Effect of the different substrates on root pruning of *Acacia auriculiformis* A. Cunn. ex Benth. and *Acacia mangium* Willd. seedling in the nursery.

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1 SUMMARY

The effects of different substrates and genotypes on root pruning were estimated in order to recommend a technical plan for the production of *Acacia spp.* in the nursery. Two randomized tests-- were conducted on *Acacia auriculiformis* A. Cunn. ex Benth and *Acacia mangium Willd.*, in six types of substrates made of humus soil, crushed coal and fine sawdust in different proportions. After sprouting, young seedlings aged three weeks were transplanted into in SAPPI trays, alveolar cells filled with substrates and observed closely until planting season. A low dose of NPK fertilizer (20 20 20) in granule formulation (30g diluted in 10l of water) was provided weekly to the plants. Seedlings heights and diameters were measured during months 1, 2 and 3 in the acclimation area. When the planting age was reached, roots dry matter mass (RDM) was estimated for both species with the aim to appreciate seedling roots pruning. Results indicate that, at the planting age, seedlings from substrates 6 (75% soil + 25% charcoal) and 4 (50% soil + 50% charcoal) had the best growth performances, followed by plants from substrates 1 (75% sawdust + 25% charcoal) and 5 (50% soil + 25% charcoal + 25% sawdust). Substrate 5, because it does not generate compaction, was selected for plants production. A variance analysis revealed a genotype effect on root pruning (0.31g vs 0.24g respectively for *Acacia auriculiformis* A. Cunn. ex Benth. and *Acacia mangium Willd*). At the plantation age, this study results showed strong correlations between diameter and height ($r = 0.65$, $P < 0.001$), diameter and (RDM) ($r = 0.44$; $P < 0.001$) on the one hand, and between height and RDM ($r = 0.47$; $P < 0.001$) on the other hand.

2 INTRODUCTION

Acacia auriculiformis A. Cunn. ex Benth and *Acacia mangium Willd* are two species native to Indonesia, Papua New Guinea and Australia (Beadle, 1981). The Acacia species, which consists of leguminous plants from the family of Fabaceae was introduced in Asia, Africa and America a few decades ago (Koutika and Richardeson, 2019). Leguminous plants are widely used in mixed crops in poor and degraded soils (Pomier *et al.,* 1986) such as the Bateke

Plateau sandy soils in Central Africa (Bulakali *et al.,* 2014). This choice is due to their ability to fix the atmospheric nitrogen in the soil (Bouillet *et al.,* 2008) that results in positive impacts on crops, biomass and litter production (Lim Meng Tsai, 1988, Mackosso and Tchimmbakala 2008, Bouillet *et al.,* 2008; Tchichelle *et al.,* 2016; Mianhai Zheng *et al.,* 2016). Many studies report that the use of leguminous plants helps to return nitrogen to soils, initially lost by cutting mature plantations or harvesting crops for example (Kaplan, 1980; Laclau *et al.,* 2010; Félipe Martino Santos *et al.,* 2016; Epron *et al.,* 2016, Koutika, 2019). The ability of leguminous plants to bind nitrogen organically, coupled with its ability to grow more rapidly than endogenous species in countries (Pomier *et al.,* 1986), means that they are often used for soil restoration and agricultural yields optimization in developing countries (Bulakali *et al.,* 2014, Koutika, 2019) or even for biomass production in forestry (Laclau, 2008). They also help with the supply of wood energy in urban centers, promotion of sedentary agriculture through the practice of agroforestry and the improvement of agricultural yields (Bisiaux *et al.,* 2009; Salim Azad *et al.,* 2016). In the Republic of Congo, the introduction of fast growing, nitrogen-fixing species dates back to the 1980 (Diangana, 1986). Of the legumes introduced, only *Acacia auriculiformis* A. Cunn. ex Benth. and *Acacia mangium Willd.* have adapted to Congolese environmental conditions (Diangana, 1986). However, these species did not benefit from sustained research programs compared to *Eucalyptus spp.* (Bernhard-Reversat *et al.,* 1993). Recent studies of Congo's coastal area have shown beneficial effects of mixed *Acacia spp.* and *Eucalyptus spp.* crops on soil restoration, biogeochemical cycle (Epron *et al.,* 2013; Koutika *et al.,* 2016 ; Koutika *et al.,* 2017a ; Koutika *et al.,* 2017b; Tchitselle, 2017), and on intensive silviculture under drought conditions (Koutika *et al.,* 2018). The results confirmed previous Congolese research recommending the use of *Acacia mangium* and *Acacia auriculiformis* in agroforestry systems. In the Republic of Congo, the production of *Acacia spp.* plants uses a substrate made with 100% humus soil, a component of the topsoil. In this kind of plant production, seedlings are produced either directly in phytocells or in germiners before being transplanted into phytocells containing 0.5 to 1 kg of humus soil as substrate. These phytocells cause enormous logistical problems when transporting seedlings from nurseries to the plantation sites. In this context, it is necessary to define a technical plan for the production of *Acacia spp.* seedling in the nurseries, using environment-friendly substrates with local components in trays alveolar cells (likely to ease logistical issues during planting). However, the good roots pruning is the major condition for this new technical plan.

3 MATERIAL AND METHODS

3.1 Plant material characteristics : The seeds of *Acacia Auriculformis* A. Cunn. ex Benth. and *Acacia mangium Willd.* were harvested three months before, in the "Patte d'Oie" urban forest in Brazzaville and kept at 4°C for 4 months. These seeds were prepared by immersion in boiling water, cooled for 24 hours and then removed to be sown in SAPPI trays (alveolar cells) of 50 ml each (Saya *et al.,* 2008) containing a fine sand substrate. Two seeds were sown per cell SAPPI trays (alveolar cells) were placed under shade with a 60% refractive capacity of solar radiation. Seedlings maintenance consisted of weekly irrigation of the SAPPI trays (alveolar cells) through misting, using a 15l sprayer until the transplantation period.

3.2 Types of substrates: In the search for an ideal substrate, two experiments were conducted successively, with young seedlings of *Acacia mangium Willd* and *Acacia auriculiformis* A. Cunn. ex Benth. Young seedlings of this two species of 3-week-old were transplanted in SAPPI trays (alveolar cells) filled with the six different substrate types. Below are the substrates composition (Table 1): The different substrates were made with humus soil, charcoal particles, and fine sawdust as shown in Table 1. Humus soil was sterilized by heat through a 4-

hour wood fire to eliminate any pathogenic germs before use, the next day after cooling. Charcoal was crushed and sieved through 6mm x 6mm mesh. After being collected in the carpentry shop, sawdust was directly mixed with the other components of the substrates.

3.3 Procedures: Three weeks after germination, young seedlings were transplanted into immovable trays (alveolar cells), in a randomized design consisting of 540 seedlings of *Acacia mangium Willd.* and 576 plants of *Acacia auriculiformis A. Cunn. ex Benth.*, placed in three repetitions of six different substrates, at a rate of 30 plants per repetition and per substrate type for the first experiment, and 32 plants per repetition and per substrate type for the second testing. Transplantation was done under shade with a 60% solar reflection capacity. The plants remained under shading for seven days before being fully exposed in the acclimation area. After seedling transplantation to germiner in SAPPI trays (alveolar cells), irrigation was supplied as a mist every 30 minutes with a knapsack sprayer. One week after transplantation, a weekly supply of low-dose foliar fertilizer and fungicides was done. The dose of fertilizer supplied was 30g of fertilizer diluted in 10l of water. Three NPK $_{(20\ 20\ 20)}$ fertilizers in granule formulation enriched with trace elements were used alternately, these are Fertigri (NPK $_{(20\text{-}20\text{-}20)}$ + 0.E), Nutriga (NPK $_{(20\text{-}20\text{-}20)}$ + 0.E, (Fe, Mn, Zn, Cu, B, Mo) and Harvest (NPK$_{(20\text{-}20\text{-}20)}$ + T.E (Mg, B, Co, Cu, Mn, Mo, Zn, Fe). The fertilizers were spread using a knapsack sprayer.

3.4 Estimated and measured parameters and data analysis : Growth parameters (height and collar diameter) were recorded at 1, 2 and 3 months after transplantation. Heights were measured using a ruler while stems' diameters were measured using a digital calliper. After destruction of the clods, the mortality rate and roots dry matter mass (RDM) were evaluated on *Acacia mangium Willd.* plants and *Acacia auriculiformis* A. Cunn. ex Benth. plants. Once out of the cells, the root systems were cleaned with water on a 2 mm mesh sieve and spread on paper towels. After a 30-minutes open-air drying process, each plant's root was recovered and then dried in an oven at 65°C for 72 hours. Once removed from the oven, each plant's roots were weighed using an OHAUS® precision scale (1/1000th sensitivity threshold) to determine the (RDM).

The variance analysis was performed using the following General Linear Models (GLM):

$$X_{ij} = \mu + sub_i + esp_j + sub_i * esp_j + \varepsilon_{ij}$$

With:

X_{ij}: phenotypic value of the sample under study;

μ: average value of all samples;

sub_i: fixed-effect of substrates i;

esp_j: fixed-effect of species j;

$sub_i * esp_j$: interaction between substrate i and species j;

ε_{ij}: residual error.

Average comparisons per substrate and per age for all ages were made according to the Scheffer test at the 5% level. Different parameters averages are presented in the form of curves or histograms.

4 RESULTS

4.1 Substrate effect on plants mortality rates: The study showed low nursery mortality rates for both species. These mortality rates were of 1.04% for *Acacia auriculiformis* A. Cunn. ex Benth. and 2.52% for *Acacia mangium* Willd. They varied according to the types of substrates used. For *Acacia auriculiformis* A. Cunn. ex Benth., substrates 4, 5 and 6 presented the highest mortality rates at 3.13% while those of the substrates 1, 2 and 3, were equal to zero. For *Acacia mangium* Willd., the highest mortality rate was observed with substrate 4 at 16.67% while the lowest mortality rate was obtained with each substrates 2, 5 and 6, at 6.67%.

4.2 Effect on nursery plants growth parameters: The variance analysis showed a highly significant substrate effect (P<0.001) on plant growth in terms of height, regardless of age in *Acacia auriculiformis* A. Cunn. ex Benth. Figures 1, 2 and 3 illustrate the growth in height at 1, 2 and 3 months for both species. Average comparison done with the Scheffer test at the 5% level showed that the best substrates at the planting age were substrates 4 and 6 with respective average height values of 19.78 cm ± 0.53 and 19.88 cm ± 0.59. On the other hand, substrate 3 is last with an average plants height of 16.95 ± 0.53 cm (Figure 3). However, in *Acacia mangium* Willd. no significant differences in height growth were observed at the first month. At two (2) and three (3) months, a substrate effect was observed ($P<0.001$) on height growth. A comparison of averages indicates that the mean plant heights on substrates 4 and 6 do not differ significantly and are respectively 19.39cm and 19.49cm, but differ significantly from averages obtained with the rest of the substrates. Substrate type 2 is last with an average plant height of 13.80 cm ± 0.67 (Figure 3). A repetition effect was noted at month 1 and the Scheffer test reveals that the average height values differ significantly between repetitions 1 and 3 as well as 2 and 3. Variance analysis showed significant substrate and repetition effects ($P <0.001$) on seed diameter growth of *Acacia auriculiformis* A. Cunn. ex Benth. between one and three months. Comparison of averages showed that average diameters of all substrates did not differ significantly at two months. Figures 4, 5 and 6 illustrate the growth in height at 1, 2 and 3 months for both species. Also, only repetitions 1 (2.45 + mm ± 0.05) and 2 (2.57+ mm ± 0.06) as well as 1 (2.45 + mm ± 0.05) and 3 (2.63 mm ± 0.05) differ significantly. Very significant substrate and repetition effects ($P <0.001$) were observed on *Acacia mangium* Willd at all three ages. Comparing averages shows that up to three months, substrates types 4 and 6 are the best, without much difference among the results. Their average values are respectively equal to 2.62 mm ± 0.08 and 2.66 mm ± 0.06. Substrate type 2 is last with an average value of 2.28 mm ± 0.06. Based on a comparison of Scheffer diameter averages, repetitions 1 (2.47mm ± 0.06) and 2 (2.43mm ± 0.05) as well as 1 (2.47mm ± 0.06) and 3 (2.45 mm ± 0.06) are significantly different.

4.3 Evolution of the growth parameters based on age: Figures 7 and 8 report both species plants growth in height and diameter based on age. Variance analysis showed an age effect on growth dynamics for both species. Comparing averages using the Scheffer test showed that seedlings height growth is in favor of *Acacia auriculiformis* A. Cunn. ex Benth. regardless of age. While the diameter growth of *Acacia mangium* Willd. seedlings are superior to that of *Acacia auriculiformis* A. Cunn. ex Benth. until about 2 months of age. *Acacia auriculiformis* A. Cunn. ex Benth. With regard to the genotype, variance analysis showed a highly significant genotype effect to the different ages ($P<0.001$) on the parameters height and diameter. According of our results, the best genotype is *Acacia auriculiformis* A. Cunn. ex Benth.

4.4 Substrate effect on roots dry matter mass (RDM): At planting age, the average RDM of nursery acacia plants differs from one substrate to another. The analysis of variance showed a very significant substrate effect ($P<0.001$) for both species. The Scheffer average comparison test at the 5% level indicates that for *Acacia auriculiformis* A. Cunn. ex Benth., substrates 4 (0.35 g ± 0.022) and 6 (0.40 ± 0.018) did not differ significantly from each other; but they are significantly different from the rest of the substrates (Figure 9). Moreover, a repetition effect has also been observed: repetition 1 (0.27g ± 0.01) and 2 (0.33g ± 0.02) as well as 1 (0.27g ± 0.01) and 3 (0.33 ± 0.02) showed significant differences. In *Acacia mangium* Willd., the average comparison test indicated that RDM average of substrate 4 are significantly different from the rest of the substrates. More roots dry biomass was observed on substrate 4 (0.36 g ± 0.022). Other dry root mass values are as follows: substrates 6 (0.30 g ± 0.021) and 5 (0.24 g ± 0.018). Substrate 3 (0.18 g ± 0.017) has the lowest RDM at 3 months (Figure 7). The repetition effect was also noted ($P <0.001$). Repetitions 1 (0.28 ± 0.01) and 2 (0.22 ± 0.021) as well as 1 (0.28 ± 0.01) and 3 (0.23 ± 0.02) are significantly different.

The genotype effect was significance at the level of 5% for the parameter RDM ($P<0.001$). The average comparison test indicated that *Acacia auriculiformis* A. Cunn. ex Benth is the best genotype. With regard to the interaction, overall, study showed a very strong interaction substrate x species (genotype) at two and three months (P <0.001) for the parameter height. On the other hand, for the diameter, this interaction was weak (P <0.01).

4.5 Study correlation parameters at the plantation age: Overall, at three months, results showed strong correlations between diameter and height (r=0.65; P <0.001), diameter and RDM (r=0.44; P <0.001) on the one hand, and between height and RDM (r = 0.47; P <0.001), on the other hand. These correlation values were from *Acacia auriculiformis* of (r=0.65; P <0.001) between diameter and height, (r=0.38, P <0.001) between diameter and RDM and (r = 0.41; P <0.001) between height and RDM. For *Acacia mangium,* correlation values were of (r=0.65; P <0.001) between diameter and height, (r=0.46, P <0.001) between diameter and RDM and (r = 0.50; P <0.001) between height and RDM

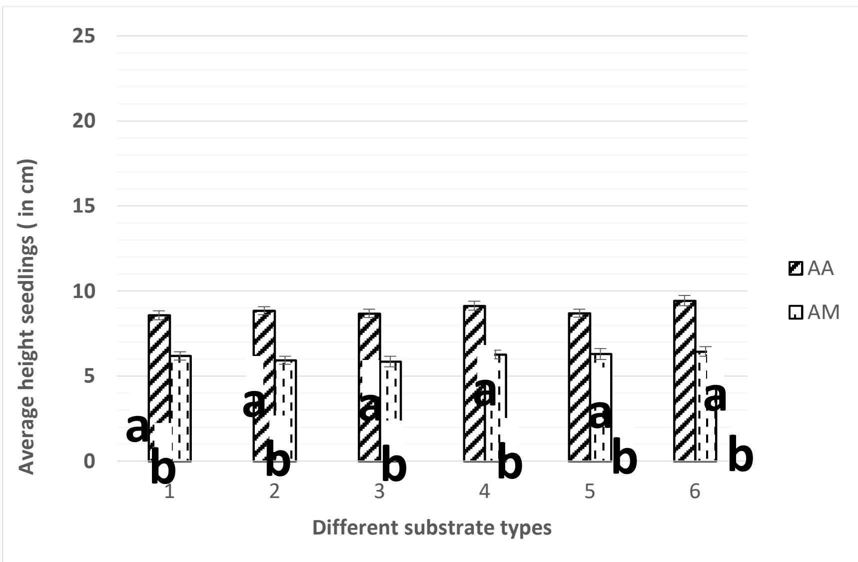

Figure 1: Average height of *Acacia auriculiformis* (AA) and *Acacia mangium* (AM) seedling at one month, in acclimation area. Mean values was based on 30X3=90 seedlings per substrate type. Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

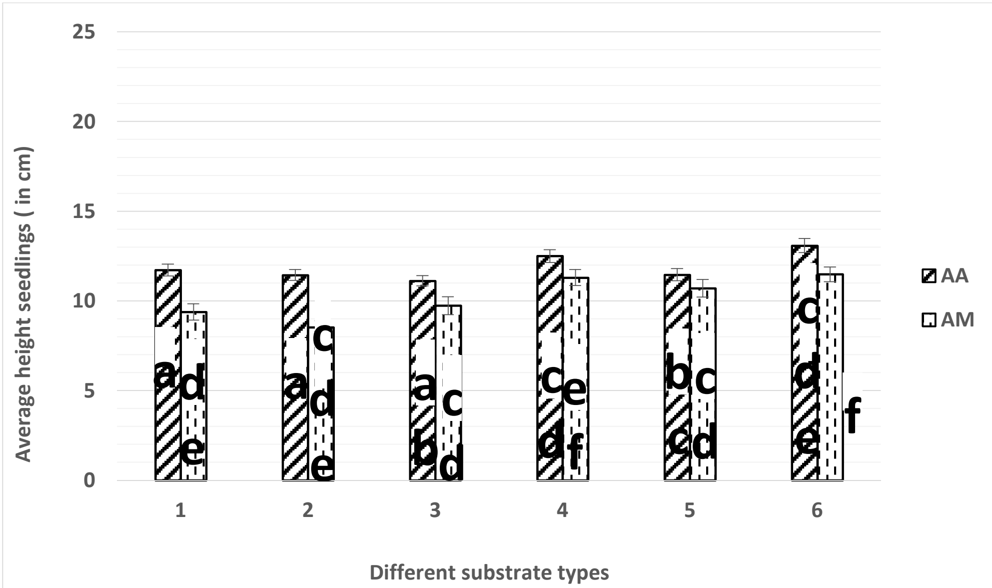

Figure 2: Average height of *Acacia auriculiformis* (AA) and *Acacia mangium* (AM) seedling at two months, in acclimation area. Mean values was based on 30X3=90 seedlings per substrate type. Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

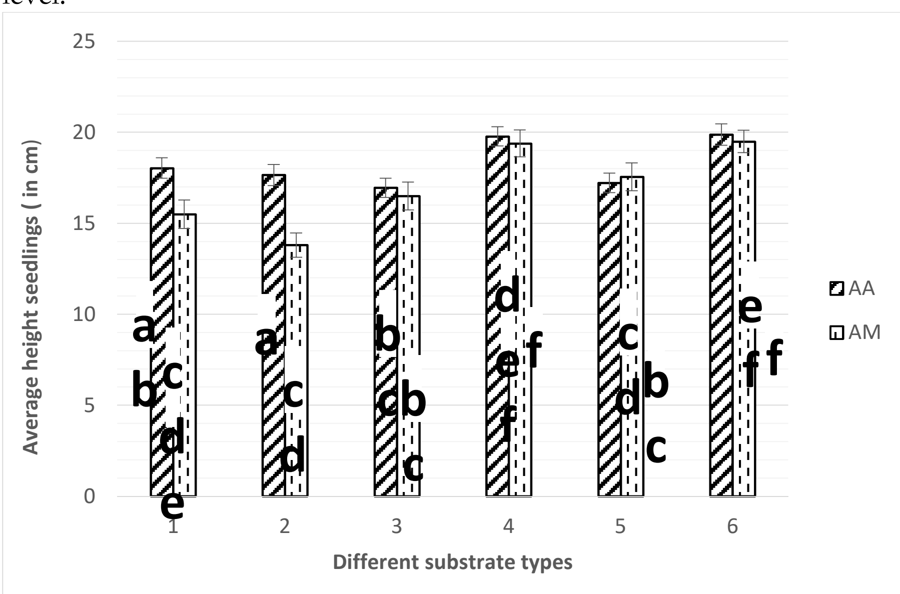

Figure 3: Average height of *Acacia auriculiformis* (AA) and *Acacia mangium* (AM) seedling at three months, in acclimation area. Mean values was based on 30X3=90 seedlings per substrate type. Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

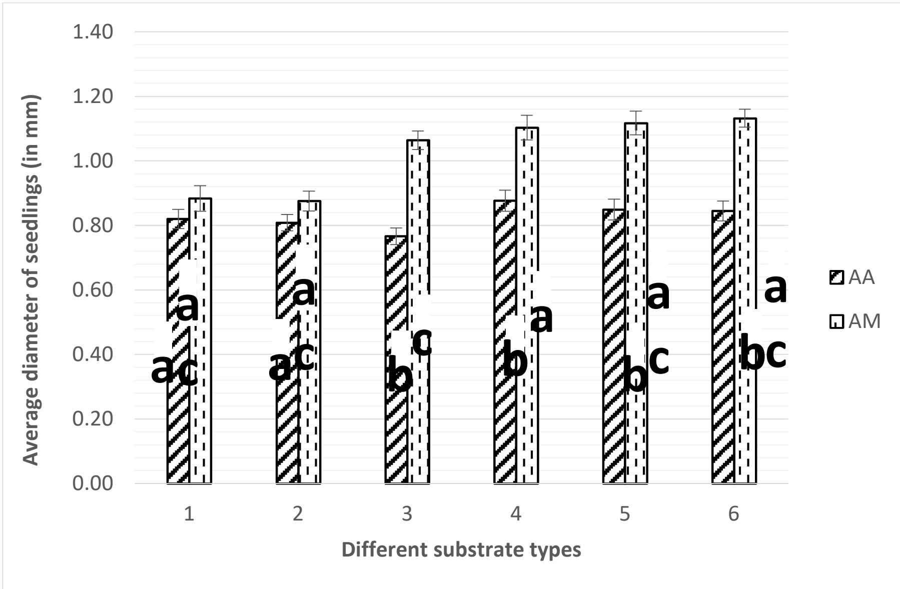

Figure 4: Average diameter of *Acacia auriculiformis* (AA) and *Acacia mangium* (AM) seedling to one month in acclimation area. Mean values was based on 30X3=90 seedlings per substrate type. Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

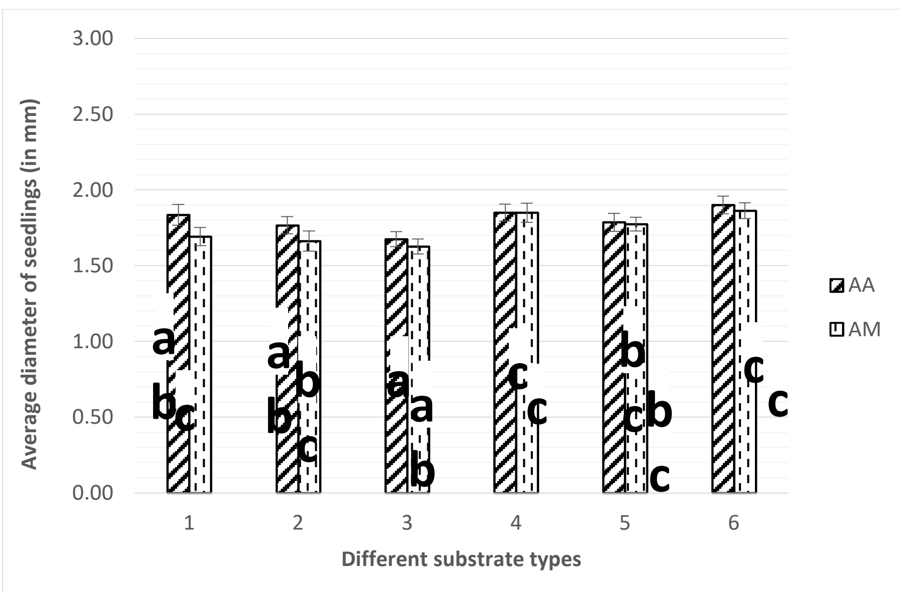

Figure 5: Average diameter of *Acacia auriculiformis* (AA) and *Acacia mangium* (AM) seedling to two months in acclimation area. Mean values was based on 30X3=90 seedlings per substrate type. Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

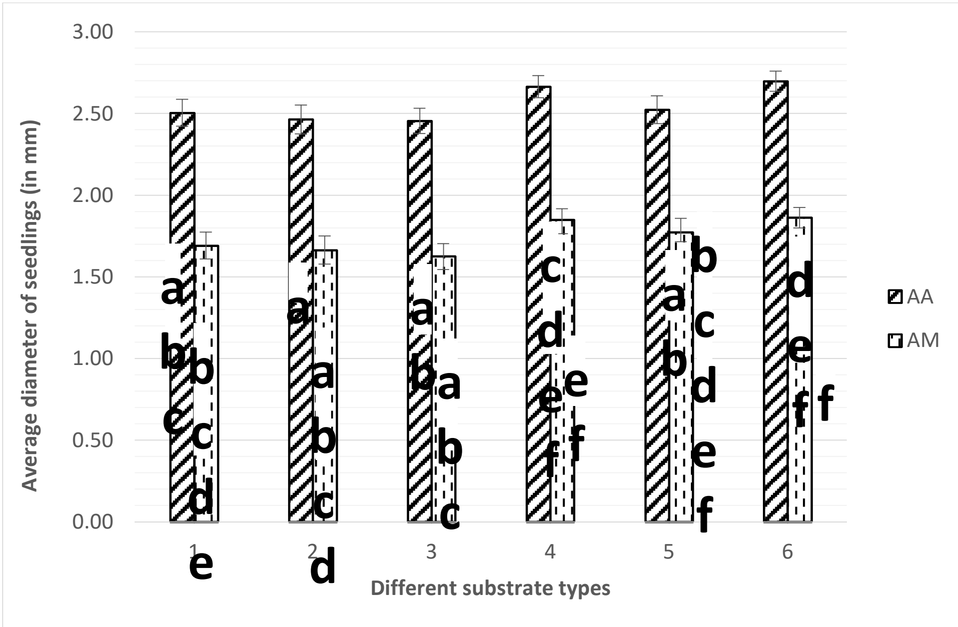

Figure 6: Average diameter of *Acacia auriculiformis* (AA) and *Acacia mangium* (AM) seedling to three months in acclimation area. Mean values was based on 30X3=90 seedlings per substrate type. Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

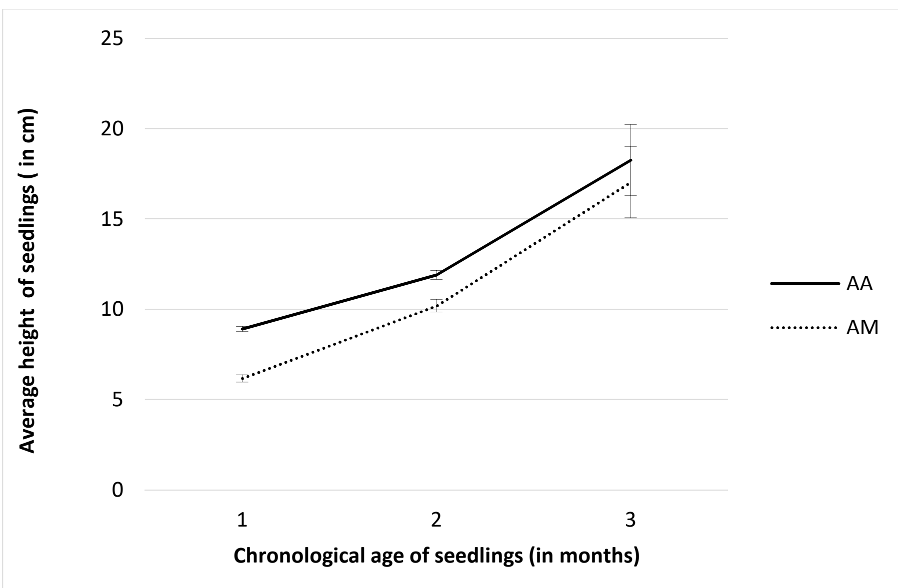

Figure 7: Average height growth recorded during the course of time for the two genotypes *Acacia auriculiformis* (AA) and *Acacia mangium* (AM). Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

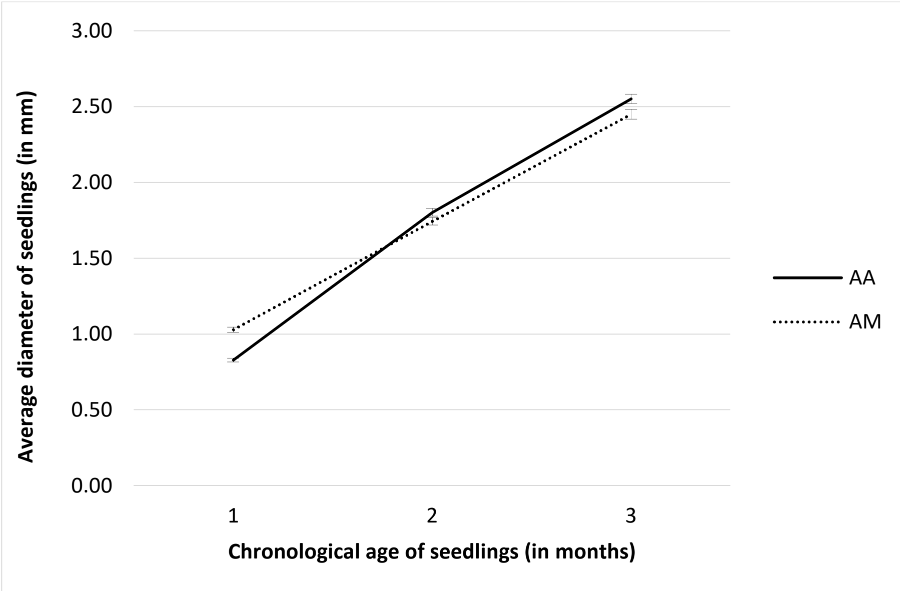

Figure 8: Average diameter growth recorded during the course of time for the two genotypes *Acacia auriculiformis* (AA) and *Acacia mangium* (AM). Bars represent confidence intervals at p=0.05 and letters distinguish means, which are significantly different at 5% level.

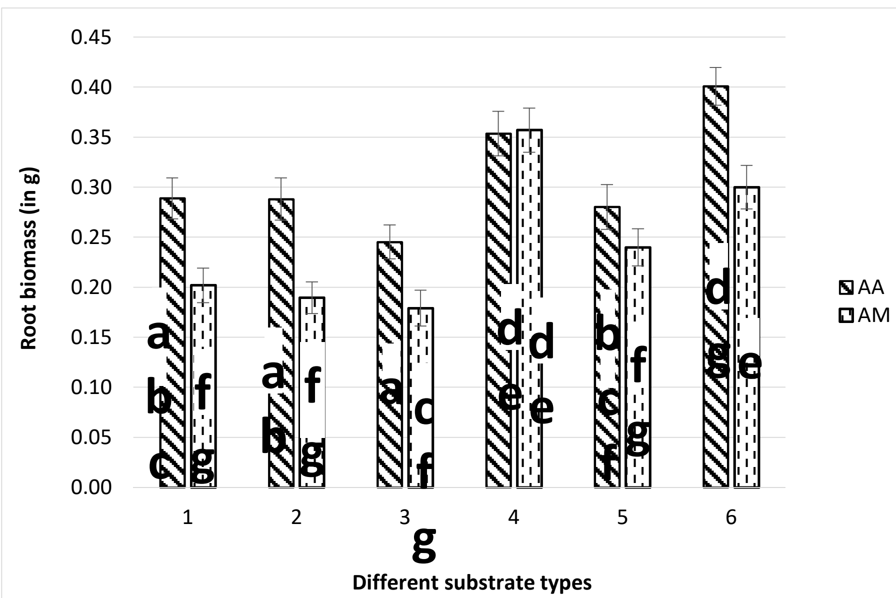

Figure 9: Average root biomass of *Acacia mangium* (AA) and *Acacia mangium* (AM) seedling at the plantation age, in function of different substrates. Mean values was based on 30X3 = 90 seedlings by substrate type. Bars represent standard deviation and letters distinguish means, which are significantly different at 5% level.

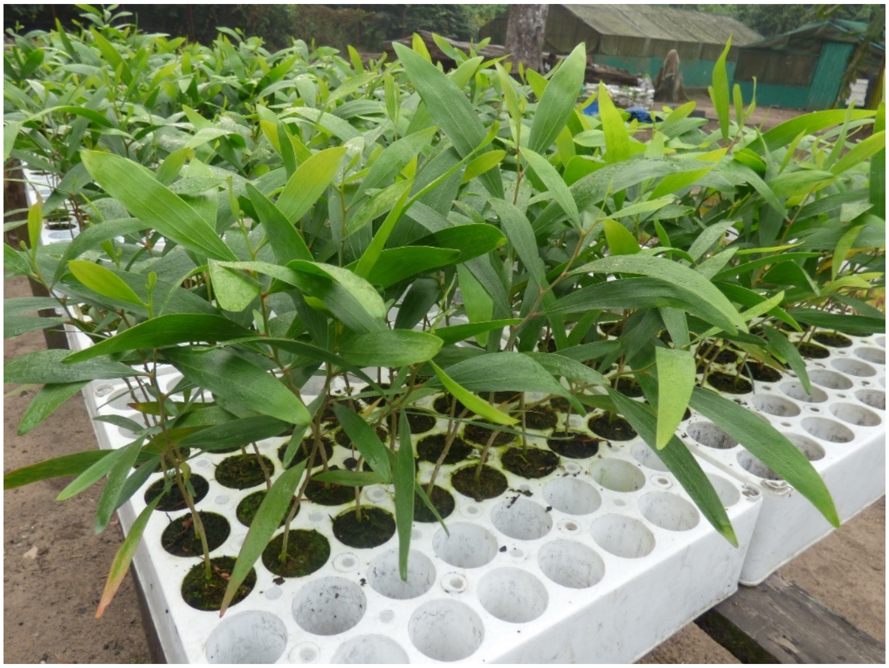

Figure 10: Sight of *Acacia mangium* seedlings in the SAPPI trays (alveolar cells).

Figure 11: Sight an ease extraction seedling from SAPPI trays alveolar cells.

5 DISCUSSION

The effects of genotype and substrate on growth parameters have been reported for a long time by many authors like Mankessi *et al.,* 2010, M'sadak *et al.,* 2013, M'sadak et al. 2014 and Amelework *et al.,* 2015. The results showed that the type of substrate 6 with a high proportion of soil had the best performance as well as regards the growth parameters (Figures 1, 2, 3, 4, 5 and 6) as of dries roots biomass (Figure 9). These performances can be explained by the fact that the growth of *Acacia spp*. is regulated by rhizobia housed in the soil (Cornet and Diem, 1982). In addition, soil, unlike sawdust, provides nutrients to young plants as soon as they start growing and stimulates nodulation through its microorganisms (Galiana *et al.,* 1990, Galiana *et al.,* 1996). Plants cultivated on sawdust substrate are stunted, a delay that may be explained by a lack of nutrients during the planting process. This lack is apparent through the yellowing of the leaves in testing. Fortunately, this yellowing has been cleared over time through light weekly doses of fertilizers coupled with the mineralization of sawdust. The genotype effect was translated in nursery by the observed difference in growth performances. The best growth performance was obtained with *Acacia auriculiformis* A Cunn. ex Benth, compared to *Acacia mangium* Willd. whilst in the field, studies report that the best performances are obtained with *Acacia mangium* Willd. (Bernhard-Reversat *et al.,* 1993).

5.1 Choosing the best substrate: Three parameters including height at planting age (H3), root biomass and mortality rate have been chosen to determine the best substrate. Root biomass explains the consolidation of seedlings in the nursery, the bigger root biomass provide the better consolidation. With regard to height at three (3) months, the planting age, the best substrates are those of types 4 (50% Humus soil + 50% Charcoal particles) and 6 (75% Humus soil + 25% Charcoal particles). These substrates are followed by substrate 1 (75% sawdust + 25% Charcoal particles) for *Acacia auriculiformis* A. Cunn. ex Benth. and substrate 5 (50% Humus soil + 25% Charcoal particles + 25% sawdust) for *Acacia mangium* Willd. in terms of root biomass, substrates 4 and 6 are also the best. Regarding clod consolidation, mixtures with a high proportion of soil (50 or 75%) caused the compaction of substrates in the SAPPI trays alveolar cells. The good root system development in the SAPPI trays (alveolar cells) caused plants extraction difficulties. Ultimately, this development caused clod break and therefore a higher risk of mortality after transplanting to the field conditions. Substrates 1 and 5, on the other hand, have good clod consolidation and facilitate easy removal of the plants from the SAPPI trays (alveolar cells) (Figures 10 and 11). With respect to growth parameters, M'sadak *et al.* (2012) reported that there are correlations between collar diameter and the different morphological parameters (height, total dry weight, root dry weight, and dry weight of the aerial part). At three months, results showed strong correlations between the study parameters. These results are close to those obtained by M'Sadak *et al.* (2012). Overall, mortality rates obtained were of 1.04% for *Acacia auriculiformis* A. Cunn. ex Benth. and 2.52% for *Acacia mangium* Willd. in the nursery. These low mortality rates are in agreement with the results of Gnahoua and Louppe (2003), which reveal that these species are relatively resistant. The experiment revealed that seedlings set in substrate type 2 showed stunted growth from the beginning compared to those in substrates 4, and 6, probably because of undecomposed sawdust and a lack of rhizobia, which explains the poor nutrients. Thus, in order to limit planting losses and to guarantee the initial growth of plants in the field, substrate 5 (50% sawdust, 25% Charcoal particles and 25% earth) is recommended to answer the problem of this study.

6 CONCLUSION

This study provides important advances for the understanding the effect of the different substrates on root pruning of *Acacia auriculiformis* A. Cunn. ex Benth. and *Acacia mangium* Willd. seedling in the nursery. These results show that whatever the species, substrates 6 (75% Humus soil + 25% Charcoal particles) and 4 (50% Humus soil + 50% Charcoal particles) are the best in terms of dry root mass production. The results also indicate the possibilities of using the following four types of substrate after a 3-weeks germination period: substrates 1, 4, 5 and 6. However, compared to substrate 1, substrate 5 has shown an ease of plants extraction from in SAPPI trays (alveolar cells) and a high average of dry root mass. Substrates with high soil proportions cause clods break, which in turn disrupts the root system and is likely to cause plants stress during transplantation, and therefore, high mortality in the field. In addition, the production of plants in SAPPI trays (alveolar cells) instead of phytocells alleviates logistical issues at the time of planting. The prepared substrate is easily made with local materials compared to the use of humus soil alone. It also helps recycle fine sawdust, usually considered a waste in the Republic of the Congo.

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