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Impact of mycorrhization on transplanting stress and the juvenile growth of an Ivorian forest species *Guibourtia ehie* (Fabaceae, (A. Chev.) J. Leonard)

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Ivorian forest has been experiencing degradation for several decades despite reforestation efforts. The main cause of the failure of reforestation policies is an important mortality at the time of planting (transplanting stress). To remedy this problem, mycorrhization technology based on the beneficial effects of arbuscular mycorrhizal fungi (AMF) could provide a sustainable solution. The objective of this study was to evaluate the effects of AMF inocula (local and commercial inoculum) on the juvenile growth of a Côte d'Ivoire forest species (*Guibourtia ehie*). Vegetative growth parameters and mineral nutrition (N, P, K, and Ca) were evaluated. After 150 days of cultivation, the plants treated with the local inoculum had the highest mycorrhization frequencies (75%) and intensities (21.23%). Also for growth parameters (height, number of leaves, leaf area, and crown diameter) and for nitrogen and potassium contents, the plants treated with the local polyspecific inoculum had the highest values compared to the plants treated with the commercial inoculum and the control plants. Mycorrhization improved mineral nutrition as well as vegetative growth of *G. ehie* seedlings. The integration of mycorrhizal inoculation from local strains in reforestation policies could be a sustainable solution for the recolonization of degraded forests by endangered species.

Key words: *Guibourtia ehie*, inoculum, mycorrhization mineral nutrition, vegetative growth, Ivory Coast.

INTRODUCTION

Tropical forests harbour many forest species with high economic value (Parmentier et al., 2007; Schroeder et al., 2010; Slik et al., 2015). However, anthropogenic activities including agriculture and extractive activities have been responsible for the degradation of these ecosystems (Ghazoul and Sheil, 2010; Maystadt et al., 2020). The overexploitation of forest resources mainly leads to the degradation of the ecological characteristics of the ecosystems, the impoverishment of the vegetation cover and especially the extinction of important forest species such as *Guibourtia ehie* (Gone et al., 2013). Indeed, of the 43 local forest species commonly recorded,

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25 are considered by the IUCN as vulnerable, and at risk (Kouadio, 2012). *G. ehie* is a slow-growing plant (Oteng-Amoako and Essien, 2011) whose trees are susceptible of burning (Hall and Swaine, 1981). It is common in West African tropical forests (Keay, 1990), but now threatened by overexploitation because of its wood quality (Hawthorne and Jongkind, 2006; Tosso et al., 2017). In Côte d'Ivoire, reforestation efforts have been undertaken by the forest development company (SODEFOR) to recolonize environments with extinct or endangered species. However, very few forests have been reconstituted and the keystone species that were the pride of the Ivorian forests are continuing to disappear. The main reason for the failure of reforestation policies is significant mortality at transplantation crisis (Ouahmane et al., 2007). This mortality would be linked to the difficult adaptation of plant species to the new unfavorable ecological conditions of degraded ecosystems. To remedy this difficulty, mycorrhizal symbiosis could be a promising and sustainable alternative. Arbuscular mycorrhizal fungi could play an essential role in the adaptation and survival of plant species in adverse environments. These mycorrhizal fungi provide the host plant with better phosphate, nitrogen and water nutrition. In addition to its nutritional contribution, the symbiosis confers on plants a high resistance to abiotic stresses (Aroca et al., 2017) through the improvement of plant nutritional status (Colla et al., 2008), the dilution of the toxic effects of ions (Audet and Charest, 2006; Kapoor and Bhatnagar, 2007) and the modification of plant physiology (Kumar et al., 2010). In temperate regions, ectomycorrhizal fungi have improved the growth and mineral nutrition of trees (Smith and Read, 2008). They also allow trees to better resist certain root diseases and to better exploit water resources. In the tropics, the primordial role of endomycorrhizal fungi in reforestation has already been demonstrated in the Sahel. Mycorrhizal inoculation has allowed the successful reforestation of more than 5,104 ha out of 11,106 ha planned (Duponnois et al., 2010). Thus, the evaluation of the mycorrhizal status of forest species and the integration of this data in the realization of nurseries and then in the growth and development of seedlings in plantation could be a sustainable solution for the colonization of forests degraded by endangered species. In this study the main objective is to obtain by the mycorrhization technique balanced, vigorous, and healthy *G. ehie* seedlings able to withstand the stress of transplantation in degraded ecosystem conditions.

MATERIALS AND METHODS

Inoculum 1 characteristics

Inoculum 1 production

Inoculum 1 (Local inoculum) was produced by trapping AMFs in the soil of the INP-HB (Institut National Polytechnique Félix Houphouet-Boigny) forest. The trapping technique is a bioassay, which allows to obtain AMF propagules in quality and quantity to initiate inoculation tests (Morton et al., 1993). Cowpea, which has a 60- to 70-day cycle, was chosen as the host plant. Cowpea seeds disinfected with 12°-10% bleach and rinsed once for 2 min 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/cm^2, 3 h) and 150 g of forest soil serving as inoculum (2 plants per pot).

Arbuscular mycorrhizal fungal (AMF) spore identification in inoculum 1

After 3 months, the number of AMFs propagules (spores) from trap culture was established. Spores were extracted by wet-sieving and decanting (Gerdemann and Nicolson, 1963) using sieve with different sizes (45, 90, 125 and 500 µm) and the modified sucrose density gradient centrifugation method (Walker et al., 1982). For AMF spore's identification, healthy spores were mounted on glass microscope slides and stained with polyvinyl alcohollacto-glycerol (1 v/v PVLG) mixed with and without Melzer's reagent (Morton et al., 1993; Brundrett et al., 1994). Spores were cracked open to allow spore substructure characteristics under an optic microscope (EUROMEX Holland CSL/CKL) at a magnification of ×400. AMF spore morphotyping was based on Oehl et al. (2011) and the revision of *Glomeromycota* genera proposed by Redecker et al. (2013). The number of AMFs propagules (spores) in inoculum 1 (substrate in the pots) was estimated to be 700 spores per gram.

Inoculum 2 characteristics

Inoculum 2 is a commercial monospecific inoculum of *Glomus intraradices* produced by Myke Pro whose density has been estimated by the manufacturer at 3000 propagules/g.

Collection of G. ehie seedlings

G. ehie seedlings of about 10 cm high at the four-leaf stage were collected in Yamoussoukro (Côte d'Ivoire) in the arboriculture of the Institut National Polytechnique Félix Houphouët-Boigny (INPHB). This forest created in 1989 is full of essential forest species such as *Milicia excelsa* (Moraceae), *Mansonia altissima* (Sterculiaceae), *Pterygota marcrocarpa* (Sterculiaceae), *G. ehie* (Fabaceae), *Triplochiton scleroxylon* (Sterculiaceae), and *Terminalia ivorensis* (Combretaceae). To harvest a seedling, furrows were made around the seedling with a daba, then it was dug up with the clod of soil present on the roots. The roots were then cleaned of the soil clod and rinsed thoroughly with water to remove any surface microorganisms. On each plant, at the lateral roots, a sample of the finest roots likely to be colonized by native AMF were pulled out for a colonization check according to Trouvelot et al. (1986). Only seedlings with 0% colonization were retained for the study.

Inoculation process of G. ehie 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 of sterilized potting soil (autoclaved at 110°C, 2 kg/cm^2, 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/pot). The roots of the seedlings were placed in direct contact with the inoculum to optimize mycorrhizal

Table 1. Mycorrhization intensity and frequency.

Treatment	Mycorrhization intensity (%)	Mycorrhization frequency (%)
Control	00±0.00	00±0.00
Inoculum 1	21.23^{a}±4.37	75^{a}±3.77
Inoculum 2	5.40^{b}±1.77	14.5^{b}±2.30

Means within the same column followed by the same letter are not significantly different at the P = 0.05 level of probability based on Tukey's HSD statistics

colonization. The seedlings in the control bags were carried in 2000 g of sterilized potting soil + 200 g of sterilized substrate (autoclaved at 110°C, 2 kg/cm^2, 3 h). Each bag was watered with 500 ml of water every 3 days until the end of the experiment.

Experimental design

The experiment took place in the open area at the edge of the experimental forest of the INP-HB. The design is completely randomized and includes one (1) plant species (*G. ehie*), three (3) treatments and 30 replications. The treatment factor has three levels: Inoculum 1 (local inoculum), Inoculum 2 (commercial inoculum) and Control. A total number of 90 seedlings were used, 30 seedlings per treatment.

Assessment of root colonization

Fine roots were sampled at 150 days of cultivation with three replicates per treatment. Each treatment contained three plants. Roots were rinsed and cut into 1 cm fragments. These roots fragments were cleared by boiling in 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue in lactoglycerol according to Phillips and Haymann (1970) method. Ten pieces of roots per plant were placed in glycerol (50%) between slide and coverslip (Kormanik and McGraw, 1982) and observed under an optical microscope. Root colonization was evaluated through two parameters: mycorrhization intensity and mycorrhization frequency. The 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. The colonized roots were observed and evaluated according to Trouvelot et al. (1986).

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 by means of 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 stock solutions obtained were stored in flasks and thus ready for the determination of mineral elements. Nitrogen was determined by the Kjeldahl method (Bremner, 1960) with mineralization in the presence of glucose to avoid nitrate losses and catalysts (SO_4K_2, SO_4Cu, and Selenium). Phosphorus was determined by phopho-vanado-molybdate colorimetry (Pansu and Gautheyrou, 2006); potassium and calcium by flame photometry after ion exchange; magnesium by the complexometric method.

Collection of growth data

Measurements were made on the first day of transplantation (D1), on the 30th day (D30), on the 60th day (D60), on the 90th day (D90), on the 120th day (D120) and on the 150th day (D150) corresponding to the number of days necessary to judge the resistance to transplantation stress. The data collected concerned the survival rate, the height of the seedlings, the diameter at the collar and the leaf area. The survival rate was determined according to the following formula:

Survival rate (%) = (Number of surviving plants)/(Number of replicates) × 100

Plant height was measured with a 30 cm ruler. The diameter at the neck of the plants was measured with a Vernier caliper. The number of leaves was obtained by counting. Total leaf area for each individual plant sampled per treatment was determined as follows: leaves were classified into "large" (L) and "small" (S) batches according to whether they had reached maximum growth or not. 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 (SFT) 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.

Statistical analysis of the data

The data obtained in this study were processed by a single 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 which means actually differed when the analysis of variance revealed a significant difference. The Tukey HSD test also allowed for multiple comparisons of means to form homogeneous groups.

RESULTS

Mycorrhizal colonization rate of *G. ehie* roots at 150 days

At 150 days of cultivation, roots of plants treated with inoculum 1 showed higher mycorrhization intensities and frequencies than those treated with inoculum 2. Roots of control plants showed no mycorrhizal structure (Table 1).

Impact of mycorrhization on plant mineral nutrition

The average mineral element contents of aerial parts are

Table 2. Mineral contents of aerial parts of *Guibourtia ehie* seedlings.

Treatment	Nitrogen (%)	Phosphorus (ppm)	Potassium (cmol.kg^{-1})	Calcium (cmol.kg^{-1})	Magnesium (cmol.kg^{-1})
Control	1.68^{b}±0.15	0.22^{a}±0.05	0.81^{b}±0.15	1.46^{a}±0.25	0.4^{a}±0.22
Inoculum 1	2.25^{a}±0.21	0.23^{a}±0.03	1.89^{a}±0.07	1.45^{a}±0.23	0.4^{a}±0.27
Inoculum 2	2.01^{ab}±0.18	0.22^{a}±0.04	0.98^{b}±0.18	1.43^{a}±0.20	0.38^{a}±0.18

Means within the same column followed by the same letter are not significantly different at the P = 0.05 level of probability based on Tukey's HSD statistics

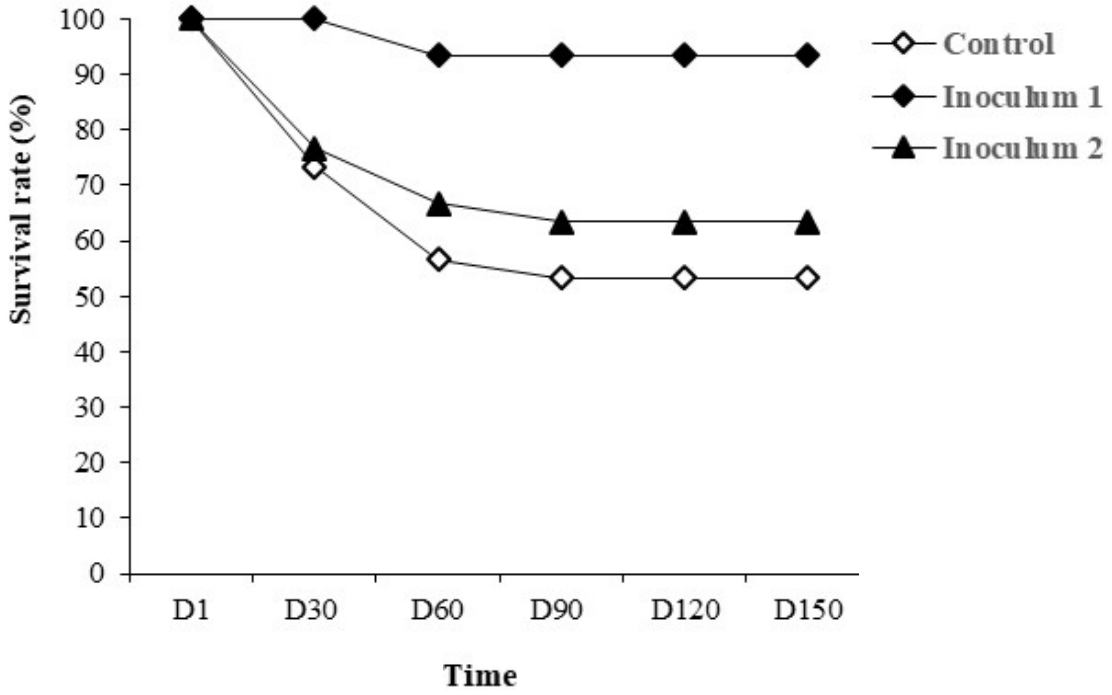

Figure 1. Survival rate of *Guibourtia ehei* seedlings as a function of treatments and growth duration.

presented in Table 2. The mineral nitrogen content of the leaves of plants treated with inoculum 1 was higher (2.52%) than those obtained with the control (1.68%). Similarly, the potassium content of the leaves of plants treated with inoculum 1 is higher (1.89 cmol.kg^{-1}) than those obtained with inoculum 2 (0.98 cmol.kg^{-1}) and the control (0.807 cmol/kg^{-1}). On the other hand, for phosphorus, calcium and magnesium contents, no significant difference was noted between the three different treatments with Tukey's HSD test (p≤ 0.05).

Adaptation of mycorrhizal seedlings to transplant stress

Survival of seedlings under transplanting stress

The survival rate of seedlings to transplantation stress was assessed from day 1 to day 150 (Figure 1). For seedlings treated with inoculum 1, the survival rate was 100% for the first 30 days of transplantation. From the 60th day, the survival rate decreased to 93.33% and then stabilized at this value until the 150th day. With the plants treated with inoculum 2, a continuous decrease from day 1 to day 90 of transplantation from 100 to 63.33% was observed, then this rate was maintained until day 150. In the case of plants that received no inoculum (controls), the survival rate dropped from 100 to 53.33% from day 1 to day 90 of transplantation, then the rate stabilized until day 150.

Impact of mycorrhization on the growth of G. ehie seedlings

The height of the plants as a function of time is as shown in Figure 2A. All plants have a continuous height growth from day 1 to day 150. The plants treated with inoculum 1

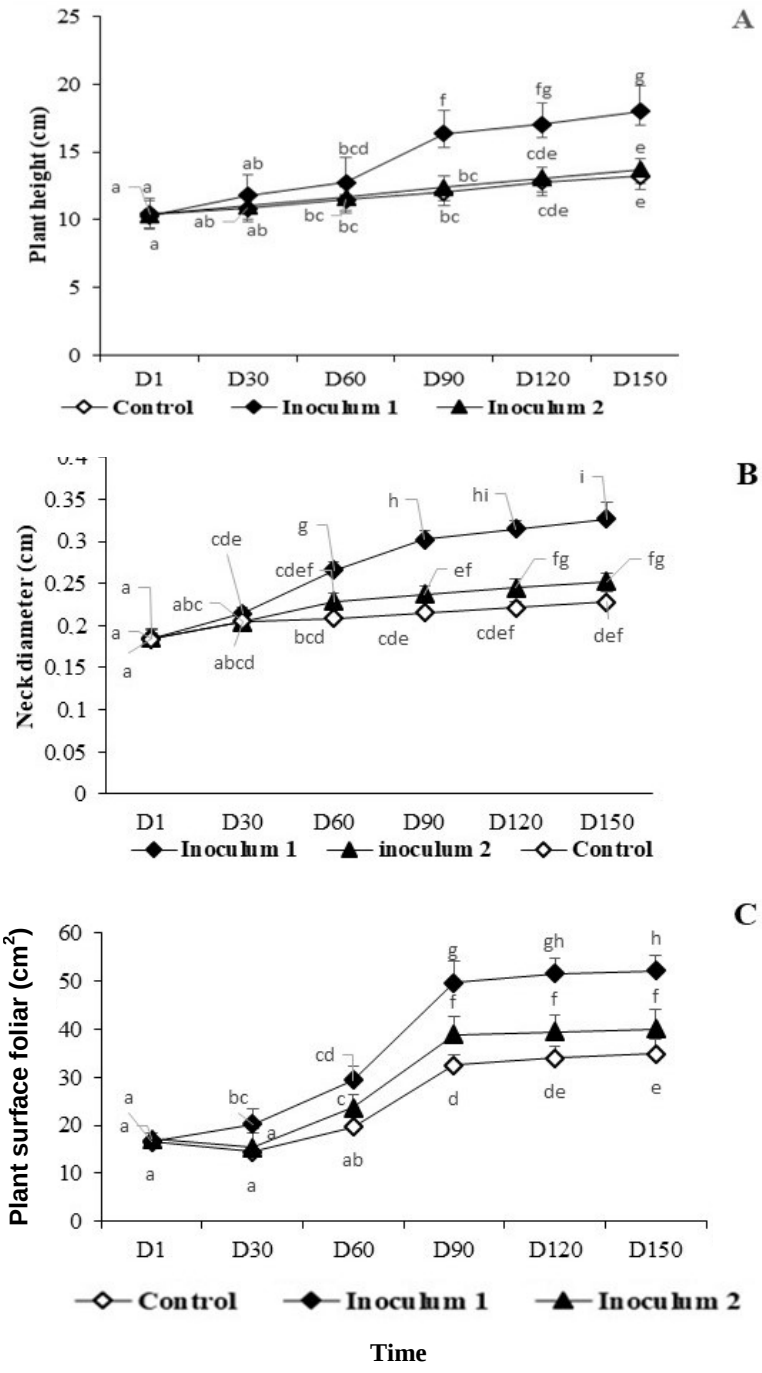

Figure 2. Evolution of growth parameters of *Guibourtia ehie* plants according to treatments and time. A. Average height; B. Average collar diameter; C. Average total leaf area. Means within the same column followed by the same letter are not significantly different at the P = 0.05 level of probability based on Tukey's HSD statistics.

show the greatest growth in height. Indeed, with inoculum 1 the growth of the plants is faster. However, no significant difference was observed between the height growth of the control plants and the plants treated with inoculum 2 until day 105.

Evolution of the diameter at the neck of the plants as a function of time was evaluated (Figure 2B). All curves show an increasing trend. However, the diameter of the plants treated with inoculum 1 evolved faster than those treated with inoculum 2. Also, the diameter of the plants

treated with inoculum 2 evolved faster than that of the control plants. Indeed, as early as 30 days after transplanting, a significant difference was observed between the diameter of the plants treated with inoculum 1 and the initial diameter of the seedlings at T0. It is from 60 days that a significant difference is noted between the diameter of the plants treated with inoculum 2 and the initial diameter at T0. It is only after 90 days that a significant difference is noted between the diameter of the control plants and the initial diameter at T0 of the control seedlings.

Evolution of the average total leaf area of *G. ehie* plants is as shown in Figure 2C. In control plants and inoculum 2, a decrease in leaf area was observed during the first 30 days followed by an increase in leaf area from day 30 to day 150. On the other hand, inoculum 1, the growth of leaf area is continuous from time T0 to 150 days. Also, the growth of the SFT of the plants treated with inoculum 1 is faster than that of the control plants and the plants treated with inoculum 2. As early as 60 days, there is a significant difference between the total leaf areas of the plants treated with inoculum 1 and those of the seedlings at T0. The growth of plants treated with inoculum 2 was faster than that of the control plants. However, it is from 90 days that a significant difference is observed between the plants treated with inoculum 2 and the seedlings at T0.

DISCUSSION

This study was conducted with the objective of evaluating the effect of mycorrhization on adaptation to transplanting stress, mineral nutrition and growth of young shoots of *G. ehie.* It is a proposal for sustainable solutions to the difficulties encountered by reforestation policies in a context of climate change and forest ecosystem degradation. This study shows that the mycorizogenic power of inoculum 1, that is, the local polyspecific inoculum, is more important on *G. ehie* seedlings than that of inoculum 2, that is, the exotic monospecific inoculum. The climatic and edaphic environment would influence the ability of arbuscular mycorrhizal fungi to colonize plant roots (Casazza et al., 2017; Melo et al., 2019). Thus, the exotic inoculum (inoculum 2) would have difficulty developing under local ecological conditions. The same conclusion has been drawn by several authors (Copeman et al., 1996; Berruti et al., 2016). In fact these authors proved that in general, local inocula colonize plants better than inocula of foreign origin. It was also obtained during this study that mycorrhization, especially with the local polyspecific inoculum, improved the nitrogen and potassium contents of the aerial parts on the other hand no improvement in phosphorus, magnesium and calcium contents was noted compared to the control. These results are contrary to some studies dealing with the impact of mycorrhization on plant mineral nutrition, which concluded that mycorrhization acted mainly by improving phosphate nutrition (Walder and van der Heijden, 2015; Shi et al., 2021). In contrast, most studies on tropical soils reach the same conclusion as ours. Namely, that mycorrhization has more effects on nitrogen and potassium nutrition than on phosphorus nutrition (Osonubi et al., 1995; Séry et al., 2016).

The mycorrhized seedlings had higher survival percentages than those that received no inoculum. That is, 63.33% survival with inoculum 2 and up to 93.33% survival with inoculum 1, compared to 53.33% with the control plants. The mycorrhized seedlings were more resistant to transplant stress despite the pre-transplant removal of the thinner roots. This important ability of mycorrhization to improve plant adaptation to stressful conditions or changing ecosystems has been cited several times (Smith and Read, 2008; Sinclair et al., 2014). Indeed these studies showed that mycorrhization improved plant adaptation to harsh conditions through its positive action on soil structure (Rillig and Steinberg, 2002; Zhang et al., 2017), inhibition of some soil pathogens (Elsen et al., 2003; Chen et al., 2018; Diagne et al., 2020) and mobilization of essential mineral elements.

Also, during this study, mycorrhization improved the overall growth parameters of *G. ehie* seedlings compared to control plants. Indeed, the increase in total leaf area of treated seedlings was greater than that of untreated seedlings. However, only inoculum 1 resulted in a greater growth in height and an increase in the diameter at the collar of the seedlings compared to the control plants. Overall, inoculum 1 appeared to perform better than inoculum 2 (N and K nutrition, transplant stress resistance, seedling height, and total leaf area). This can be explained by the greater root colonization with inoculum 1 compared to inoculum 2. Indeed, the benefits brought to the plant by mycorrhization are proportional to the root colonization rate (Campo et al., 2020). These results are consistent with the majority of studies that have compared 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 (Ortas and Ustuner, 2014; Kouadio et al., 2017).

Conclusion

Anthropogenic activities such as agriculture and overexploitation of species are at the origin of the disappearance of important forest species. The quality of the timber and the use of secondary metabolites in pharmaceutical and cosmetic industries make *G. ehie* a highly sought after plant. This study proved that mycorrhization could provide solutions to the reforestation difficulties of this plant. Indeed, adaptation

to transplanting stress, mineral nutrition and vegetative growth of *G. ehie* seedlings were improved by mycorrhization. However, for a better efficiency of the mycorrhization technology on reforestation and for a sustainable commercial exploitation of this plant, it will be necessary to develop an ecological engineering from local strains. This technology will consist of identification and selection of best strains for the production of efficient inoculums.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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