Scientific Research Publishing

American Journal of Plant Sciences, 2022, 13, 1209-1226
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Development of a Technical Itinerary for the Production of Avocado (*Persea américana* Mill.) Seedlings with Biofertilizers

Somo Toukam Gabriel Mahbou[1*], Godswill Ntsomboh-Ntsefong[1], Aminatou Mongoue Fanche[2], Félix Tchio[2], Fallone Dongmo[1], Gaston Onana Etoga[2], Emmanuel Youmbi[1]

[1]Department of Plant Biology, Faculty of Science, University of Yaounde I, Yaounde, Cameroon
[2]Institute of Agricultural Research for Development (IRAD), Yaounde, Cameroon
Email: *mahbousomo@gmail.com

How to cite this paper: Mahbou, S.T.G., Ntsomboh-Ntsefong, G., Fanche, A.M., Tchio, F., Dongmo, F., Etoga, G.O. and Youmbi, E. (2022) Development of a Technical Itinerary for the Production of Avocado (*Persea américana* Mill.) Seedlings with Biofertilizers. *American Journal of Plant Sciences*, **13**, 1209-1226.
https://doi.org/10.4236/ajps.2022.139082

Received: June 12, 2022
Accepted: September 17, 2022
Published: September 20, 2022

Abstract

The cultivation of fruit trees generally requires a nursery phase during which the young seedlings are monitored and treated to improve their ability to adapt to the environment. This leads to the production of seedlings that are used to create orchards. It consists of four essential steps or operations: 1) The germination phase of the seeds in germinators for the production of rootstocks; 2) Transplanting into pots or bags; 3) Fertilisation in order to obtain seedlings of a satisfactory vigour (stem diameter) ready for grafting. The nursery phase requires a good understanding and mastery of plant regeneration and fertilisation techniques. In Cameroon, the demand for avocado (*Persea americana*) fruits is increasing, but the supply is not keeping up with this demand. After a summary monograph on the production practices of avocado seedlings in the Yaounde area, this work aims to optimise the aforementioned three steps in order to obtain seedlings of sufficient sizes for grafting. Three factors are considered in this study: 1) The substrate (Substrate), whose effects are evaluated by the germination rate (GR), the daily average germination (DAG) and the root volume of seedlings (RootV). 2) The transplanting date (TransD), determined by considering three dates including 40 (Trans40), 65 (Trans65) and 75 (Trans75) days after sowing, and 3) Fertilisation using biological fertilisers, evaluated by testing four fertilisation levels, Fert1 (10 gr of 20-10-10 plus 10 gr fowl droppings), Fert2 (*Acaulospora tuberculata*), Fert3 (*Gigaspora margarita*) and Fert4 (Mixed mycorrhizal strains of *Gigaspora margarita* and *Acaulospora tuberculata*). This third factor is evaluated by growth parameters including leaf area (LeafA), chlorophyll index (ChlorInd), gain in Plant height (GainPltH) and plant diameter (GainPltD). The trial took place in the First Seed company, a seed production unit located in the Sim-

bock district of Yaounde for the field phase, and the Biological Control Laboratory of the Institute of Agricultural Research for Development (IRAD), Nlolbisson, Yaounde. Two trials were conducted, the first with the objective of determining the best substrate with a completely randomized block design in 2 replications, three substrates/replication. The second trial was done with a factorial design (Split plot) with three replicates, the main factor being the Transplanting Date (TransD) and the second factor the biological fertilizer. Data were separated using least significant difference at 5% treshhold. Results indicate a highly significant effect of substrate on RootV ($p = 5.00E{-}03$). This effect translated by an increase of 49.42% and 19.53% of root volume on sawdust respectively to sand and soil. Sawdust (100%) and soil (98%) affect germination by 8 days reduction over sand and the germination rate on these two substrates is higher than the one on sand (92%). The early transplanting (TransD40) allows a better growth of the seedlings in terms of stem length and the collar diameter. The only observation variable that stands out for the early nursery stage fertilisation is leaf area, which shows significant differences between the 4 fertilisation formulae tested. The chlorophyll index and leaf area are also strongly correlated with the seedling growth parameters. Our results show that the early transplanting stage (40 days after planting) combined with a germination on white sawdust should be proposed to reduce the production cycle of grafted seedlings in association with early application of biofertilisers or organic fertilizer.

Keywords

Persea americana, Nursery, Mycorrhizae, *Acaulospora tuberculata*, *Gigaspora margarita*, Germination Substrate, Transplanting Date, Growth

1. Introduction

Avocado (*Persea americana* Mill.) is a plant of Lauraceae family. Its fruit is considered to be the world's most nutritive fruit [1]. It is also considered to be the world's most energy value fruit [2]. This plant is a tropical species that adapts perfectly to subtropical climates with mild and tropical winters [3]. Mexico, with a production of 2,300,889 tonnes is the world's leading producer of avocado, followed by the Dominican Republic (661,626 tonnes) and Peru (535,911 tonnes). Kenya is the leading African producer with a production of 364,935 tonnes (FAOSTAT, 2019). Avocado imports in 2020 grew by 6.9% to around 2.3 million tonnes. The main importing countries, the United States and the European Union, absorbed some 48% and 25% of world exports respectively (FAOSTAT, 2020).

Cameroon, which has all the assets for the massive production of fruit trees, is not a competitor on the world market. The development of orchards is limited by the lack of quality seedlings. Supply of agricultural inputs is not appropriate. The regulations, even if they exist on paper, are not observed in most cases.

There are no manufactured substrates for agriculture on the market as is the case in developed countries. The production of avocado seedlings is done in most cases according to a production itinerary that includes a germination phase in the germinator followed by transplantation in the nursery. The substrates used in the germinator are soil, sawdust and sand.

The role of the substrate in the germination is documented. Some results are contradictory. For example, wood sawdust is reported to negatively affect the germination of Sunflower (*Helianthus annus* L.) [4] while Singh *et al.* [5] state the contrary. Among the soil microbial communities, mycorrhizal fungi are a "key" component in plant-soil relationships. These fungi, present in the soils of most ecosystems, form symbiotic associations with the roots of many terrestrial plant species (about 80%) [6] [7]. They play a major role in the amelioration of soil fertility and organic agriculture [8]. In exchange for the carbon resources received from the host plant, mycorrhizal fungi improve plant nutrient uptake and transport (mainly phosphorus) with very low mobility [9]. Biological fertilizers are commonly used in seedling or crop production processes [1] [10] [11]. The sowing date is known to influence the yield of plant such as onion [12] [13] [14] [15], rice [16] [17], strawberry [18] and fennel [19].

In the tropics, biotic and abiotic pressures are such that successful orchard establishment requires very vigorous seedlings at planting. The technical itineraries used in the production of avocado plants are not scientifically documented. This study aims to optimise the use of these materials to produce vigorous avocado plants from biofertilizers. This objective is realised by identifying the best substrate for the germinator, the most appropriate transplanting date and the biological fertilisation in the nursery that could guarantee the production of the most vigorous avocado seedlings. The three specific objective of the study are to: 1) Evaluate the effect of different substrates currently used by seedling producers of the region on germination; 2) Determine the best stage for transplanting germinated seeds from the germinator to the nursery; and 3) Measure the effect of different biofertilizers on growth in the nursery.

2. Materials

2.1. Study Site

The experiment was conducted in Yaounde in the Centre region, Mfoundi administrative Division, more precisely in the locality of Simbock (Latitude: 3˚49'13.76" Longitude 11˚28'13.52", Altitude: 694 m. The average annual rainfall varies between 1500 and 2000 mm/year. The average annual temperature is between 23˚C and 27˚C, and the relative humidity and average humidity are above 80%.

2.2. Biological Material

For plant material, we used: Avocado seeds consisting of an accession of the species *Persea Americana* obtained from vendors in the town of Mbouda in western Cameroon.

2.3. Organic Fertilisers and Mycorrhizae Strains

Two types of fertiliser were used in the study: 1) organic fertilizer and 2) biological fertilizer. The Aburcular Mycorrhizae Fungi (AMF) species used in this study are *Gigaspora margarita* and *Acaulospora tuberculata*.

2.4. Experimental Set-Up

In a germinator: The experimental set-up chosen was the randomised complete bloc design with two repetitions. The studied factor is the substrate with three modalities (soil, white wood sawdust and sand), each consisting of 100 seeds, with a total of 600 seeds (Figure 1).

In the nursery: The experimental design used is the split-plot factorial design with two factors and three repetitions including transplanting date (TransD/ main factor) and biological fertilisation (Fert/secondary factor). Transplanting date included: Trans40 (40 days after sowing), Trans 65 (65 days after sowing) and Trans75 (75 days after sowing) and fertilisation Fert1: control (soil + sand + fertilizer treatment); Fert2 (T1 + *Gigaspora margarita*); Fert3 (T1 + *Acaulospora tuberculate* and Fert4 (T1 + *Gigaspora margarita* and *Acaulospora tuberculate*, that is Fert2 + Fert3). Three repetitions of 10 plants/repetition, giving (3 × 4 × 10) × 3 plants; that is 360 plants in total (Figure 2).

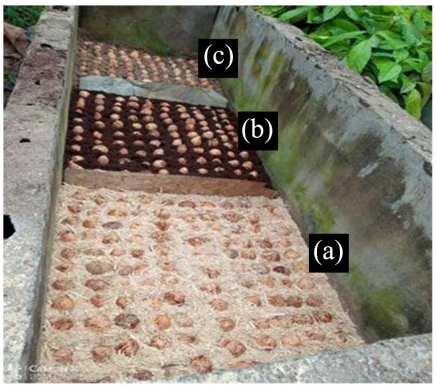

Figure 1. Germination of avocado seeds on different substrates. (a) Sawdust, (b) Soil and (c) Sand.

Figure 2. Seedlings transplantation at transplanting date 40 (TransD40). (a) Sowing; (b) Nursery.

3. Methods

3.1. Preparation of the Substrate

The substrate used for the germplasm experiment in each compartment consisted of three basic elements that were used to prepare the substrates. These were: the S1 substrate made up of fine sand; the second substrate S2 consisted of black humus soil. The third, S3 substrate was white wood sawdust. The soil was taken from the locality of Etoudi in the city of Yaounde to a depth of 15 cm and then sieved. The wood sawdust was taken from a saw mill and the sand from a sand deposit and then sieved to eliminate residues. The different tubs of the germinator were filled with each of the substrates.

3.2. Preparation of the Grains

After removing the seeds from the fruits, they were cleaned to remove the fruit tissue and then washed with tap water before disinfection by soaking in a solution containing a mixture of insecticide (Cigogne 360 EC, pyrethroid insecticide; 50 ml/15L of water); and fungicide (Mancomax bleu 800 WP 80 g/15L of water) for 15 minutes.

3.3. Maintenance of the Germinator

The germinator was established in a sunny place, sheltered from the wind. The maintenance of the germinator consisted in watering every day in the evening or very early in the morning, with a watering can to maintain moisture in the substrate; and removing weeds that compete with the plants. Once the seeds germinated, they were transplanted.

3.4. Setting up the Trial in the Nursery

3.4.1. Preparation of the Substrate

The substrate used for the experiment was a mixture of black soil and fine sand and fowl droppings. The loam soil used was dried, sieved to obtain uniform particle sizes and mixed with sieved fine sand and fowl droppings in proportions of 1/2, 1/4 and 1/4 respectively to obtain good drainage, permeability, as well as better water retention capacity. The characteristics of this soil are indicated in Table 1.

3.4.2. Filling the Bags

The 70 micron bags (30 by 21 cm) were filled completely so that the walls of the bag could not be folded over the free surface of the substrate.

3.4.3. Transplanting

Once the seeds have germinated in the germinator containing only white wood sawdust as substrate, the seedlings were extracted from the germinator and transplanted. The mycorrhizae were applied to the roots of the seedlings (by coating them) before transplanting into the bags, at 2/3 depth in the substrate.

Table 1. Physical and chemical properties of the soil used for the experiments.

Class	(%)
Loamy soil	
Soil acidity	
pH-H_2O	6.77
pH-KCl	5.45
ΔpH	−1.32
Organique mater	
OC (%)	3.48
OM (%)	6.00
Total nitrogen (%)	0.13
C/N	27
Echangeable Cations (meq/100g)	
Calcium (Ca^{2+})	6.00
Magnesium (Mg^{2+})	0.40
Potassium (K^{+})	0.20
Sodium (Na^{+})	0.03
Sum of bases	6.62
Cationic exchange capacity (meq/100g)	
CEC	22.95
Base Saturation (%)	29
Assimilable phosphorus	
Bray II (mg/kg)	21.36

3.5. Measurement of Agronomic Parameters

3.5.1. Evaluation of the Effect of the Substrate on Germination in the Germinator

On each block of the germinator containing the different substrates (black soil; sand and white wood sawdust). Three parameters for this evaluation were recorded including the germination rate (GR, in percentage of seeds), the daily average germination (DAG) and the root volume (RooTV) of avocado seeds sown on the different substrates. Germination Rate (GR) indicates the number of seeds that are likely to germinate in a given period (Germination power). It is given by GR = Number of germinated seeds/Total number of seeds sown*100.

The average daily germination rate (DAG) was calculated according to Osborne and Mercer [20]. DAG = Final germination percentage/Number of days to final germination. Ratings were recorded every four days over a period of 65 days, for a total of 11 ratings.

Root volume (RootV) was measured with a measuring cylinder, by dipping all the roots from each substrate separately in a graduated cylinder containing water. The volume of water displaced gives the root volume. Root volume = Final volume of water displaced − Initial volume of water. A total of 20 × 3 seedlings were used for this evaluation, 20 seedlings from each substrate.

3.5.2. Evaluation of Different Transplanting Dates (TransD) and Fertilisations (Fert) on the Growth of Seedlings in the Nursery

The two factors evaluated here are transplanting dates (TransD40; TransD60; TransD75) and the biological fertilisation applied. Fert1 (10 gr 20-10-10 plus 10 gr chicken droppings), Fert2 (*Acaulospora tuberculata*), Fert3 (*Gigaspora margarita*) and Fert4 (Mixed mycorrhizal strains of *Gigaspora margarita* and *Acaulospora tuberculata*) were analysed for growth in the nursery. Four variables were used for their evaluation: the gain in plant height (GainPltH) and diameter (GainPltD), the leaf area (LeafA) and the chlorophyll index (ChlorInd). The GainPltH and GainPltD were calculated as follows:

Gain = Final data − Initial data/Initial data. The gain was calculated because the plants were not of the same size at transplanting. The sizes of the seedlings were measured with a tape at one-week intervals.

The leaf area (LeafA) of the seedlings was obtained by measuring length and width with a tape measure and calculating using the formula:

$$S = 2/3(L + l);$$

S: leaf area, *L*: length and *l*: width.

The chlorophyll index of the different plants was measured with a Chlorophyll meter (SPAD 502plus) at the 11th week.

3.6. Root Colonisation in Relation to the Different Treatments

3.6.1. Root Isolation

In order to verify the effectiveness of mycorhyzae colonisation in each treatments, 10 plants were selected randomly and the soil was crumbled to obtain roots. The roots obtained (the finest) were cut into fragments of 1 to 2 centimetres in length.

3.6.2. Root Staining and Observation for Mycorrhizae Colonisation

Root staining was done according to the modified Grace and Stribley [21] method. Arbuscules and vesicles of mycorrhizae were observed using 10×, 20× and 40× objectives of a light microscope.

3.7. Statistical Analysis

The collected data were subjected to analysis using the SPSS version 20 software and the means were separated with the Least Significant Difference (LSD) at 5% threshold. The graphical representations of the data were made with Microsoft Excel 2016. The model used is a univariate general linear model whose dependent factors are the treatment and the date of transplanting of the seedlings. The

relationship between growth parameters and chlorophyll index was established using Pearson correlation coefficient at 5%.

4. Results and Discussion

4.1. Results

4.1.1. Substrates versus Germination Rate

Seedling germination started on the 10th day after sowing and reached its maximum between the 40 - 50th day. This is illustrated on Figure 3 on which the sigmoid shape of the germination can be observed. Results here indicate that there is a difference between germination rates of seedlings on different types of substrates. The germination rate is lower in sand (83.64%) than on soil (98%) and wood sawdust (100%) after 65 days of germination.

4.1.2. Substrates versus Daily Average Germination (DAG) and Root Volume (RootV)

Results indicated on Table 2 and Table 3 reveal a significant effect of substrate ($p = 4.80E{-}02$) over daily average germination (DAG) and root volume of

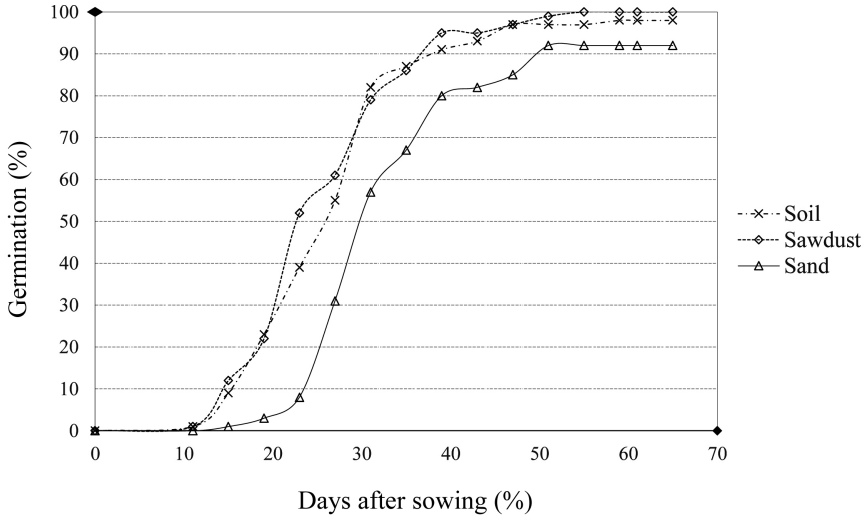

Figure 3. Evolution of the germination of avocado seeds on soil, sawdust and sand.

Table 2. ANOVA of impact of substrate on Daily Average Germination (DAG).

Sources of variations	Type III Sum of Squares	df	Mean Square	F	Significance
Corrected Model	3.11	2	1.6	3.3	4.80E−02
Intercept	111	1	111.2	234.0	0.00E+00
Substrate	3	2	1.6	3.3	4.80E−02*
Error	20	42	0.5		
Total	134	45			
Corrected Total	23.06	44			

seedling (RootV; p = 5.00E−03) (Figure 4). This is illustrated on Figure 4 by a gain of 8 days (43 - 35) when seeds are germinated on wood sawdust or soil in comparison with those germinated on sand. This results to a gain of 49.42% of root volume on wood sawdust over sand, and 19.53% of wood sawdust over soil (Figure 5 and Table 4).

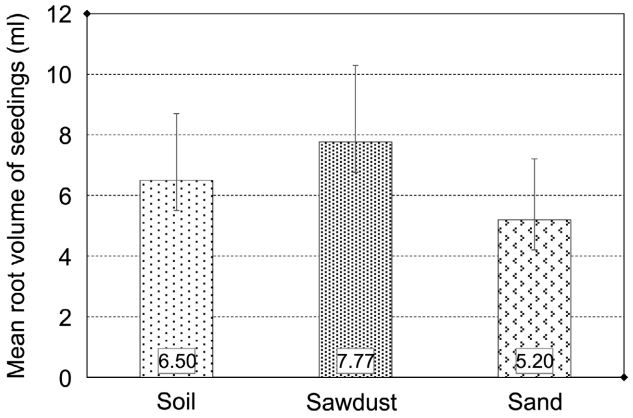

Figure 4. Root volume of seedlings from different substrates (in cubic millimetres).

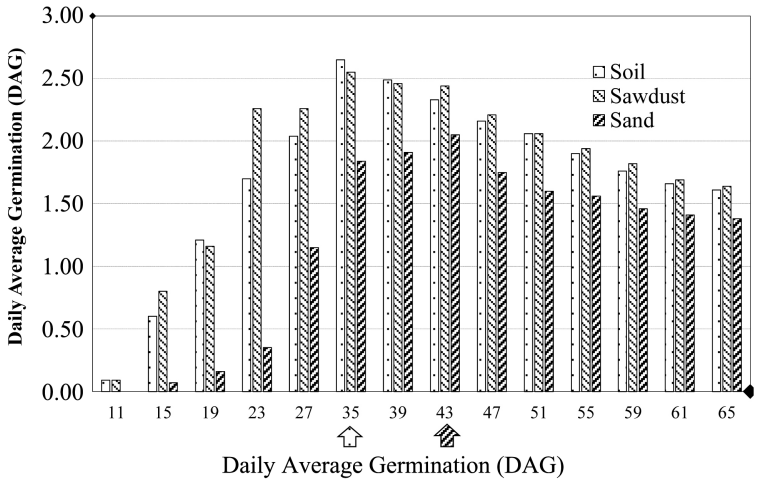

Figure 5. Daily average germination (DAG) of avocado seeds on soil, sawdust and sand. from the 11th to the 65th day after sowing.

Table 3. ANOVA of impact of substrate on Root Volume (RootV) of seedlings.

Sources of variations	Type III Sum of Squares	df	Mean Square	F	significance
Corrected Model	62.5	2	31.3	5.8	5.00E−03
Intercept	2509.1	1	2509.1	463.7	0.00E+00***
Substrate	62.5	2	31.3	5.8	**5.00E−03*** **
Error	308.4	57	5.4		
Total	2880.0	60			
Corrected Total	370.9	59			

R Squared = 0.72 (Adjusted R Squared = 0.65).

Table 4. Multiple comparison of substrate effect on Root Volume (RootV) based on LSD indicating a differenceof root volume in relation with type of substrate. Sawdust versus sand (p= 0.001) and sand versus soil (p= 0.042).

	(I) Substrat	(J) Substrat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
						Lower Bound
LSD	**Sawdust**	**Sand**	2.50	0.71	**0.001**	1.08
		Soil	1.03	0.71	0.150	−0.38
	Sand	Sawdust	−1.30	0.71	**0.001**	−3.92
		Soil	−1.47	0.71	**0.042**	−2.88
	Soil	Sawdust	−1.03	0.71	0.150	−2.45
		Sand	1.47	0.71	**0.042**	0.05

Based on observed means. The error term is Mean Square (Error) = 7.607*. The mean difference is significant at the 0.05 level.

4.1.3. Transplanting Date and Biological Fertilisation versus Growth Parameters

The combined effects of the transplanting dates and the biological fertilisation used are indicated on Table 5. It can be seen from Table 5 that the transplanting date has a very significant influence on studied parameters. Gain in plant height (GainPltH; $p < 0.0001$), Gain in plant diameter (GainPltD; $p < 0.050$), Leaf area (LeafA); $p < 0.0001$) and Chlorophyll index (ChlorInd; ($p = 0.021$). Fertilisation had a significant effect on leaf area (SurtF) ($p = 0.013$). No significant interactions were detected. The separation of the means between the different transplanting dates is indicated in the Table 6. Transplanting dates have a very significant influence on seedling growth parameters. The 40-day duration was the one that resulted to the most vigorous plants appropriate for grafting.

4.1.4. Fertlisation versus Growth Parameters

A significant fertilisation effect was detected on the leaf area variable (Table 5). Biofertilisers reduced the leaf area from 5% - 10% compared to the treatment with manure (Table 7).

4.1.5. Verification of the Effectiveness of Avocado Seedling Symbiosis

The presence of hyphae and vesicles was observed in the plants of treatments Fert2, 3 and 4, in contrast to the roots of the plants of treatment Fert1 where no mycorrhizal symbiosis was observed under the microscope (Figure 6).

4.2. Discussion

The development of research on the establishment of a technical itinerary is of primary interest in agronomy. The results obtained from this work have made it possible to appreciate the effect of different substrates, transplanting dates and biofertilisers on the nursery growth of avocado plants. Different substrates used for the germination of *P. americana* show different behaviours, as a function of

Table 5. ANOVA of plant growth parameter Gain in plant high (GainPltH), Gain in plant diameter (GainPltD), Leaves area (LeafA), and Chlorophyll index (ChlorInd).

Sources of variations	Dependent Variable	Type III Sum of Squares	Degree of freedom	Mean Square	F	Significance	
Corrected Model	ChlorInd	167[a]	19	8.84	1.75	0.131	
	LeafA	217[b]	19	11.45	8.09	**0.000**	***
	GainPltH	1949[c]	19	102.63	3.38	**0.009**	**
	GainPltD	18979[d]	19	998.91	1.25	0.331	ns
Intercept	ChlorInd	42540.44	1	42540.44	8432.00	**0.000**	***
	LeafA	9606.61	1	9606.61	6789.00	**0.000**	***
	GainPltH	6921.96	1	6921.96	227.69	**0.000**	***
	GainPltD	12400.68	1	12400.68	15.47	**0.001**	***
TransD	ChlorInd	49.93	2	24.96	4.95	**0.021**	**
	LeafA	167.47	2	83.73	59.17	**0.000**	**
	GainPltH	1358.20	2	679.10	22.34	**0.000**	***
	GainPltD	5836.86	2	2918.43	3.64	**0.050**	**
Ferti.	ChlorInd	20.99	3	7.00	1.39	0.283	ns
	LeafA	12.04	3	4.01	2.84	**0.051**	*
	GainPltH	35.61	3	11.87	0.39	0.761	ns
	GainPltD	2435.69	3	811.90	1.01	0.413	ns
Rep	ChlorInd	12.86	2	6.43	1.28	0.306	ns
	LeafA	16.27	2	8.13	5.75	**0.013**	**
	GainPltH	165.78	2	82.89	2.73	0.096	ns
	GainPltD	1957.14	2	978.57	1.22	0.321	ns
TransD* Fertil	ChlorInd	50.34	6	8.39	1.66	0.194	ns
	LeafA	8.94	6	1.49	1.05	0.429	ns
	GainPltH	111.73	6	18.62	0.61	0.717	ns
	GainPltD	4618.74	6	769.79	0.96	0.482	ns
Fertil*Rep	ChlorInd	33.79	6	5.63	1.12	0.396	ns
	LeafA	12.90	6	2.15	1.52	0.235	ns
	GainPltH	278.59	6	46.43	1.53	0.232	ns
	GainPltD	4130.88	6	688.48	0.86	0.545	ns
Error	ChlorInd	80.73	16	5.05			
	LeafA	22.64	16	1.42			
	GainPltH	486.41	16	30.40			
	GainPltD	12824.62	16	801.54			

Continued

Total	ChlorInd	42789.07	36
	LeafA	9846.87	36
	GainPltH	9358.29	36
	GainPltD	44204.60	36
Corrected Total	ChlorInd	248.63	35
	LeafA	240.26	35
	GainPltH	2436.33	35
	GainPltD	31803.92	35

[a]R Squared = 0.67 (Adjusted R Squared = 0.29); [b]R Squared = 0.90 (Adjusted R Squared = 0.79); [c]R Squared = 0.80 (Adjusted R Squared = 0.56); [d]R Squared = 0.59 (Adjusted R Squared = 0.12).

Table 6. Multiple comparisons of transplanting date on chlorophyll index (ChlorIndex), leaf Artea (LeafA), Gain in Plant Height (GainPltH) and Plant Diameter (GainPltD).

Dependent variable	(I) Transplanting date	(J) Transplanting date	Mean difference (I – J)	Std. Error	Significance		95% Confidence Interval	
							Lower Bound	Upper Bound
ChlorInd	1	2	1.58	0.92	0.104		−0.36	3.52
		3	2.88	0.92	**0.006**	**	0.94	4.82
	2	1	−1.58	0.92	0.104		−3.52	0.36
		3	1.3	0.92	0.176		−0.64	3.24
	3	1	−2.88	0.92	**0.006**	**	−4.82	−0.94
		2	−1.3	0.92	0.176		−3.24	0.64
LeafA	1	2	5.27	0.49	**0.000**	***	4.24	6.3
		3	2.93	0.49	**0.000**	***	1.9	3.96
	2	1	−5.27	0.49	**0.000**	***	−6.3	−4.24
		3	−2.35	0.49	**0.000**	***	−3.38	−1.32
	3	1	−2.93	0.49	**0.000**	***	−3.96	−1.9
		2	2.35	0.49	**0.000**	***	1.32	3.38
GainPltH	1	2	14.12	2.25	**0.000**	***	9.35	18.89
		3	11.56	2.25	**0.000**	***	6.79	16.33
	2	1	−14.12	2.25	**0.000**	***	−18.89	−9.35
		3	−2.56	2.25	0.273		−7.33	2.22
	3	1	−11.56	2.25	**0.000**	***	−16.33	−6.79
		2	2.56	2.25	0.273		−2.22	7.33

Continued

GainPltD	1	2	28.2	11.56	**0.027**	**	3.7	52.7
		3	25.64	11.56	**0.041**	**	1.14	50.14
	2	1	−28.2	11.56	**0.027**	**	−52.7	−3.7
		3	−2.56	11.56	0.828		−27.06	21.95
	3	1	−25.64	11.56	**0.041**	**	−50.14	−1.14
		2	2.56	11.56	0.828		−21.95	27.06

**Correlation is significant at the 0.01 level (2-tailed).

Table 7. Influence of fertilisation on leave surface (Leaf A).

Fertilisation	Composition	Leaf A	Loss (%)
Fert1	10 gr 20-10-10 plus 10 gr of poultry manure	17.24	
Fert2	*Acaulospora tuberculata*	16.44	4.9
Fert3	*Gigaspora margarita*	15.89	8.5
Fert4	Mixture of mycorrhizal strains of *Gigaspora margarita* and *Acaulospora tuberculata*	15.77	9.3

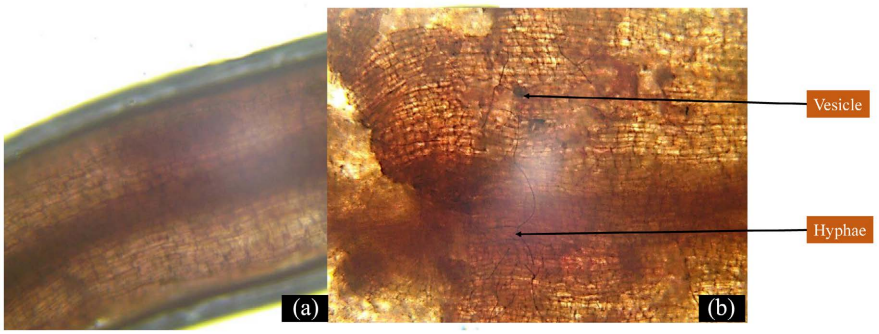

Figure 6. Verification of the effectiveness of avocado seedling symbiosis with non mycorhyzed (a) and mycorhyzed seedlings (b).

their varying structures and compositions. A higher germination rate (100%) was obtained in the wood sawdust growth substrate, followed by soil (98%) and finally sand substrate (92%).

The 65-day mean germination rate was higher in the wood sawdust (1.79), followed by soil (1.72) and finally sand (1.28). This difference in germinative behaviour of avocado seeds shows that wood sawdust by its ability to retain moisture and its mineral composition, favours the development of avocado seeds. As regards the soil and sand substrates, these results corroborate those of Sounon *et al.* [22] who worked on the best substrate for nurseries and showed that the ferralitic substrate presented the best results on the growth of *Artemisia annua* plants compared to the sand substrate. The wood sawdust contains moisture and mineral elements [23] while sand is siliceous in nature, with granules that are hard and heavy (http://umc.edu.dz, 2015); it is therefore assumed that

these characteristics can cause dehydration of the seeds by preventing the passage of oxygen and water to the seeds. Similarly, the relatively low root volume (5.20) and higher in wood sawdust (7.77) which is the best substrate, could explain the high germination rate in wood sawdust, as a large root volume would would favour the development of the plants and therefore their germination thanks to the wood sawdust with available water and mineral elements. In an unconstrained environment, a few roots can be sufficient to meet the plant's water and nutrient requirements [24].

The variation in transplanting dates of avocado seedlings significantly influenced the plant's gain in height, diameter, leaf area of the seedlings and the chlorophyll index. At the end of our experiment, we observed that the TransD40 transplanting stage had the best results in terms of leaf area and chlorophyll index than TransD65 and TransD75, which had better results in terms of plant height and diameter gain; this could be due to the fact that the late transplanted plants required a longer adaptation phase due to the trauma suffered by the roots. It is noted that the white sawdust substrate used in the germinator is rich in mineral elements with a high humidity and oxygenation rate, which are favourable to root development. These results corroborate those of Satapathy *et al.* [25], Tahir *et al.* [26] and Goita *et al.* [16] who found that paddy yield decreased with delaying the transplanting time.

Plenchette and Morel [27] stated that whether plants are mycorrhized or not, they all feed from the same phosphorus (P) pool since the ions dissolve in the soil solution; in other words, the P released from the NPs can be used by mycorrhised or non-mycorrhised plants. On the other hand, the control treatment effect shows a significant difference in leaf area, with results significantly higher than those of the mycorrhizal treatments in the case of leaf area. This confirms the work of He and Cui [28] who observed no significant difference in avocado biomass when applying AMF on sterile and non-sterile soil. Indeed, the AMF should perform their function through the strongly branched outer hyphae which increase the plant's absorption capacity [29]; this absorption capacity which is conditioned by the type of crop grown and the nature of the soil can influence the AMF [30]. In our case, the nursery study in bags could reduce the exploration zone of the AMF and therefore their action. This could explain why the mycorrhizal fertilisation effect did not show any significant difference on the growth parameters. Viera *et al.* [31] working on native mycorrhizae of the rhizosphere, established a correlation between the growth of avocado seedlings and the amount of accumulated phosphorus. The only significant difference for the fertilisation effect was noted only for the leaf area parameter, knowing that nitrogen plays a major role in the multiplication of chloroplasts (green foliage) and also in the increase of the leaf surface and that the Fert1 treatment composed of fowl droppings, very rich in nitrogen and phosphorus, increases the protein synthesis and the phosphorylated compounds in the plants, decreases the content of soluble sugars in the roots, and consequently the rate of mycorrhizal colonisation [32]. Phosphorus input in the soil decreases the mycorrhization rate

of the host plant [27]. The organic amendment applied to the Fert1 non-mycorrhizal treatment possibly favoured the development of endogenous soil fungi and their symbiosis with avocado roots. Our results corroborate with those of Okur *et al.* [33] who stated that soil amendment with organic fertilizers increases the activity of the soil microbial biomass, hence competition when a mycorrhizal amendment is added such as mycorrhizal fertilizer.

5. Conclusion

The general objective of this research work was to define a technical itinerary for the production of avocado seedlings using biofertilizers. With the aim of producing organic avocado seedlings, this work had a two-fold objective: 1) The evaluation of the effect of the substrate on the germination and the vigour of the avocado seedling on the one hand and 2) on the other hand the evaluation of the effect of the organic fertilization (based on mycorrhizae and hen droppings) and of the stage of transplanting on the development of the avocado seedlings. At the end of this work, it appears that: 1) Wood sawdust being the best substrate compared to sand increases the root volume by 49.42%. This gain in root volume is 19.53% compared to soil. Soil increases root volume by 25% compared to sand. Also, the germination rate is lower in sand (83.64%) and higher in wood sawdust (93.64%). 2) Early transplanting dates (TransD40) significantly influence the growth (diameter and height) of the seedling and therefore better for rootstocks; 3) The non-mycorrhizal treatment shows a gain in leaf area, although the beneficial effects of the mycorrhizal treatments do not yet translate into a significant difference in growth parameters. The use of mycorrhizal treatments in the field on young seedlings could allow a better discussion of the results obtained in the nursery. These results are to be used to elaborate a technical sheet for the production of avocado seedlings with bio-fertilisers.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sukamto, L.A., Lestari, R. and Putri, W.U. (2014) The Effect of Bio-Fertilizers on Plant Growth and Growth Rate of Grafted Avocado (*Persea americana* Mill.). *International Journal on Advanced Science, Engineering and Information Technology*, **4**, 205-214. https://doi.org/10.18517/ijaseit.4.4.402

[2] Rajendiran, S. and Chandrakant, M.A. (2019) Nutrient Management for Avocado (*Persea americana* Miller). *Journal of Plant Nutrition*, **43**, 138-147. https://doi.org/10.1080/01904167.2019.1659322

[3] Shen, Q., Yang, M., Li, L. and Cheung, H.Y. (2014) Graphene/TiO_2 Nanocomposite Based Solid-Phase Extraction and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for Lipidomic Profiling of Avocado (*Persea americana* Mill.). *Analytica Chimica Acta*, **852**, 153-161. https://doi.org/10.1016/j.aca.2014.09.022

[4] Yerima, B.P.K., Tiamgne, Y.A., Tziemi, T.C.M.A. and Van Ranst, E. (2015) Effect of Substrates on Germination and Seedling Emergence of Sunflower (*Helianthus annuus* L.) at the Yongka Western Highlands Research/Garden Park, Bamenda-Cameroon. *Tropicultura*, **33**, 91-100.

[5] Singh, A., Sarkar, S., Kaur, J., Gundlee, D., Hussain, A. and Kalmodiya, Y. (2021) Gertmination of Sunflower Seed in Different Media. *Plant Archives*, **21**, 2566-2571. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.419

[6] Strullu, D.G. (1991) Les mycorhizes des arbres et plantes cultivées. Lavoisier, Paris, 26-55.

[7] Van der Heijden, M.G., Boller, T., Wiemken, A. and Sanders, I.R. (1998) Different Arbuscular Mycorrhizal Fungal Species Are Potential Determinants of Plant Community Structure. *Ecology*, **79**, 2082-2091. https://doi.org/10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2

[8] Dubey, S.K. and Verma, S.K. (2021) Plant, Soil and Microbes in Tropical Ecosystems. Springer Nature, Berlin. https://doi.org/10.1007/978-981-16-3364-5

[9] Bolan, N.S. (1991) A Critical Review of the Role of Mycorrhizal Fungi in the Uptake of Phosphorus by Plants. *Plant and Soil*, **134**, 189-207. https://doi.org/10.1007/BF00012037

[10] Mahmood, K.A., Jumma, A.I. and Mahmood, D.B. (2022) Effect of Biofertilizer and Biostimulators on Seeds Germination and Seedlings Growth of Albizia Lebbeck L. *Tikrit Journal for Agricultural Sciences*, **22**, 119-133.

[11] Ronga, D., Biazzi, E., Parati, K., Carminati, D., Carminati, E. and Tava, A. (2019) Microalgal Biostimulants and Biofertilisers in Crop Productions. *Agronomy*, **9**, Article 192. https://doi.org/10.3390/agronomy9040192

[12] Ali, M., Rab, A., Ali, J., Ahmad, W. and Muhammad, H. (2021) Influence of Transplanting Dates and Population Densities on the Growth and Yield of Onion. *Pure and Applied Biology*, **5**, 345-354. https://doi.org/10.19045/bspab.2016.50045

[13] Khan, N.H., Khan, S.M., Khan, N.U., Khan, A., Farid, A., Khan, S.A. and Ali, S. (2019) Flowering Initiation in Onion Bulb Crop as Influenced by Transplanting Dates and Nitrogen Fertilizer. *Journal of Animal and Plant Sciences*, **29**, 772-782.

[14] Aboukhadrah, S.H., El-Alsayed, A.W.A.H., Sobhy, L. and Abdelmasieh, W. (2017) Response of Onion Yield and Quality to Different Planting Date, Methods and Density. *Egyptian Journal of Agronomy*, **39**, 203-219. https://doi.org/10.21608/agro.2017.1203.1065

[15] Sharma, D. and JariaL, K. (2017) Effect of Different Varieties and Planting Time on Kharif Onion Production in Lower Shivalik Hills of Himachal Pradesh. *Current Agriculture Research Journal*, **5**, 74-80. https://doi.org/10.12944/CARJ.5.1.09

[16] Goita, O., Traore, K., Diwara, B., Coulibaly, M.M., N'diaye, M.K., Guindo, S.S. and Sako, D. (2017) Etude de l'effet de la date de semis et de l'age des plantules au repiquage sur le rendement de trois varietes de riz adoptees dans les perimetres irrigues villageois des regions de Tombouctou et de Gao. *Agronomie Africaine*, **29**, 11-17.

[17] Pasuquin, E., Lafarge, T. and Tubana, B. (2008) Transplanting Young Seedlings in Irrigated Rice Fields: Early and High Tiller Production Enhanced Grain Yield. *Field Crops Research*, **105**, 141-155. https://doi.org/10.1016/j.fcr.2007.09.001

[18] Cocco, C., Andriolo, J.L., Erpen, L., Cardoso, F.L. and Casagrande, G.S. (2010) Development and Fruit Yield of Strawberry Plants as Affected by Crown Diameter and Plantlet Growing Period. *Pesquisa Agropecuária Brasileira*, **45**, 730-736. https://doi.org/10.1590/S0100-204X2010000700014

[19] Raj, H., Panghal, V.P.S., Lal, M., Duhan, D.S. and Kumar, K. (2016) Effect of Sowing Dates and Planting Methods on Quality of Different Order Umbels in Fennel, Fennel Quality as Effected by Different Planting Methods and Time. *International Journal of Environment, Agriculture and Biotechnology*, **1**, 577-580. https://doi.org/10.22161/ijeab/1.3.40

[20] Osborne, J.M., Fox, J.E.D. and Mercer, S. (1993) Germination Response under Elevated Salinities of Six Semi-Arid Bluebush Species (Western Austrailia). In: Lieth, H. and Al-Masoom, A., Eds., *Towards the Rational Use of High Salinity Tolerant Plants*: *Vol.* 1: *Deliberations about High Salinity Tolerant Plants and Ecosystems*, Springer, Berlin, 323-338. https://doi.org/10.1007/978-94-011-1858-3_35

[21] Grace, C. and Stribley, D.P. (1991) A Safer Procedure for Root Staining of Vesicular Arbuscular Mycorrhizal Fungi. *Mycological Research*, **95**, 1160-1162. https://doi.org/10.1016/S0953-7562(09)80005-1

[22] Sounon, M., Kakai, R.G., Avakoudjo, J., Assogbadjo, A.E. and Sinsin, B. (2009) Tests de germination et de croissance de *Artemisia annua* L. anamed sur différents substrats au Bénin. *International Journal of Biological and Chemical Sciences*, **3**, 337-346. https://doi.org/10.4314/ijbcs.v3i2.44505

[23] Benyoucef, S. and Harrache, D. (2015) Caractérisation de la microstructure de sciure de bois de pin sylvestre "*Pinus sylvestris*" [Microstructure characterization of scots pine "*Pinus sylvestris*" sawdust]. *Journal of Materials and Environmental Science*, **6**, 765-772.

[24] Maertens, C., Blanchet, R. and Puech, J. (1974) Influence des différents régimes hydriques sur l'absorption de l'eau et des éléments minéraux par la culture. I. Régimes hydiques, systèmes racinaires et modalités d'alimentation en eau. *Annales Agronomiques*, **25**, 575-586.

[25] Satapathy, S.M., Srivastava, V.K., Majhi, P.K. and Gond, S. (2021) Effect of Delayed Time of Planting on Grain Yield and Agromorphological Traits of Elite Rice (*Oryza sativa* L.) Varieties. *International Journal of Plant & Soil Science*, **33**, 68-79. https://doi.org/10.9734/ijpss/2021/v33i1630524

[26] Tahir, M.A., Arain, M.A., Durrani, S., Shakoor, A., Bilal, A., Ali, N., Ishfaq, M., Farooq, U., Ahmed, S. and Irfan, M. (2018) Evaluating the Optimum Transplanting Time for Different Coarse Rice Genotypes under Semi-Arid Conditions of Faisalabad. *Agricultural Sciences*, **9**, 69-77. https://doi.org/10.4236/as.2018.91006

[27] Plenchette, C. and Morel, C. (1996) External Phosphorus Requirement of Mycorrhizal and Non-Mycorrhizal Barley and Soybean Plants. *Biology and Fertility of Soils*, **21**, 303-308. https://doi.org/10.1007/BF00334907

[28] He, W.M. and Cui, Q.G. (2009) Manipulation of Soil Biota in Ecological Research. *Web Ecology*, **9**, 68-71. https://doi.org/10.5194/we-9-68-2009

[29] Noda, Y. (2009) Las Micorrizas: Una alternativa de fertilización ecológica en los pastos. *Pastos y Forrajes*, **32**, 1-10.

[30] Hijri, I., Sýkorová, Z., Oehl, F., Ineichen, K., Maeder, P., Wiemken, A. and Redecker, D. (2006) Communities of Arbuscular Mycorrhizal Fungi in Arable Soils Are Not Necessarily Low in Diversity. *Molecular Ecology*, **15**, 2277-2289. https://doi.org/10.1111/j.1365-294X.2006.02921.x

[31] Viera, W., Campaña, D., Gallardo, D., Vásquez, W., Viteri, P. and Sotomayor, A. (2017) Native Mycorrhizae for Improving Seedling Growthin Avocado Nursery (*Persea americana* Mill.). *Indian Journal of Science and Technology*, **10**, 1-13. https://doi.org/10.17485/ijst/2017/v10i25/110415

[32] Le Tacon, F., Le Tacon, T., Mauron, V., Rousseau, Y., Backer, M. and Bouchard, D.

(1999) Fertilisation raisonnée et mycorhize. 4*ème rencontre de la fertilisation raisonée*, Blois, Novembre-Décembre 1999, 211-222.

[33] Okur, N., Altindİşlİ, A., Çengel, M., Göçmez, S. and Kayikçioğlu, H.H. (2009) Microbial Biomass and Enzyme Activity in Vineyard Soils under Organic and Conventional Farming Systems. *Turkish Journal of Agriculture and Forestry*, **33**, 413-423. https://doi.org/10.3906/tar-0806-23