, the branches number and the fresh weight of roots taken from a fragment of 5cm from the collar, observations were recorded at 28, 70 and 119 days after transplanting.

Mycorrhizal rate inside the roots

The roots were separated from soil by sieving through 1.0 mm mesh sieve and washing several times in water. Then the roots were segmented into 1.0 cm pieces which were cleared with 10% KOH and few drops of hydrogen peroxide (H_2O_2) for 45 min at 90° C then washed with tap water and stained with Cresyl Blue for 15 min at 90° C in a water bath[17].

Thirty fragments were selected randomly and percentage of root length containing fungal hyphae, vesicles and arbuscules were determined microscopically. The mycorrhizal frequency and intensity were quantified using the technique of Phillips and Hayman[17], as modified by Koske and Gemma[18]. The frequency and the intensity of arbuscules and vesicles of AMF inside the root bark were measured by assigning an index of mycorrhization from 0 to 5 [19,20].

The mycorrhizal frequency (M.F. %) reflects the importance of the colonization of the root system and was calculated using the following formula :

M.F. % = 100 × (N - n0) / N

N: Number of observed fragments,

n0: Number of non-mycorrhizal fragments.

The mycorrhizal intensity (M.I. %) (Cortex colonized estimated proportion from the entire root system and expressed in %) was determined as follows:

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

The numbers n5, n4, n3, n2, and n1 denote the number of recorded fragments 5, 4, 3, 2 and 1 estimating the proportion of root colonized by mycorrhizae according to the scale.

Spores extraction

Mycorrhizal spores were separated from soil by wet sieving and decanting method of Gerdemann and Nicolson[21], the isolated spores were identified basing on their morphological characters.

Statistical Analysis

The statistical treatment of the obtained results focused on the analysis of variance with a single classification criterion (ANOVA1).

RESULTS

The mycorrhizal rates inside roots and observations on different growth parameters of Casuarina plants were recorded at 28, 70 and 119 days after transplanting.

1. **Mycorrhizal rates inside roots of Casuarina plants**

Mycorrhizal frequency and intensity, arbuscular and vesicular content in the roots of inoculated plants and the spore number in the Casuarina rhizospheric soil can be seen respectively in figure 1, 2, 3.

None of the mycorrhizal plants was infected by any mycorrhizal fungus the first days of experiment; mycorrhiza was recorded on the 10th week.

The spore number in the rhizospheric soil of mycorrhizal plants is very low, 6 spores/100g of soil belonging to the genera *Glomus* and *Acaulospora* with a dominance of the genus *Glomus* after 119 days after transplantation; namely, 5 indeterminate spores of the genus Glomus and 1 indeterminate spore of *Acaulospora* genus; However, control plants were not infected by any mycorrhizal fungi.

Different structures characterizing AMF were observed: vesicles, arbuscules, intracellular hyphae. We noted the presence of endophitic fungal structures.

2. **Growth of plants**

The figures 6, 7, 8 showed the effect of the endomycorrhizal inoculation on different growth parameters of the Casuarina plants recorded at 28, 70 and 119 days after transplanting. The influence of AMF on seedling height growth and fresh weight of roots taken from a fragment of 5cm from the collar after 17 weeks transplantation was limited, respectively 89,3cm and 1.4g in the inoculated plant and 98,6cm and 2.7g in the control, however the aerial part of inoculated plants was very dense compared to the control, the leaves average number of mycorrhizal plants is considerably higher compared to control respectively 28.5 and 14 leaves counted on a fragment of 10cm.

It has been observed that plants of both treatments have approximately the same average stem diameter and branch number on the 17th week after transplantation, respectively, 6 mm and 2 in the inoculated plants and 6, 2 mm and 1.8 in the control ones.

3. **Formation of cluster roots and spontaneous infection of plants by actinorhizal nodules**

After 4 weeks after transplantation, cluster roots were observed in all of the plants in each treatment mycorrhizal and non mycorrhizal plants, and were more abundant in the control plants, the roots were fine and branched, and showed high frequency of lateral root formation (Fig.10).

Both roots of mycorrhizal and non mycorrizal plants were found to have spontaneous actinorhizal nodules 17 weeks after transplantation, the average number of nodules found under mycorrhizal plants is 19 and 28 under non mycorrhizal plants.

DISCUSSION

From this study it has been observed that the effect of inoculation with AMF was very limited when compared to the control after 17weeks after transplantation, in terms of stem diameter, and branch number. Seedling height growth and fresh weight of roots taken from a fragment of 5cm from the collar were greater in the non inoculated plants and the spore's number in the rhizospheric soil of inoculated plants was very low 6 spores/100g of soil. These results are probably explained by the development of cluster roots the first month of inoculation and the formation of spontaneous actinorhizal nodules in both mycorrhizal and non mycorrhizal plants.

Lamont[22] and Skene[23], noted that species bearing cluster roots are rarely mycorrhizal and can grow in soils with poorly available nutrients, Lamont[22] hypothesized that cluster roots have an adaptive advantage in exploiting nutrients in litter and humus layers that accumulate on nutrient-impoverished soils in strongly seasonal environments. Some members of the Casuarinaceae are an exception and form both cluster roots and mycorrhizae[23,24].

According to Reddell *et al*[25] mycorrhizal colonisation of roots declined with increasing in P supply, it was highest at 0 and 10 mg of P per kg soil and no mycorrhizae formed at or above 100 mg P per kg soil.

The result of this study support previous work regarding the mycorrhizal status of casuarina plants, according to Reddell *et al*,[25] the occurrence of cluster roots in samples from 18 out of 20 natural populations of *C. cunninghamiana* that were surveyed in north Queensland showed that Cluster roots were found at 90% of the sites surveyed. By contrast, Arbuscular mycorrhizae were observed at only 45% of the field sites and the extent to which they colonised *C. cunninghamiana* proved to be very low by comparison with levels generally encountered in other woody native species. Zhang *et al*,[26] observed a limited influence of AMF on Casuarina seedling height growth but the effects of AMF on total biomass increment were very significant, and it was noted that AMF exerted more influences on root biomass than shoot biomass[26], although, Ducousso *et al*,[27] noted that Arbuscular mycorrhiza of *Casuarina* spp. remains low in frequency and intensity, except for *Casuarina cunninghamiana* plants grown in nurseries.

However, the results of *C. equisetifolia* and *C. junghuhniana* showed that inoculation with ectomycorrhizal fungus significantly improved the diameter and height of seedlings, there was also variation among seed lots in response to the inoculation[28,29] and The mycorrhiza of actinorhizal plants is essential to obtain higher yields especially when the plants grow in the phosphorus deficient soils and coastal saline sandy soils[30].

Rose[15] noted the presence of arbuscular mycorrhizal structures in roots of *C. cunninghamiana* obtained from sand woodlands of Florida and Japan and in roots of *C. equisetifolia* from coastal area, The genus of the AM *Glomus* was the dominant native species found in field surveys in southern China et Zhong *et al*.[28] have identified the genus of arbuscular mycorrhizal fungi *Glomus*, *Acaulospora*, *Gigaspora*, and *Scutellospora* in the rhizosphere of Casuarina, two unidentified *Glomus* are particularly common in their observations.

In the absence of P, inoculation of AM cultures influenced *Casuarina equisetifolia* seedlings growth significantly. Among them, G. fasiculatum was found to be highly effective[31]. In our study the mycorrhizal infection was very low and we noted a dominance of the genus Glomus; this domination was also reported in several studies conducted in Morocco in the rhizosphere of the olive tree[32,33], the oleaster [34], date palm[35],Carob tree[36,37], poplar[38], Juncus[39] and Lycium europaeum[40].

The results of this study lead us to hypothesize that a significant effect of inoculation with AMF can be observed over time, indeed, no effect was observed in the growth parameters of the inoculated plants when compared to the control and none of the mycorrhizal plants was infected by any mycorrhizal fungus the first months of experiment. In spite of this, due to their tolerance to adverse soil and climate conditions and their ability to form symbiotic associations with ecto- and endomycorrhizal fungi and the nitrogen-fixing actinomycete Frankia in addition to the cluster roots formation, Casuarina trees are a biological tool for rehabilitation of degraded road slope and forestation programmes.

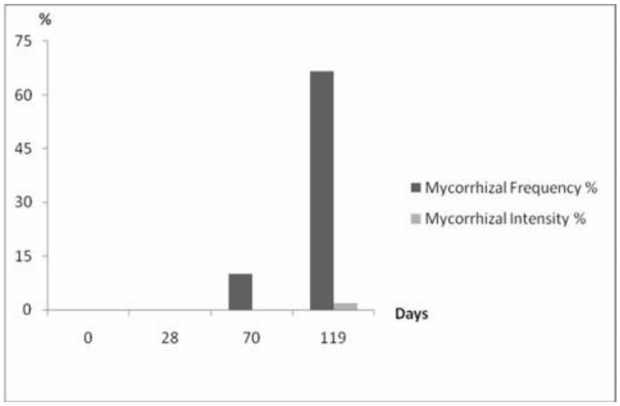

Fig. 1
Mycorrhizal frequency and intensity of the inoculated Casuarina plant roots

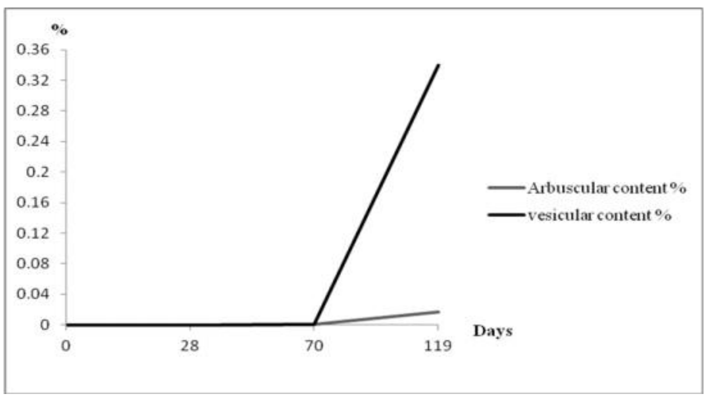

Fig. 2
Arbuscular and vesicular content of the inoculated Casuarina plant roots

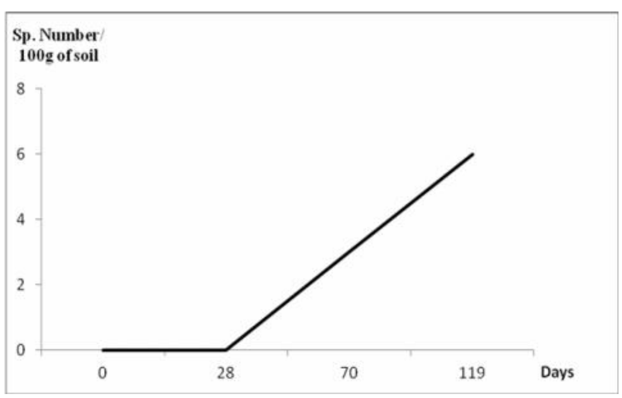

Fig. 3
Spores number in the rhizospheric soil of *Casuarina* plant

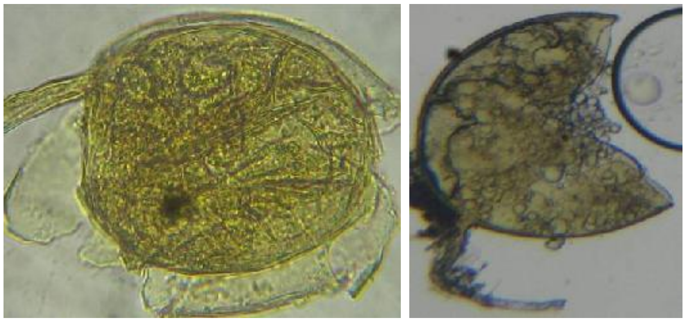

Fig. 4
Mycorrhizal fungi species isolated from the rhizosphere of *Casuarina* plants (A): *Glomus* sp1; (B): *Acaulospora* sp1.

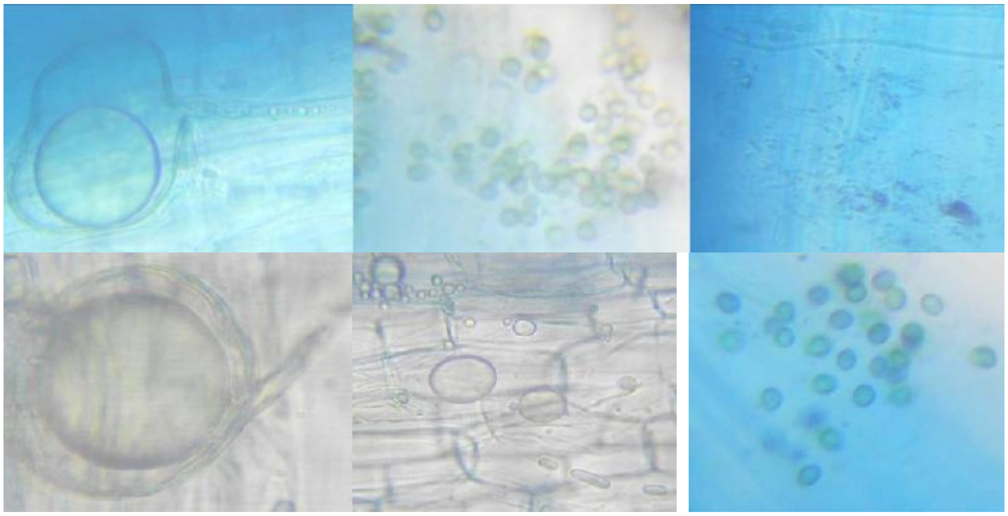

Fig. 5
Endomycorrhizal and endophytic fungal structures in the roots of *Casuarina*
arbuscule (a); endophytes: (e); internal hyphae (ih); vesicule (v); (G. ×400)

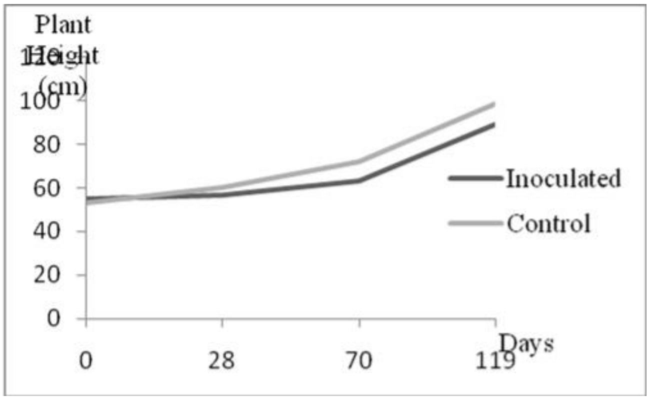

Fig. 6
Effect of arbuscular mycorrhizal fungi (AMF) on Casuarina plants height (cm)

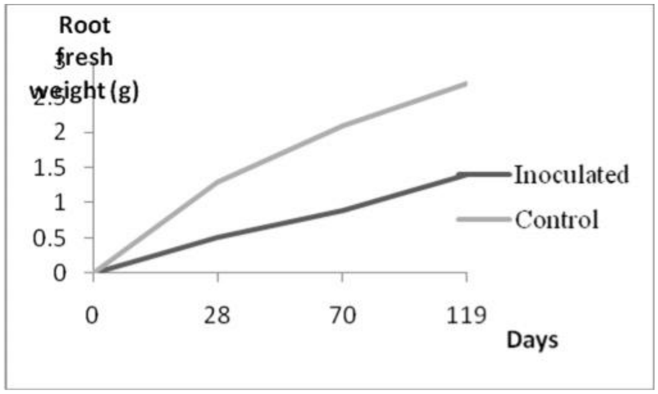

Fig. 7
Effect of arbuscular mycorrhizal fungi (AMF) on fresh weight of roots taken from a fragment of 5cm from the collar (g)

Fig.8
Effect of AMF on the aerial part of Casuarina plant in the 17th weeks after transplantation

Fig.9
Effect of AMF on the root system of Casuarina plant in the 17th weeks after transplantation

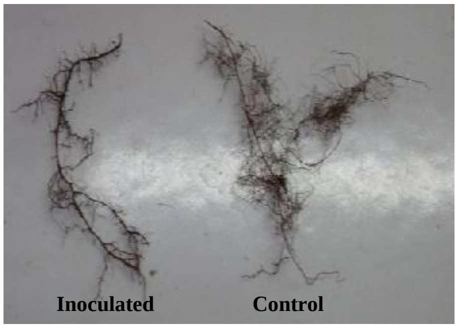

Fig.10
Cluster roots observed in the root system of Casuarina plant in the 8th weeks after transplantation

Fig.11
Spontaneous formation of actinorhizal nodules in the root system of Casuarina plant in the 17th weeks after transplantation

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