



Improved *Sclerocarya birrea* (A. Rich.) Hochst. growth by mycorrhizal inoculation

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Abstract

Sclerocarya birrea is a multipurpose species which is recognized as a plant species of commercial, medicinal and cultural importance in Africa. However, it remains in the wild and its production and existence are dependent on the vagaries of the weather. This study was carried out to improve this plant growth by mycorrhizal inoculation. In this study, *Sclerocarya birrea* was grown in the greenhouse for nine months and inoculated with three mycorrhizal inocula. The height and the collar diameter were measured at three and nine months after sowing. The shoot, root and total biomass as well as the relative growth rate in height and the collar diameter were evaluated at nine months after sowing. The results of this study show that the roots of *Sclerocarya birrea* are relatively mycorrhized by arbuscular mycorrhizal fungi (AMF) and the M1 inoculum appears to be the best of all the inocula used. This inoculum improves height growth by 31.72% (9th month after sowing), collar diameter by 77.27% and 80.15% (respectively at 3rd and 9th month after sowing) and relative growth rate in height by 71.43%, shoot biomass of *Sclerocariat birrea* by 59.95%, root biomass by 101.75% and total biomass by 66.99% compared to the control.

Keywords: *Sclerocariat birrea*, Inoculation, Arbuscular mycorrhizal fungi, Burkina Faso

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1. Introduction

Sclerocarya birrea (A. Rich.) Hochst., subspecies *caffra* (family: Anacardiaceae) is an important food, commercial, cultural and ethnomedicinal plant in Africa (Ojewole et al., 2010). It's a deciduous tree that produces abundant yellowish green and plum shaped fruit, that contain sour sweet pulp covered by a tough skin (Ndhlala et al., 2007). The tree is highly valued in southern Africa for its delicious fruit and ethnomedicinal properties, and has received great attention in terms of domestication and commercialization (Viljoen et al., 2008). In Burkina Faso *Sclerocarya birrea* occurs in all climatic zones, sometimes in pure and dense populations (Bationo/Kando et al., 2008). The plant is the subject of multiple uses in this country and it's considered by the populations of certain areas of the Sahel as one of the priorities woody species (Ouedraogo and Belem, 1999). It's one of the most widely used spontaneous woody plants for human consumption (Belem et al., 2008) and the main

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product used is the fruit (Bationo/Kando et al., 2009). Despite its importance, this plant has remained essentially wild, hence the interest in its domestication both to protect the tree as a resource and to allow its exploitation in a sustainable way. However, in Burkina Faso as in many countries, plant growth faces a number of constraints such as poor soil and drought. However, previous studies (Muok and Ishiim, 2006) have mentioned the ability of *Sclerocarya birrea* to associate with arbuscular mycorrhizal fungi (AMF). In addition, the ability of AMF to improve the growth of their host plants even when they grow on soils relatively poor in mineral elements has already been shown for certain plants (Haro et al., 2012 and 2016; and Haro and Sanon, 2020). This study was initiated with the aim to improve the *Sclerocarya birrea* growth in order to contribute to its domestication.

2. Materials and methods

2.1. Plant and fungal materials

The local seeds of *Sclerocarya birrea* were used. These seeds were tramped in tap water for 24 h and then washed thoroughly with water. They were surface disinfected by soaking in 96% ethanol for 3 min, rinsed thoroughly with sterile distilled water and then disinfected in calcium hypochlorite solution (CaCl_2O_2 at 3.3%, w/v) for 3 min and finally rinsed thoroughly with sterile distilled water before sowing. These seeds were sown at a rate of two seeds per pot.

The fungal material was composed of three inocula of AMF including an efficient local mixed inoculum isolated from the rhizosphere of cowpea cultivated in Burkina Faso [*Scutellospora* sp., *Gigaspora* sp., *Glomus* sp. (M1)] (Haro, 2016) and two fungal inoculum from the collection of Laboratoire Commun de Microbiologie (LCM) of Senegal [*Glomus aggregatum*, (M2) and *Rhizophagus irregularis* (M3)]. The inocula were obtained by multiplication of AMF (Haro et al., 2012). The inoculum constituted of spores, mycorrhizal root fragments and soil.

2.2. Culture substrate

The growing substrate was a sterilized soil of Ouagadougou and its physico-chemical characteristics were as follows: clay (%) 3.92, total silt (%) 5.88, total sand (%) 90.2, total organic matter (%) 0.331, total carbon (%) 0.192, total nitrogen (%) 0.016, C/N 12, total phosphorus (mg.kg⁻¹) 172.52, available phosphorus (mg.kg⁻¹) 1.74 and pH H₂O: 6.44. Culture substrate was homogenized, sieved with a 2 mm sieve and sterilized at 121 °C for 1 h.

2.3. Greenhouse experiment

The experiment was conducted for nine months in 4 L pots containing 4 kg of sterilized culture substrate. The inoculation was carried out at the sowing time with 10 g of inocula [each inoculum constituted of spores, mycorrhizal root fragments and soil and was kept at room temperature (about 25 °C)] (Haro et al., 2017) for each inoculated treatment. The inoculation consisted to place in the middle of each pot containing the culture substrate, 10 g of inoculum at 2 cm or 3 cm deep. Control pots weren't inoculated. There were 10 replicates per treatment (n = 3 mycorrhizal inocula + 1 control x 10 replicates = 40 plants). *Sclerocarya birrea* was sown at the rate of two seeds per pot and a wedge was carried out two weeks after the plants emergence to allow only one plant per pot. The experimental design used was a simple randomization complete block design.

To estimate the effect of mycorrhizal inoculation on *Sclerocarya birrea*, the height, the collar diameter, the rate of relative growth in height and the rate of relative growth of the collar diameter were calculated at the 3rd and at the 9th month after sowing. Height growth was measured from the growing tip to the base of the stem. The relative growth rate in height (RGRh) was calculated according to the following formula:

$$RGRh = \frac{\ln(h_2) - \ln(h_1)}{T_2 - T_1}$$

with h : height, T : time, $_1$: initial and $_2$: final.

The collar diameter was measured using a caliper at the separation zone between the root system and the aerial part at the 3rd and at the 9th month after sowing. The relative growth rate of the collar diameter (TCRdc) was calculated by the following formula:

$$RGRD_c = \frac{\ln(Dc_2) - \ln(Dc_1)}{T_2 - T_1}$$

with Dc: Collar diameter, T: time, ₁: initial and ₂: final.

2.4. Shoot, root and total biomass measurement

At nine months after sowing, each plant was carefully removed in order to recover the aerial part and all the roots of the plants. All these parts were dried in an oven at 70 °C for 72 h for the measurement of shoot, root and total biomass. After the biomass measurement, the roots were used for the mycorrhizal infection study.

2.5. Staining for mycorrhizal colonization

About 10 g of roots from each treatment were thoroughly washed and placed in falcon tubes and then cleared using 10% KOH. They were heated in 90 °C water bath for 1 h. The roots were washed with tap water. Staining was then done by adding 0.05% trypan blue in lactic acid and heating in 90 °C water bath for 30 min (Phillips and Hayman, 1970) and the observation was done under microscope (OLYMPUS BH-2) (magnification = 10x). The mycorrhizal frequency and intensity were estimated by Trouvelot et al. (1986) method.

2.6. Data analysis

Data were statistically analyzed using a one-way analysis of variance (ANOVA) with XLSTAT 2018 statistical software and the means were compared using the Newman-Keuls test ($p < 5\%$).

3. Results and discussion

3.1. Mycorrhization parameters measurements

Sclerocarya birrea mycorrhization varies according to the mycorrhizal inoculum (Figure 1). The frequency of mycorrhization is generally high while the intensity of mycorrhization is medium. Statistical analyzes show significant differences between the different treatments and the highest values were obtained with the M1 inoculum for both the frequency (85.71%) and the intensity (28.06%) of mycorrhization. These results could be

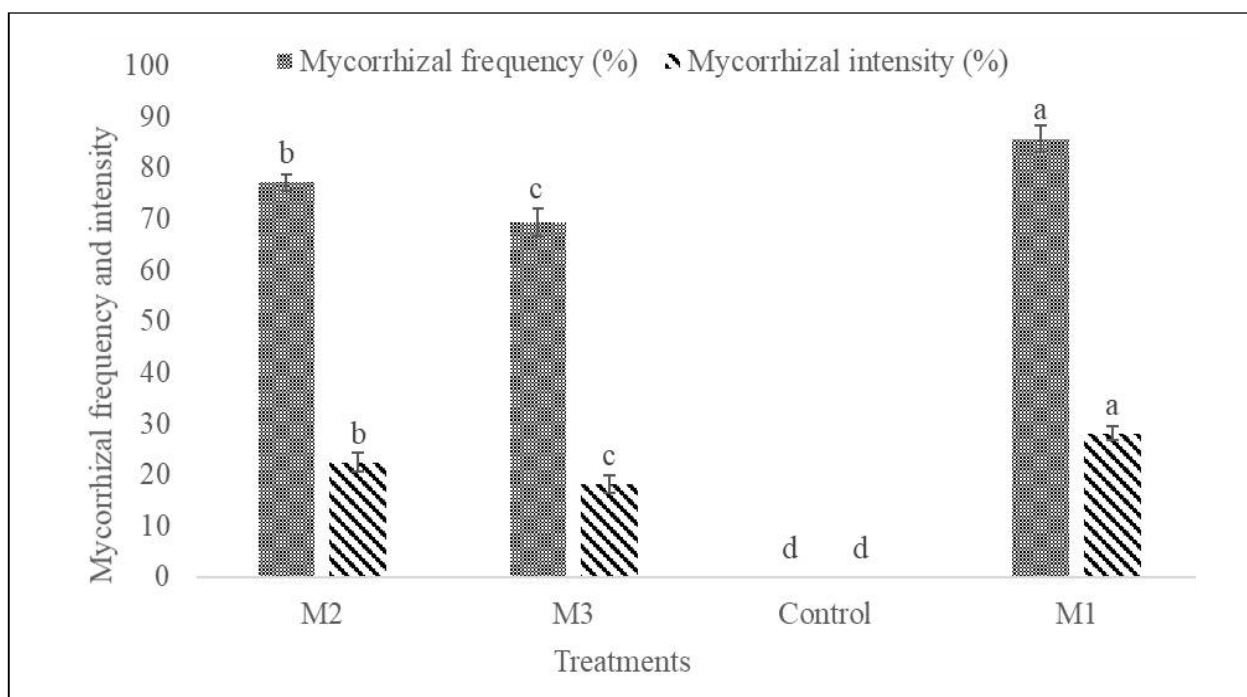


Figure 1: The mycorrhizal frequency and intensity of *Sclerocarya birrea* plants inoculated or not with (M1, M2 and M3)

Note: Values of the same parameter (mycorrhizal frequency or intensity) with the same letter are not significantly different according to the Newman-Keuls test ($p < 0.05$); Significance level for mycorrhizal frequency and intensity ($p < 0.0001$); and Standard error of the mean ($n = 10$).

explained by the fact that *Sclerocarya birrea* is a mycotrophic plant. These results are in agreement with those of Muok and Ishiim (2006) who showed that *Sclerocarya birrea* had a root colonization of AMF reaching 48.5%. However, the roots of the control treatments aren't mycorrhizal. The absence of mycorrhizal infection on the controls roots could be justified that the treatments are free from any endomycorrhizal contamination. These results corroborate those of Muok and Ishiim (2006) who found no mycorrhization on the roots of uninoculated *Sclerocarya birrea*. The growth and biomass parameter stimulation between the different treatments compared to the control could be attributed to the effect of inoculated AMF. The growth parameter results show that inoculation significantly improves *Sclerocarya birrea* height and collar diameter.

3.2. Growth and biomass production parameters measurements

The growth parameters measurements are presented in Table 1. The statistical analyzes show significant differences ($p < 0.05$) between the different treatments for all the growth parameters except the height measured at three months after sowing ($p > 0.05$).

At three months after sowing, no significant difference was statistically observed for the height of *Sclerocarya birrea* inoculated treatment or not. The inoculation did not improve the *Sclerocarya birrea* height. These results could be justified by the fact that the culture substrate contains the necessary nutrients and directly accessible by the plant roots. As a result, the roots of the plant manage to supply it with adequate nutrients, hence the ineffectiveness of mycorrhizal inoculation. However, the collar diameter was significantly improved by mycorrhizal inoculation compared to the control. This improvement varies according to the mycorrhizal

Table 1: Plant height, the collar diameter, the rate of relative growth in height and the rate of relative growth of the collar diameter of *Sclerocarya birrea* inoculated or not with 3 mycorrhizal inocula (M1, M2 and M3)

| Treatments | Height 1 (cm) | Height 2 (cm) | RGRh (cm/day.cm) | Diameter 1 (mm) | Diameter 2 (mm) | TCRDc (mm/day.mm) |
|--------------------|---------------------------|---------------------------|-------------------------------|--------------------------|--------------------------|------------------------------|
| Control | 29.75 ± 1.66 ^a | 33.2 ± 1.43 ^b | 0.0007 ± 0.00011 ^b | 3.08 ± 0.3 ^c | 4.08 ± 0.34 ^c | 0.0014 ± 0.0002 ^c |
| M1 | 35.5 ± 1.47 ^a | 43.73 ± 1.73 ^a | 0.0012 ± 0.00009 ^a | 5.46 ± 0.19 ^a | 7.35 ± 0.24 ^a | 0.0017 ± 0.0001 ^b |
| M2 | 34.36 ± 1.79 ^a | 41.79 ± 1.77 ^a | 0.0011 ± 0.00007 ^a | 4.69 ± 0.14 ^b | 7 ± 0.18 ^{ab} | 0.0022 ± 0.0002 ^a |
| M3 | 34.89 ± 2 ^a | 39.36 ± 1.93 ^a | 0.0007 ± 0.00099 ^b | 5.19 ± 0.2 ^{ab} | 6.29 ± 0.23 ^b | 0.0011 ± 0.0001 ^c |
| Significance level | NS | 0.0005 | 0.0001 | <0.0001 | <0.0001 | 0.0001 |

Note: For the same parameter, data followed by the same letters are not significantly different according to the Newman-Keuls test ($p < 0.05$); Standard error of the mean ($n = 10$); Height 1 and 2: height measured respectively at 3 and 9 months after sowing; Diameter 1 and 2: collar diameter measured respectively at 3 and 9 months after sowing; NS: not significant.

Table 2: *Sclerocarya birrea* shoot, root and total biomass 9 months after the sowing of *Sclerocarya birrea* inoculated with 5 mycorrhizal inocula (M1, M2 and M3)

| Treatments | Shoot biomass (g) | Root biomass (g) | Total biomass (g) |
|--------------------|---------------------------|---------------------------|---------------------------|
| Control | 8.59 ± 0.33 ^c | 1.71 ± 0.16 ^c | 10.3 ± 0.37 ^c |
| M1 | 13.74 ± 0.56 ^a | 3.45 ± 0.14 ^a | 17.2 ± 0.69 ^a |
| M2 | 13.49 ± 0.42 ^a | 3.13 ± 0.13 ^{ab} | 16.62 ± 0.53 ^a |
| M3 | 11.51 ± 0.51 ^b | 2.78 ± 0.15 ^b | 14.29 ± 0.64 ^b |
| Significance level | <0.0001 | <0.0001 | <0.0001 |

Note: For the same parameter, data followed by the same letters are not significantly different according to the Newman-Keuls test ($p < 0.05$); Standard error of the mean ($n = 10$).

inoculum and the highest values were obtained with the M1 inoculum (5.46 mm). This inoculum improved the collar diameter by 77.27% compared to the control. For this parameter, the plant seems to have a more pronounced nutritional need than for height. The content of mineral elements in the culture substrate would no longer allow the plant roots to adequately ensure its nutrition. Therefore, the plant uses mycorrhizae to boost its mineral nutrition, hence the effectiveness of mycorrhizal inoculation. These results corroborate those of Haro and Sanon (2020) who reported the effectiveness of mycorrhizal inoculation at the height of *Sesamum indicum*.

At nine months after sowing, the statistical analyzes showed significant differences ($p < 0.05$) for the height, the collar diameter, the rate of relative growth in height and in collar diameter (Table 1) as well as the biomass production (Table 2). The highest values were obtained with the M1 inoculum for the height (43.73 cm), the collar diameter (7.35 mm), for the relative growth rate in height (0.0012 cm/jour.cm), for shoot (13.74 g), root (3.45 g) and total biomass (17.2 g). This inoculum improved the height by 31.72%, the collar diameter by 80.15%, the relative growth rate in height by 71.43%, the shoot biomass by 59.95%, the root biomass by 101.75% and the total biomass by 66.99% compared to the control. These could be justified by the better efficacy of the inoculum M1 in improving the *Sclerocarya birrea* mineral absorption. These results corroborate those of Muok and Ishiim (2006) who found that AMF can be used to improve the *Sclerocarya birrea* growth in arid and semi-arid areas. Similar results were found by Haro et al. (2016) on cowpea. These authors reported that mycorrhizal inoculation improves *Vigna unguiculata* growth and biomass production.

For the relative growth rate in collar diameter, the highest values were obtained with the inoculum M2 (0.5%) which improves it by 35.14%. Thus, the inoculum M2 which seems more effective while the best collar diameter is obtained with the inoculum M1. This further confirms the effectiveness of the M1 inoculum on improving the *Sclerocarya birrea* growth.

4. Conclusion

The objective of this study was to improve the *Sclerocarya birrea* growth has shown that this plant responds well to mycorrhizal inoculation. From this study, it emerges that the M1 inoculum appears to be the best of all the inocula used. The results of this study are promising and deserve to be deepened by planting *Sclerocarya birrea* inoculated in situ.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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