Original Research Article

Impact of endomycorrhization on the nursery growth of seedlings of a threatened Ivorian forest species *Pterygota macrocarpa* K. Schum (Malvaceae)

.

ABSTRACT

Aims: *Pterygota macrocarpa*, a common species in the forests of Côte d'Ivoire, is threatened with extinction due to overexploitation. Protective measures for *P. macrocarpa* could consist in the integration of arbuscular mycorrhizae in the reforestation of this species. The objective of this study was to evaluate the impact of arbuscular mycorrhizae inoculation on the resistance and development of *P. macrocarpa* plants.
Study design: The design is completely randomized and includes one (1) plant species (*Pterygota macrocarpa*), three (3) treatments (local inoculum 1, commercial inoculum 2 and non-inoculated control) and 20 seedlings per treatment.
Place and Duration of Study: The experimental study was set up at the border of the experimental forest of the Northern site of INP-HB (National Polytechnic Institute Houphouët-Boigny, Yamoussoukro, Côte d'Ivoire) from February to May 2018.
Methodology: Thus, from seedlings collected in the arboriculture of the INP-HB of Yamoussokro, the effects of mycorrhization through treatments on the mineral nutrition and on the growth parameters of *P. macrocarpa* were evaluated during 120 days of culture in nursery.
Results: The mycorrhised plants survived 100% while the control plants had 90% survival rate. The mycorrhizal intensity of the roots was 19.21% for inoculum 1 and 10.40% for inoculum 2. The plants treated with inoculum 1 had the highest mineral content, especially phosphorus (0.3 ppm) and nitrogen (2.6%). The vegetative growth of inoculum 1 treated plants was more accelerated than that of the other two treatments. Local inoculum 1 was more effective than commercial inoculum 2.
Conclusion: The integration of local mycorrhizal inocula in the reforestation of *P. macrocarpa* seedlings could be a sustainable solution for the restoration of degraded forests.

Keywords: Côte d'Ivoire; inoculum; forests, Pterygota macrocarpa, vegetative growth

***(Note:** 1. 3. Research Papers and Short Notes should follow the structure of Abstract, Introduction, Methodology, Results and Discussion, Conclusion, Acknowledgements, Competing Interests, Authors' Contributions, Consent (where applicable), Ethical approval (where applicable), and References plus figures and/or tables.)*

1. INTRODUCTION

The timber industry played an important role in the economy of Côte d'Ivoire from 1950 to 1980. Timber was (along with coffee and cocoa) one of the essential pillars of the national economy: through the foreign exchange earned from its export, through its industrializing power and through its role in land-use planning [1]. But because of the overexploitation of

forest species, the extension of cash crop agriculture and the galloping demography, this industry is threatened. Timber harvesting has dropped from 5.321 million m3 in 1977 to 2 million m3 in 2018. The scarcity of timber species directly handicaps the wood industry sector, which is nevertheless a provider of employment [2]. Indeed, almost all of the most exploited Ivorian forest species are present on the IUCN red list of threatened species [3].
Pterygota macrocarpa common a species of the Ivorian forests is on the IUCN Red List in the "vulnerable" category due to the loss of its environment and overexploitation. In 1995, the country exported 2,000 m³ of peeled *P. macrocarpa* veneer at a price of US$ 406/m³ and 2,000 m³ of sliced veneer at a price of US$ 963/m³, but also 5,000 m³ of logs at an average price of US$ 567/m³. In 2004, Côte d'Ivoire exported 32,000 m³ of sawn timber from *P. macrocarpa* at US$ 397/m³, and in 2005, 25,000 m³ at US$ 439/m³. Also, in Côte d'Ivoire, this tree is perfectly integrated in the agroforestry system in cocoa production. It is kept as a shade tree in cocoa plantations, its large leaves protect cocoa plants, especially the youngest ones, from excessive sunlight and high temperatures. Unfortunately, despite the overexploitation of *P. macrocarpa*, other species such as *Milicia excelsa, Tectona grandis* or *Triplochiton scleroxylon* are favored in reforestation campaigns in Ivorian classified forests. The exploitation of *P. macrocarpa* wood should therefore be regulated and protective measures implemented. These measures could consist of developing an effective reforestation policy for this species by reintroducing young seedlings into degraded forests. Taking into account the context of climate change, the pedoclimatic requirements of the plant and the natural biological interactions that it could maintain in its ecosystem. Generally in Côte d'Ivoire, these last aspects are not integrated in reforestation policies. This explains the failures in the attempts to reconstitute degraded forests initiated by SODEFOR (Forestry Development Society). Also, previous works have shown that the rate of natural regeneration by seeds of forest species commonly exploited in Côte d'Ivoire is low [4;5]. Thus, very few forests have been restored [6]. Biological interactions beneficial to plants include mycorrhizae in general and arbuscular and vesicular mycorrhizae in particular. Arbuscular mycorrhizae (AM) result from symbiosis between plant roots and fungi belonging to the phylum Glomeromycota [7]. AM fungi are considered to have been essential for the colonization of terrestrial habitats by green plants [8]. Their primary function is to contribute to plant mineral and water nutrition, particularly phosphorus (P) [9], which is often a limiting resource in soils [10;11]. They also allow trees to better resist certain root diseases and make the best use of water resources. In addition to the above functions, AM fungi are involved in the organization and structuring of plant communities [12] and soil microbiota communities [13; 14]. Also in the tropics, the primary role of endomycorrhizal fungi in reforestation has already been demonstrated in the Sahel [15]. Mycorrhizal inoculation has allowed the successful reforestation of more than $5{,}10^4$ ha out of $11.7{,}10^6$ ha planned. Thus the integration of arbuscular mycorrhizae in the reforestation of Ivorian forest species could be a solution for the sustainable recovery of degraded forests. The main objective of the study was to evaluate the impact of arbuscular mycorrhizal inoculation on the growth of *Pterygota macrocarpa* seedlings. More specifically, it was to evaluate the effects of mycorrhization on plant mineral nutrition and to assess the impact of mycorrhization on the growth parameters of *P. macrocarpa.*

2. MATERIAL AND METHODS

2.1 Characteristics of mycorrhizal inocula

2.1.1 Characteristics of inoculum 1

Inoculum 1 (local inoculum) was produced by trapping arbuscular mycorrhizal fungi (AMF) in the forest soil of the INP-HB (National Polytechnic Institute Houphouët-Boigny) in Yamoussoukro. Yamoussoukro region, in the center of Côte d'Ivoire, is located between 6°15 and 7°35 North latitude and 4°40 and 5°40 West longitude. The trapping technique is a

bioassay, which allows to obtain AMF spores in quality and quantity to initiate inoculation tests [16]. Cowpea, which has a 60-70 day cycle, was chosen as the host plant. Cowpea seeds disinfected with 12°-10% bleach and rinsed once for 2 minutes with sterile water were pre-germinated. Plants of the same size were selected and sown in 2-l plastic pots containing a mixture of 700 g of gardener's potting soil + sand (1v/1v) previously sterilized (110°C, 2 kg/cm2, 3 h) and 150 g of forest soil serving as inoculum (2 plants per pot).

After 3 months, the number of AMF propagules (spores) from the trap culture was established. Spores were extracted by wet sieving and decanting [17] using sieves of different sizes (45, 90, 125, and 500 µm) and the modified sucrose density gradient centrifugation method (Walker et al. 1982). For identification of AMF spores, healthy spores were mounted on microscope slides and stained with polyvinyl alcoholhollacto-glycerol (1vol/1vol) (PVLG) mixed with and without Melzer's reagent [16; 18]. The morphotype of AMF spores was based on Oehl description [19] and the revision of Glomeromycota genera [7]. The number of AMF propagules (spores) in inoculum 1 (substrate in pots) was estimated to be 700 spores per gram.

2.1.2. Characteristics of inoculum 2

Inoculum 2 is a commercial monospecific inoculum of *Glomus intraradices* produced by the Canadian company Myke Pro whose density was estimated by the manufacturer at 3000 propagules per gram.

2.2. Collection of *Pterygota macrocarpa* seedlings

To harvest the seedlings, furrows were made around the plant with a daba, then it was dug up with the clod of soil present on the roots. The roots were then removed from the clod and rinsed thoroughly with water to remove surface microorganisms. On each plant, at the lateral roots, a sample of the finest roots likely to be colonized by native AM was removed for a colonization check according to [20]. Only seedlings with 0% colonization were retained for the study.

2.3. Inoculation process of *Pterygota macrocarpa* seedlings

Seedlings of the same size (about 10 cm high, about 0.185 mm in diameter) at the 4-leaf stage were selected for planting in 5 l plastic bags containing a mixture of 2000 g sterilized potting soil (autoclaved at 110°C, 2 kg/cm2, 3 h; characteristics: pH = 6. 8; organic matter = 2.57%; total nitrogen = 0.16%; available phosphorus = 75 mg/kg; cation exchange capacity = 7.4 cmol.kg-1) and 200 g of inoculum (1 plant per pot). The roots of the seedlings were placed in direct contact with the inoculum to optimize mycorrhizal colonization. Seedlings in control bags were grown in 2000 g of sterilized potting soil + 200 g of sterilized substrate (autoclaved at 110°C, 2 kg/cm2, 3 h). Each bag was watered with 500 ml of water every 3 days until the end of the experiment.

2.4. Experimental design

The experiment took place in an open area at the edge of the experimental forest at INP-HB. The design is completely randomized and includes one (1) plant species (*Pterygota macrocarpa*), three (3) treatments and 20 seedlings per treatment. The treatment factor has three levels: Inoculum 1 (local inoculum), Inoculum 2 (commercial inoculum) and Control. A total number of 60 seedlings were used.

2.5. Assessment of root colonization

Fine roots were sampled at 150 days of culture, with three replicates per treatment. Each treatment contained three plants. Roots were rinsed and cut into 1 cm fragments. These root fragments were cleaned by boiling in 10% (w/v) KOH and stained with 0.05% (v/v) trypan

blue in lactoglycerol using the method of [21]. Ten pieces of roots per plant were placed in glycerol (50%) between a slide and a coverslip [22] and observed under a light microscope. Root colonization was assessed by two parameters: mycorrhization intensity and mycorrhization frequency. Mycorrhization intensity indicates the rate of mycorrhizal structures in a colonized root fragment. Mycorrhization frequency represents the percentage of root fragments with mycorrhizal structures out of the total number of fragments observed. Colonized roots were observed and evaluated according to [19].

2.6. Measurement of mineral nutrition parameters

After drying in an oven at 60°C for 5 days, the samples of aerial parts (leaves and stems) were reduced to fine powder with a mortar. Then the mineralization of the powders was performed in a muffle furnace at 500°C. The ashes were solubilized with HCl. The extracts obtained were then filtered on ash-free filter paper and made up to 50 ml with distilled water. The resulting stock solutions were stored in vials and thus ready for the determination of the mineral elements. Nitrogen was determined by the Kjeldahl method [23] with mineralization in the presence of glucose to avoid losses of nitrate and catalysts (SO4K2, SO4Cu, and Selenium). Phosphorus was determined by phopho-vanado-molybdate colorimetry [24]; potassium and calcium by flame photometry after ion exchange; magnesium by the complexometric method.

2.7. Collection of growth data

Measurements were made on the first day of transplantation (D0), the 30th day (D30), the 60th day (D60), the 90th day (D90), the 120th day (D120) corresponding to the number of days necessary to judge the resistance to transplantation stress. Data collected were survival rate, seedling height, collar diameter and leaf area. The survival rate was determined according to the following formula:

Survival rate (%) = (number of growing plants)/(number of initial number of plants) X 100.

Plant height was measured with a 30 cm ruler. Plant collar diameter was measured with a Vernier caliper. The number of leaves was obtained by counting. The total leaf area of each individual plant sampled per treatment was determined as follows: leaves were classified into "large" (L) and "small" (S) batches based on whether they had reached maximum growth. For each batch of leaves, a sample of 2 leaves was considered for the determination of the average leaf area using the MESURIUM software. The leaf area of each batch of leaves was obtained by multiplying the number of leaves by the corresponding average area. Thus, the total leaf area (TLA) is calculated from the following formula:

SFT = STG + STP with STG = total leaf area of the "large" batch of leaves and STP = total leaf area of the "small" batch of leaves.

2.8. Statistical analysis of the data

The data obtained in this study were processed by a one-factor analysis of variance (ANOVA) with 3 modalities (Control: no inoculum, Inoculum 1: local inoculum; Inoculum 2: commercial inoculum). This analysis was performed by STATISTICA 7.1 software. Tukey's HSD test ($p \leq 0.05$) was used to identify truly different means when analysis of variance revealed a significant difference. The Tukey HSD test was also used to perform multiple comparisons of means to form homogeneous groups.

3. RESULTS AND DISCUSSION

3.1 Mycorrhizal colonization rate of *Pterygota macrocarpa* roots

The rate of mycorrhization of *Pterygota macrocarpa* roots specifically the intensity and frequency for each treatment is shown (Table I). At 120 days, the roots of the control plants showed no mycorrhizal structure. The results showed a significant difference between the different mycorrhizal inocula (Inoculum 1 and inoculum 2). Inoculum 1 recorded the highest intensity (19.21%) as well as the highest frequency (70%) of mycorrhization; whereas with inoculum 2, lower mycorrhization parameters were observed, i.e. an intensity of 10.40% for a frequency of 55%. Thus, this study revealed that *Pterygota macrocarpa* is a mycotrophic plant because mycorrhizal structures were observed in the inoculated seedlings. Arbuscular mycorrhizal fungi are able to colonize tree roots and thereby provide benefits for better growth and also adaptation to degraded ecosystems [25]. The use of arbuscular mycorrhizae is more common in agriculture to improve crop production [26]. In forestry, ectomycorrhizae are more commonly used as inoculum to promote the growth of forest species [27]. However, in tropical and subtropical regions of Africa, where botanical diversity is very high, arbuscular mycorrhizal fungi predominate [28; 29]. Moreover, studies dealing with mycorrhizal associations with tropical tree species have revealed arbuscular mycorrhizal associations in about 500 forest species [30; 31; 32]. Some of these trees are used in agroforestry systems [33; 34]. During this work, it was observed that mycorizogenic power of local polyspecific inoculum 1 was higher than that of exotic monospecific inoculum 2. Some previous studies found that polyspecific inocula were more effective than monospecific inocula [35]. Also inoculum 1 was produced from the forest soil from which the *Pterygota macrocarpa* seedlings were collected, while inoculum 2 was exotic. These results are consistent with authors who state that the behavior of mycorrhizal fungi and their efficiency would be related to their environment of origin and specifically to environmental factors in their habitat [36]. These factors could be climate and soil conditions [37; 38]. Indeed local inocula in general colonize plants better than inocula of foreign origin. It appears that mycorrhization of local strains should be preferred, as they are better adapted to environmental conditions.

Table I. Mycorrhizal colonization at 120 days

Treatment	Mycorrhization intensity (%)	Mycorrhization frequency (%)
Control	00	00
Inoculum 1	19,21±1,22[a]	70±8,27[a]
Inoculum 2	10,40±1,36[b]	55±10,41[b]
F (1, 8)	115,50	6,35
p	*0,00*	*0,03*

Values with the same letters are not statistically different at the 5% threshold of Fisher's LSD test

3.2. Impact of mycorrhization on plant mineral nutrition

The average mineral element contents of the aerial parts are represented (Table II). The proportion of mineral nitrogen in the plants was highest for plants treated with inoculum 1

(2.6%) followed by plants treated with inoculum 2 (2.24%). The lowest nitrogen proportions were obtained with the control plants (2.1%). Similarly, phosphorus levels were lowest in the control plants (0.23 ppm). There was no significant difference between the phosphorus content of plants treated with inoculum 1 (0.3 ppm) and inoculum 2 (0.27 ppm). In terms of exchangeable bases, inoculum 1 treated plants had the highest potassium (1.99 cmol/kg), calcium (1.73 cmol/kg) and magnesium (0.49 cmol/kg) contents. The potassium content of inoculum 2 treated plants (1.83 cmol/kg) and control plants (1.8 cmol/kg) were not statistically different. Control plants had lower calcium levels (1.54 cmol/kg) than inoculum 2 plants (1.66 cmol/kg). However, the inoculum 2 plants had lower magnesium content (0.42 cmol/kg) than the control plants (0.48 cmol/kg). The study demonstrated that mycorrhization, especially the local polyspecific inoculum, allows a better absorption of mineral elements from the soil by the young plants of *Pterygota macrocarpa*. This confirms that arbuscular mycorrhization could be beneficial to woody species. These results regarding the improvement of mineral nutrition and in particular phosphate nutrition by mycorrhizae are confirmed by several other previous works [9; 39; 40; 41].

Table II: Mineral content of leaves at 120 days

Traitment	Nitrogen (%)	Phosphorus (ppm)	Potassium (cmol/kg)	Calcium (cmol/kg)	Magnesium (cmol/kg)
Control	2,1±0,1^{c}	0,23±0,05^{b}	1,8±0,08^{b}	1,54±0,05^{c}	0,48±0,06^{a}
Inoculum 1	2,6±0,13^{a}	0,3±0,03^{a}	1,99±0,11^{a}	1,73±0,05^{a}	0,49±0,07^{a}
Inoculum 2	2,24±0,09^{b}	0,27±0,02^{a}	1,83±0,01^{b}	1,66±0,07^{b}	0,42±0,04^{b}
F (2, 27)	51,24	9,018	6,97	21,67	4,61
p	*0,00*	*0,00*	*0,00*	*0,00*	*0,01*

Values with the same letters are not statistically different at the 5% threshold of Fisher's LSD test

3.3. Impact of mycorrhization on plant growth parameters

3.3.1 Survival of seedlings under transplanting stress

The survival rate of seedlings under transplanting stress is presented (Figure 1). The inoculum 1 and inoculum 2 treatments resulted in 100% survival of the seedlings until day 120, while 90% of the non-inoculated control seedlings survived until day 120.

Low mortality of transplanted *Pterygota macrocarpa* seedlings was observed even with uninoculated seedlings (90% survival rate). However, in general, with forest species, significant mortality is observed at the time of transplantation due to the adaptation crisis [42]. *P. macrocarpa* seedlings would be more naturally resistant. However, inoculated plants (inoculum 1 and inoculum 2) survived 100%. *P. macrocarpa* seedlings increased their resistance to transplanting stress through mycorrhization. Concordant results were obtained with arbuscular mycorrhizae. Other authors had already achieved 100% survival of Acacia holosericea with Glomus mosseae (Glomeraceae) [43]. Arbuscular mycorrhization would therefore be able to improve plant adaptation to stressful conditions or changing ecosystems [44; 45; 46]. Inoculation would confer vigor to the seedlings and allow them to benefit early

from the advantages granted by the mycorrhizal symbiosis. Indeed; it has been shown that mycorrhization improves edaphic conditions including soil structure [47], inhibition of some soil pathogens [48; 49, 35]. And these benefits would be favorable for the survival of transplanted seedlings.

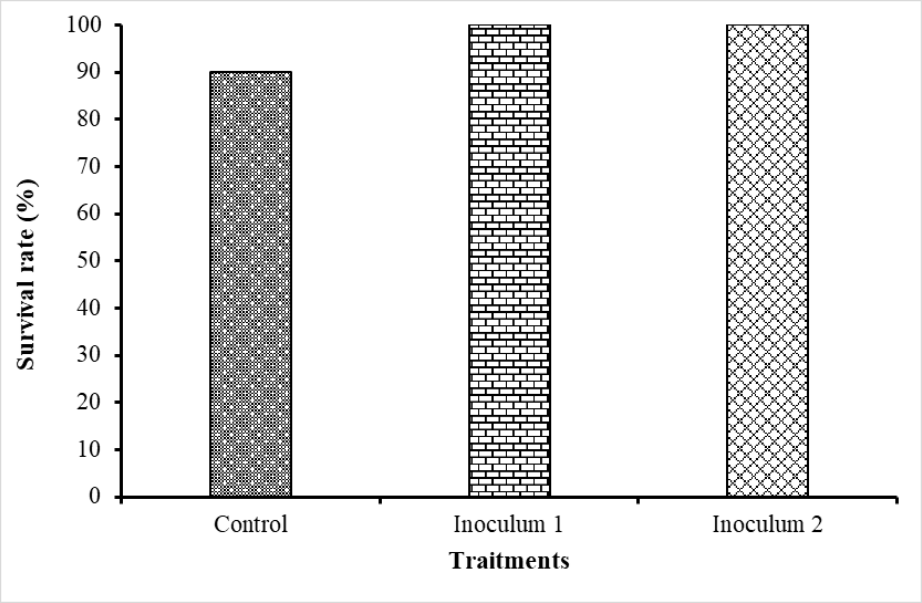

Figure 1. Survival rate of *Pterygota macrocarpa* seedlings to transplant stress according to treatments

3.3.2 Impact of mycorrhization on plant height growth

The evolution of the height of *Pterygota macrocarpa* plants as a function of time is shown (Figure 2). At time T0 the plants have the same height (about 10 cm) whatever the type of treatments applied. Then, for each treatment, there is a progressive increase of this height until reaching a maximum peak at T120. The treatment with inoculum 1 recorded the greatest height of the plants from day 60 (13.57 cm) to day 120 (17.53 cm), followed by the plants treated with inoculum 2 whose heights evolved from 12.86 to 15.86 cm from day 60 to day 120. The lowest heights were obtained with the control plants whose heights from day 30 to day 120 ranged from 10.83 to 14.34 cm.

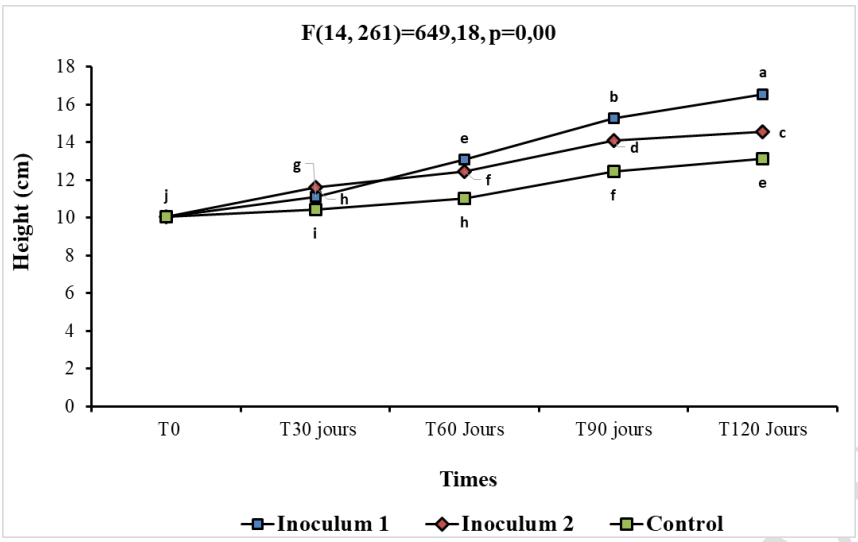

Figure 2: Evolution of the height of *Pterygota macrocarpa* plants as a function of time
Values with the same letters are not statistically different at the 5% threshold of Fisher's LSD test.

3.3.3. Impact of mycorrhization on the evolution of the diameter at the crown of the plants

The evolution of the diameter at the crown of *Pterygota macrocarpa* plants as a function of time is presented (Figure 3). At time T0 the plants have almost the same diameter (about 0.29 cm) whatever the treatment. Then, a progressive increase of this height is observed until T120 according to the different treatments applied. The treatment with inoculum 1 recorded the largest diameter of the plants from day 60 (0.34 cm) to day 120 (0.37 cm) followed by inoculum 2 whose plants reached an average diameter of 0.35 cm at day 120. The control plants had the lowest diameters, 0.32 cm at day 120.

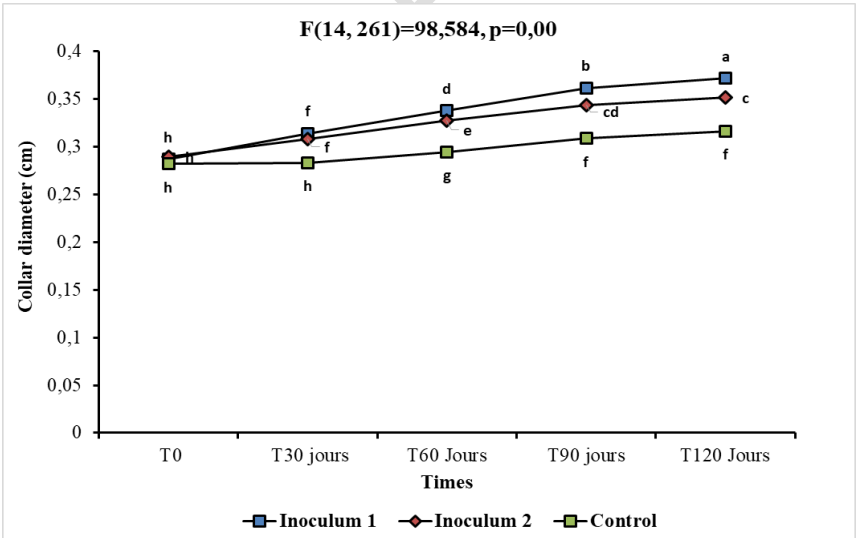

Figure 3. Evolution of the diameter at the collar of *Pterygota macrocarpa* plants as a function of time
Values with the same letters are not statistically different at the 5% threshold of Fisher's LSD test.

3.3.4. Impact of mycorrhization on the evolution of the total leaf area of the plants

The evolution of the total leaf area of *Pterygota macrocarpa* plants as a function of time is presented (Figure 4). At time T0 the plants have almost the same leaf area (about 11.9 cm2) regardless of the treatment. Then there is a progressive increase of this area until T120 for each different treatment applied. No significant difference was observed between plants treated with inoculum 1 and those treated with inoculum 2 from T0 to T60. However, the treatment with inoculum 1 recorded the largest leaf area of the plants from day 90 (36.54 cm2) to day 120 (60.31 cm2). The control plants had the smallest leaf area between 11.84 and 28.09 cm2 from day 30 (36.54 cm2) to day 120.

In relation to a better absorption of mineral elements, but especially of phosphorus and nitrogen, the inoculated plants experienced an accelerated vegetative development compared to the control plants. Indeed, from the first 30 days, the height, the diameter at the collar and the leaf surface of the mycorrhized plants were higher than those of the control plants. Also, local inoculum 1 was more efficient for the growth of *Pterygota macrocarpa* seedlings compared to exotic inoculum 2. The integration of arbuscular mycorrhization technology into the reforestation of *P. macrocarpa* would be crucial for the recovery of lost forest space and thus the removal of this forest species from the IUCN red list. Overall, inoculum 1 appears to perform better than inoculum 2. These results are consistent with the majority of studies comparing the efficiency of local and exotic inocula. These studies have shown that native multispecific inocula have a better impact on plant nutrition and growth compared to exotic monospecific commercial inocula [50; 35].

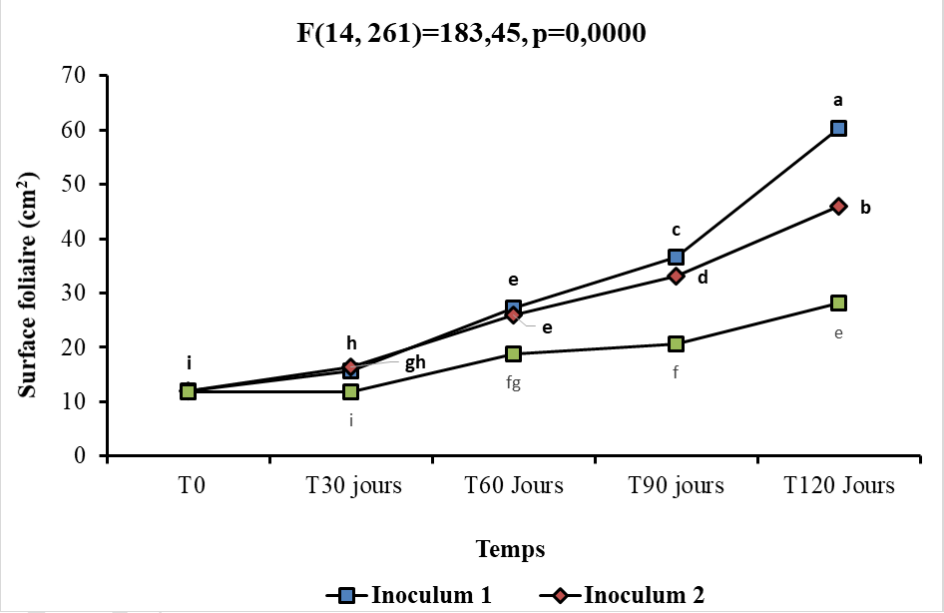

Figure 4: Evolution of the total leaf area of *Pterygota macrocarpa* plants as a function of time

Values with the same letters are not statistically different at the 5% threshold of Fisher's LSD test.

4. CONCLUSION

The present study showed that *Pterygota macrocarpa* is a mycotrophic plant capable of establishing symbiosis with arbuscular mycorrhizal fungi. The inoculated *P. macrocarpa* seedlings obtained 100% survival rate, better mineral uptake which induced a more accelerated vegetative growth especially by inoculum 1. The local polyspecific inoculum 1

was more efficient than the exotic monospecific inoculum 2. Thus, the development of a technology based on local mycorrhizal inocula is necessary. The integration of these inocula, first in the realization of nurseries and then in the growth and development of young plants in plantation could be a solution for the reconstitution of degraded forests and by this fact a sustainable source of raw material for the wood industry in difficulty.]

CONSENT (WHERE EVER APPLICABLE)

No applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

No applicable

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

REFERENCES

1. Arnaud J-C, Sournia G. The forests of Ivory Coast: an endangered natural wealth. In: Overseas notebooks. 1979; 127: 281-301. French.

2. Vroh BTA, Ouattara D, Tiebre MS, Kpagui K, N'guessan K. A case of ecological compensation in the mining sector. The Dékpa forest reserve (Côte d´Ivoiore) to the aid of forests and local populations. Sci Techn waste. 2016; 7: 1-9. French. doi: 10.4267 / waste-sciences-techniques.3511

3. IUCN (2012). IUCN Red List of Threatened Species, Version 2012, 2, Accessed 20 July 2018. Available at http://www.iucnredlist.org.

4. Ettien DT. Regeneration potential of commercial forest species by seed germination, in the classified forest of Haut Sassandra (Center-West of the Ivory Coast). 3rd cycle doctoral thesis, UFR Biosciences, Abidjan-Cocody University, (Abidjan, Cote d'Ivoire). 2005. French.

5. Kouadio K, Kouassi KE, Kouamé NF, Traore D. Impact of thinning on the natural regeneration of the main species, in the classified forest of Bomessatié (Ivory Coast). Sci Nat. 2007, 4 (1): 27-35. French

6. Gone BZB, Kouame D, Kone I, Adou Y.Y.C. Plant diversity and conservation value for the Biodiversity of Mont Péko National Park, a protected area threatened with extinction in Côte d'Ivoire. J Appl Biosci. 2013, 71: 5753– 5762. doi: 10.4314 / JAB.V71I1.98820

7. Redecker D., shussler A., Stockinger H, Sturmer S.L., Morton J.B., Walker C. An evidence- based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza, 2013, 23 (7) : 515-531. http://dx.doi.org/10.1007/s00572-013-0486-y.

8. Helgason T, Fitter A. The ecology and evolution of the arbuscular mycorrhizal fungi. Mycologist. 2005, 19 : 96-101. https://doi.org/10.1017/S0269-915X(05)00302-2.

9. Jakobsen I, Smith SE, Smith FA. Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. In: van der Heijden MGA, Sanders IR, eds. Mycorrhizal ecology. Heidelberg, Germany: Springer Verlag. 2002. https://doi.org/10.1007/978-3-540-38364-2_3.

10. Clark RB, Zeto, SK. Mineral acquisition by arbuscular mycorrhizal plants. *J Plant Nutr*. 2000, 23: 867–902. https://doi.org/10.1080/01904160009382068

11. Leigh J, Hodge A, Fitter AH. Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to the host plant from organic material. *New Phytol.* 2008, 181: 199-207. doi:10.1111/j.1469-8137.2008.02630.x.

12. van der Heijden MGA, Bardgett, RD, van Straalen, NM The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestial ecosystems. *Ecol Lett.* 2008, 11: 296–310. doi :10.1111/j.1461-0248.2007.01139.x

13. Rillig MC, Mummey DL, Ramsey PW, Klironomos JN, Gannon, JE. Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. *FEMS Microbiol*. 2008, 57: 389–395. doi: 10.1111/j.1574-6941.2006.00129.x

14. Toljander J. F., Lindahl B. D., Paul L. R., Elfstrand M. & Finlay R. Influence of arbuscular mycorrhizal exudates on soil bacterial growth and community structure. *FEMS Microbiol.* 2007 *;* 61: 295–304. doi:10.1111/j.1574-6941.2007.00337.x

15. Duponnois R, Hafidi M, Ndoye I, Ramanankierana H, Bâ AM. Symbiotic fungi against desertification of Mediterranean, tropical and island ecosystems. IRD Editions. Marseille, France. 2013. French.

16. Morton JB, Bentivenga SP, Wheeler WW. Germ plasma in the International Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation and storage. *Mycotaxon*. 1993; 48, 491 – 528.

17. Gerdemann JW, Nicolson TH. Spores of endogone species from soil by wet sieving and decanting. *Trans Br mycol soc*. 1963 ; 46 : 235 - 244.

18. Brundrett MC. Coevolution of roots and mycorrhizas of land plants. *New phytol*. 2002; 154(2), 275-304. https://doi.org/10.1046/j.1469-8137.2002.00397.x.

19. Trouvelot A, Kough JL, Gianinazzi-Pearson V. Measurement of the rate of VA mycorrhization of a root system. Search for estimation methods with functional significance. In: physiology and genetics aspects of mycorrhizae. Gianinazi-Pearson V and Gianinazzi S (Eds), 1st ESM, INRA Press, Paris, France. 1986. French.

20. Oehl F, Sieverding E, Palenzuela J. Ineichen K, da Silva GA. Advances in Glomeromycota taxonomy and classification. IMA Fungus. 2001; 2: 191–199 https://doi.org/10.5598/imafungus.2011.02.02.10 21.

Phillips JM, Haymann DS. Improved proceeding for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br mycol soc*. 1970; 69: 275.

22. Kormanik PP, Mc Graw AC. Quantification of vesicular arbuscular mycorrhizal in plants roots. In Methods and pricipes of mycorrhizal research (Ed) American phytopatological society, Minnesota (USA). 1982

23. Bremner JM. Determination of nitrogen in soil by the Keldjahl method. J Agric Sci. 1960 ; 55 (1) : 11-33.

24. Gautheyrou J, Pansu M. Analysis of mineralogical, organic and mineral soil, 1st edition, Fondation Springer Poulignac, Paris (France), 2003. French.

25. Kivlin SN, Hawkes CV, Treseder KK. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biol Biochem. 2011 ; 43: 2294–2303. doi:10.1016/j.soilbio.2011.07.012

26. De-Carvalho AMX, de Castro-Tavares R, Cardoso IM, Kuyper TW. Mycorrhizal Associations in Agroforestry Systems. In P. Dion (ed.), Soil Biology and Agriculture in the *Tropics, Soil Biology* 21. Springer-Verlag Berlin Heidelberg. 2010. https://doi.org/10.1007/978-3-642-05076-3_9.

27. Smith SE, Read DJ. Mycorrhizal symbiosis. Academic Press (3ième ed). Londres. 2008.

28. Onguene NA, Kuyper TW. Importance of the ectomycorrhizal network for seedling survival and ectomycorrhiza formation in rain forests of south Cameroon. Mycorrhiza. 2002 ; 12(1):13-7. doi: 10.1007/s00572-001-0140-y

29. Onguene NA, Kuyper TW. Growth response of three native timber species to soils with different arbuscular mycorrhizal inoculum potentials in South Cameroon. For Ecol Manag. 2005 ; 210(s 1–3):283–290. https://doi.org/10.1016/j.foreco.2005.02.038.

30. Zangaro W, Nisizaki SMA, Domingos JCB, Nakano EM. Mycorrhizal response and successional status in 80 woody species from south Brazilian. J Trop Ecol. 2003 ; 19:315–324. doi:10.1017/S0266467403003341.

31. Silveira AD, Gomes VFF. Micorrizas emplantas frutıferas tropicais. In: Silveira APD,Freitas SS (eds) Microbiota do solo e qualidade ambiental. Instituto Agronoˆmico, Campinas. Brazil. 2007. Portuguese

32. Siqueira JO, Soares CRFS., Santos JGD, Schneider J, Carneiro MAC. Mycorrhizas and soil degradation: characterization, effects and recovery action. In: Ceretta CA, Silva LS, Reichert JM (eds) Topics in soil science, vol 5. Sociedade Brasileira de Ciencia do Solo, Viçosa, Brazil. 2007. Portuguese

33. Cardoso IM, Guijt I, Franco FS, Carvalho AF, Ferreira NPS. Continual learning for agroforestry system design university, NGO and farmer partnership in Minas Gerais, Brazil. Agri Syst. 2001 ; 69:235–257. doi : 10.1016/s0308-521x(01)00028-2

34. Siqueira LC. Floristic and ethnobotanical survey of the arboreal stratum in natural and agroforestry systems, Araponga. Magister Scientiae dissertation, Federal University of Viçosa, Viçosa, Minas Gerais. Brazil, 2008. Portuguese.

35. Sery DJ-M, Kouadjo ZGC, Voko-Bi DRR., Zeze A. Selecting native arbuscular mycorrhizal fungi to promote cassava growth and increase yield under field conditions Front Microbiol. 2016 ; 7(2063) :1-13. doi : 10.3389/fmicb.2016.02063.

36. Djatta MB, Manzo OL, Diouf PM, Diop T. Effects of mycorrhizal inoculation on sesame (Sesamum indicum L.) under natural conditions. Int J Biol Chem Sci. 2013; 7 (5): 2050-2057. doi: 10.4314 / ijbcs.v7i5.22 French.

37. Voko Bi R-DR .., Ahonzo-Niamke SL, Zeze A. Impact of the physicochemical properties of cassava growing soils on the abundance and diversity of communities of arbuscular mycorrhizal fungi in the agroecological zone of Azaguié, South-eastern Côte d'Ivoire. Agron Afri. 2013; 25 (3): 251-264. French

38. Berruti A, Lumini E, Balestrini R, Bianciotto V. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front Microbiol. 2016 ; 6(1559) : 1-13. doi: 10.3389/fmicb.2015.01559.

39. Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature. 2005 ; 435 : 819-823. https://doi.org/10.1038/nature03610

40. Meddich A, Oihabi A, Jaiti F, Bourzik W, Hafidi M. Role of arbuscular mycorrhizal fungi in the tolerance of date palm (Phoenix dactylifera) to vascular fusarium wilt and water deficit. Botany. 2015 ; 93 (6): 369-377. French

41. Walder F, van der Heijden MGA. Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. Nat Plants. 2015 ; 1: 15159. https://doi.org/10.1038/nplants.2015.159

42. Ouahmane L, Hafidi M, Thioulouse J, Ducousso M, Prin Y, Galiana A, Boumez-Zougha A, Duponois R. Improvement of Cupressus atlantica Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. J Appl Microbiol. 2007 ; 103: 683-690.

43. Cornet F, Diem HG, Dommergues YR. Effect of inoculation with Glomus mosseae on the growth of Acacia holosericea in the nursery and after transplantation in the field. In: Mycorrhizae: biology and use Ed. INRA Publ. Les Colloques de l'INRA, n ° 13. 1982. French.

44. Duponnois R, Bâ AM, Prin Y, Baudoin E, Galiana A, Dreyfus B. Mycorrhizal fungi: major component in the biological processes governing the stability and productivity of tropical forest ecosystems. Project report: the major African project of the Great Green Wall.pp.42-440. 2010. French.

45. Sinclair G, Charest C, Dalpé Y, Khanizadeh S. Influence of colonization by arbuscular mycorrhizal fungi on three strawberry cultivars under salty conditions. Agric food sci. 2014 ; 23 (2): 146–158.

46. Trouvelot S, Wipf D, Lauvergeat V, Bonneau L, Dal F, van Tuinen D, Courty PE. Ecosystem services rendered by mycorrhizal to arbuscular symbiosis to the vineyard. Review of Oenologists and Vitivinicultural and Oenological Techniques. 2021; 178: 27-29. French

47. Rillig MC, Steinberg PD. Glomalin production by an arbuscular mycorrhizal fungus : a mechanism of habitat modification? Soil Biol Biochem. 2002 ; 34:1371–1374. https://doi.org/10.1016/S0038-0717(02)00060-3.

48. Elsen A, Baimey H, Swennen R, De Waele D. Relative mycorrhizal dependency and mycorrhiza-nematode interaction in banana cultivars (Musa spp.) differing in nematode susceptibility. Plant Soil. 2003 ; 256 : 303-313. https://doi.org/10.1023/A:1026150917522.

49. Thygesen K, Larsen J, Bodker L. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by Aphanomyceseuteiches using oospores as pathogen inoculum. Eur J Plant Pathol. 2004 ; 110 : 411-419. https://doi.org/10.1023/B:EJPP.0000021070.61574.8b

50. Ortas O, Ustuner O. The effects of single species, dual species and indigenous mycorrhiza inoculation on citrus growth and nutrient uptake. Eur J Soil Biol. 2014 ; 63, 64–69. doi: 10.1016/j.ejsobi.2014.05.007.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

AM: Arbuscular mycorrhizae
AMF: Arbuscular mycorrhizal Fungi,
SODEFOR: Forestry Development Society
INP-HB: National Polytechnic Institute Houphouët-Boigny