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Use of Palm Male Inflorescence and River-Sand as Acclimatization Substrate for Plantain (*Musa sp.*) Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EYM and LLD designed the study. Authors EYM, JEN and APN carried out the experiments. Author EYM performed the statistical. Author EYM wrote the first draft of the manuscript. Authors EYM and JEN managed the analyses of the study. Authors EYM and JEN managed the literature searches. Authors JEN, LLD and APN revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this work was to investigate the use of Palm Male Inflorescence (PMI) and river-sand as substrate for the acclimatization of plantain. Plantlets from three plantain cultivars (Batard, Ebanga and French Clair) were obtained after 16 weeks of tissue cultures and the plantlets were subjected to routine acclimatization under screen house conditions using two different substrates mixed in different ratios (100% Sand, 100% PMI, 75% PMI, 60% PMI and 50% PMI). The experiment was arranged in a completely randomized design with ten (10) replications; each replicate consisting of one micro-pot. The different substrates used significantly influenced the performance of the cultivars. The best medium for acclimatization for French Clair was 60% PMI in terms of percentage survival of plantlets (96.88%), plantlet height (6.03 cm), diameter (0.60 cm), number of leaves (4.42 leaves), leaf area (20.23 cm^2), leaf emergence rate (1.64), number of roots

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(7.70 roots), and root length (18.86 cm). Ebanga plantlets had the best results with 75% PMI in terms of percentage survival of plantlets (96.88%), plantlet height (6.18 cm), diameter (0.62 cm), number of leaves (4.39 leaves), leaf area (20.48 cm^2), leaf emergence rate (1.76), and total fresh weight (10.05 g). Meanwhile with Batard cultivar, 50% PMI was the best substrate in terms of percentage survival of plantlets (96.88%), plantlet height (4.41 cm), diameter (0.55 cm), number of leaves (4.55 leaves), leaf area (12.96 cm^2), leaf emergence rate (1.55), and number of roots (5.73 roots). This study clearly show that PMI can be a viable substrate to use with sand in plantlet acclimatization; however, the different plant cultivars had optimal result at different proportions of PMI.

Keywords: Plantain plantlets; ebanga; french clair; batard; palm male inflorescence; sand; acclimatization.

1. INTRODUCTION

Plantain (*Musa sp.*) is a major staple food and the most important food crop in many countries of humid West and Central Africa [1]. The cultivation of plantain with banana plays an important role in the economic and community development of the world because it is an important source of energy and revenue for the local farmers [2]. The number of local farmers and farming area keep increasing in order to meet the increasing demand for plantain as population increases [1].

Plantain is also known as plantain-banana because it closely resembles bananas, but the fruits are consumed cooked (ripe or unripe) as the starch component of a dish [3]. There are many plantain-banana species, but the most commonly planted plantains species are parthenocarpic in nature (lack seeds) where the use of conventional methods for breeding improvement of plantains is difficult to practice [4]. For this reason, the main method of propagation is vegetative propagation using sucker which is considered a slow process [4].

Multiplication using micro-propagation technique or tissue culture offers an efficient method to produce clonal populations of diverse species in a short time. This technique also provides a high rate of multiplication of genetically uniform, pest and disease-free planting materials [5,6]. However, the application of this technology is hampered by high mortality rate usually observed when micro-propagated plantlets are transferred to ex-vitro conditions [7]. During tissue culture prior to the plantlet transfer, plantlets grow under special environmental conditions in relatively air-tight vessels where humidity is higher and irradiance is lower than in conventional culture. The micro-shoots produced, upon transfer to ex-vitro conditions are exposed to abiotic stress such as altered temperature, light intensity, and humidity, and also to biotic stress such as soil microflora [8]. Hence acclimatization is needed to lessen the stress the plantlets will face during this transfer.

During acclimatization, the quality of nursery potting substrate contribute immensely to the successful growth of plantlets [9]. It is recommended that the best potting medium for acclimatizing in vitro should have a balance between good water-holding capacity and drainage characteristics [10]. This will allow the roots of plantlets to be in contact with sufficient amounts of water without drowning. The microbial property of the soil is equally important. The potting substrate constitutes the first source by which plantlets can easily be contaminated and infested by diseases such as root knot nematodes and root rots [11].

Traditionally, the best substrates for the acclimatization of plantain plantlets are sand and peat moss [12]. However, peat moss is not available locally for farmers, relatively expensive, and shown wide fluctuation in prices worldwide [13]. Several locally organic materials (composts) such as sawdust, coconut fibre, coffee husk, rice husk etc. are mostly used because they offer great advantage over the conventional topsoil [14,15]. These organic substrates provides adequate nutrients for the seedling, better root substrate relation than conventional soil mix, and less pre-dispose to soil borne pests and diseases [14]. Lee [16] reported that a potting medium of peat moss, rice husk, and sand (1:1:1 v/v) increased the growth of banana (*Musa sp*.) plantlets compared to sand only, while sawdust can support their growth for two months.

A range of crop residues, organic wastes, and other industrial by-products can be used as organic medium but the selected medium should largely be determined by availability, economics, and physical and chemical characteristics of the

substrate [14]. Sawdust has been the most commonly and widely used residue in agriculture for potting mixture [17]. However, its decomposition causes nitrogen deficiency as microflora deplete available nitrogen during decomposition [18]. During this decomposition, sawdust compacts, increasing its water holding capacity and roots rot by drowning. There is a need to have a substrate that can have good nitrogen content and is cheaply available.

In most palm (*Elaeis guineensis*) plantations, plantain is grown as an intercrop during the first three years. This makes the male inflorescence which is removed and left on the farm floor available for the farmer. Palm Male Inflorescence (PMI) is light in weight, has uniform quality, resistant to decay and depletion of available nitrogen by microorganisms. PMI has the advantage of being easily incorporated into media for improved drainage and aeration.

Most plantain plantations in the South West Region of Cameroon are close to palm plantations in the region. During pruning, the male inflorescences of palm trees are removed and left to rot on the farm floor. In these areas, there is an abundance of PMI that can be valorised as potting substrate. PMI may likely have a good water holding capacity, hence a good substrate for potting. However, the use of such material as alternative substrate to peat moss is not well- documented.

The aim of this study was to evaluate the effectiveness of PMI as acclimatization substrate of tissue culture derived plantain (*Musa Sp*.) cultivars under greenhouse condition.

2. MATERIALS AND METHODS

2.1 Location

The experiment was carried out at the Biotechnology Laboratory screen house of the Institute of Agricultural Research for Development (IRAD) Ekona (4°16´44˝ N and 9°17´50˝ E) in the South West Region of Cameroon.

2.2 Planting Material

Micro-propagated 16 weeks old plantlets of three *Musa sp.* Cultivars*;* French Clair (French type), Batard (French Horn plantain) and Ebanga (a local variety, AAB, False Horn) were obtained from in vitro culture after rooting. Plants sorted and used for this study had an average height of 2 cm, and approximately possessing 2–3 leaves and 1–2 roots. Tissue culture vessels were progressively opened for one week before plantlets were taken out. These plantlets were taken to a screen house for acclimatization.

2.3 Potting Substrates

The palm male inflorescence (PMI) used in this study was obtained from the Cameroon Development Corporation (CDC) palm plantation. They were chopped with a cutlass, crushed manually, and sieved through a 0.4 cm meshed sieve to remove fibres. Fine sand of approximately 0.3 mm was obtained from River Mungo along the Muyuka subdivision, Cameroon. The substrates were either used individually or mixed in different proportions to generated five treatments as shown in Table 1.

Table 1. Incorporation ratios of PMI in sand used for potting

Treatment	Substrate	
	PMI (%)	Sand (%)
T1	0	100
T2	100	0
T3	75	25
T4	60	40
T5	50	50

PMI= palms male inflorescence

2.4 Experimental Design

The experiment was arranged in completely randomized Design (CRD) with ten (10) replications; each replicate consisted of one plantlet per micro-pot.

2.5 Acclimatization of Plantain Plantlets

The acclimatization was carried out under screen house conditions. Plantlets were removed from culture bottles and washed thoroughly to remove excess medium. Each plantlet was transferred to a perforated micro-pot containing the different substrates ratio. After transplantation, the entire pot containing the plantlet was placed in propagators overlaid with moist sawdust and each plantlet was misted with 16.5 mL of half a strength MS solution [19]. The entire propagator was completely covered with a transparent plastic to preserve moisture. The plantlets remained undisturbed during the first week of transplantation to maintain maximum percentage of relative humidity around the plantlets. During the second week, the cover was slightly opened

for 2-3 minutes every day to reduce temperature accumulation in the propagators. The plantlets were misted twice every week to maintain substrate moisture and the cover was completely removed during the third week.

2.6 Data Collection

Vegetative growth of the plantain plantlets was measured every three weeks for three months (May-July 2017) and the following parameters were assessed: plant survival recorded in percentage, plantlet height from substrate level to the last 2 open leaves using a graduated metal ruler, number of functional leaves per plant, stem diameter beneath the 1st fully expanded leaf using a Vernier Calliper, and leaf length (L) from petiole to tip and leaf width (W) at its widest part using a graduated metal ruler. The leaf length and width were used to calculate the Leaf Area (LA) following the equation LA = (L x W) x 0.8 for banana plants [20] and leaf emergence rate (LER) was measured by marking the last emerged petioles and counting the number of raised leaves.

After 12 weeks, the plants were carefully off-rooted and substrate attached to the roots were carefully washed off with running water. The number of live roots was counted and the average length (cm) of the well-developed roots taken. Four plants per treatment were harvested at random to evaluate fresh weight analysis. In order to measure the accumulated dry matter, selected plants were divided into roots, stems and leaves and placed in paper bags. Plant parts were dried in an oven at 70°C for 72 hours. These dried plants organs were weighed using an electronic balance to determine plant biomass.

2.7 Statistical Analysis

Data was analyzed using a one-way analysis of variance (ANOVA) at P = 0.05 in Minitab statistical software package version 17. The treatment means were compared and separated using Tukey's method at 5% probability level.

3. RESULTS AND DISCUSSION

3.1 The Effect of Substrate Concentration on Plantlet Survival during Acclimatization

Survival percentage is one of the parameters often used to evaluate the efficacy of a substrate. By using 50%, 60%, and 75% PMI in all the cultivars, more than 96% of the plantlets survived the acclimatization phase, except Batard and Ebanga plantlets which recorded only 80% and 93.75% survival with 60% PMI, respectively (Fig. 1). The survival percentage of plantlets in 50%, 60%, and 75% PMI substrates were not significantly different (P = 0.05) to plantlets in 100% sand substrate. The survival percentage of plantlets in 100% sand ranged between 90-94%. Sharma et al. [21] also reported 97.00% survival of in vitro developed Dwarf Cavendish plantlets in sand culture while only 2.00% loss was observed during the acclimatization of Nanicao and Grande Naine in polythene bags containing equal proportions of organic manure : soil : sand [22,23]. Overall, all the plantlet cultivars had very poor survival percentage in 100% PMI substrate, which was significantly different compared to the other substrate used (P = 0.001). The present study illustrated that there was a positive effect of substrate concentration to the survival percentage of plantain cultivars transplants under greenhouse condition, except substrate concentration of 100% PMI.

The use of PMI alone did not provide support to the plantlets during acclimatization irrespective of the cultivars; however, by using 50-75% of PMI with sand did significant improve the performance of PMI. PMI likely have very high water holding capacity, which affects the relative humidity of the plantlets, but the addition of at least 25% of sand improves the air space of the soils. Palai and Das, [24]; Molla et al. [25] and Acharjee et al. [26], in their findings concluded that good substrate media survival percentage range should be between 80 to 100% when *Musa sp.* rooted plantlets are transferred to ex vitro hardening media under greenhouse or shade house conditions.

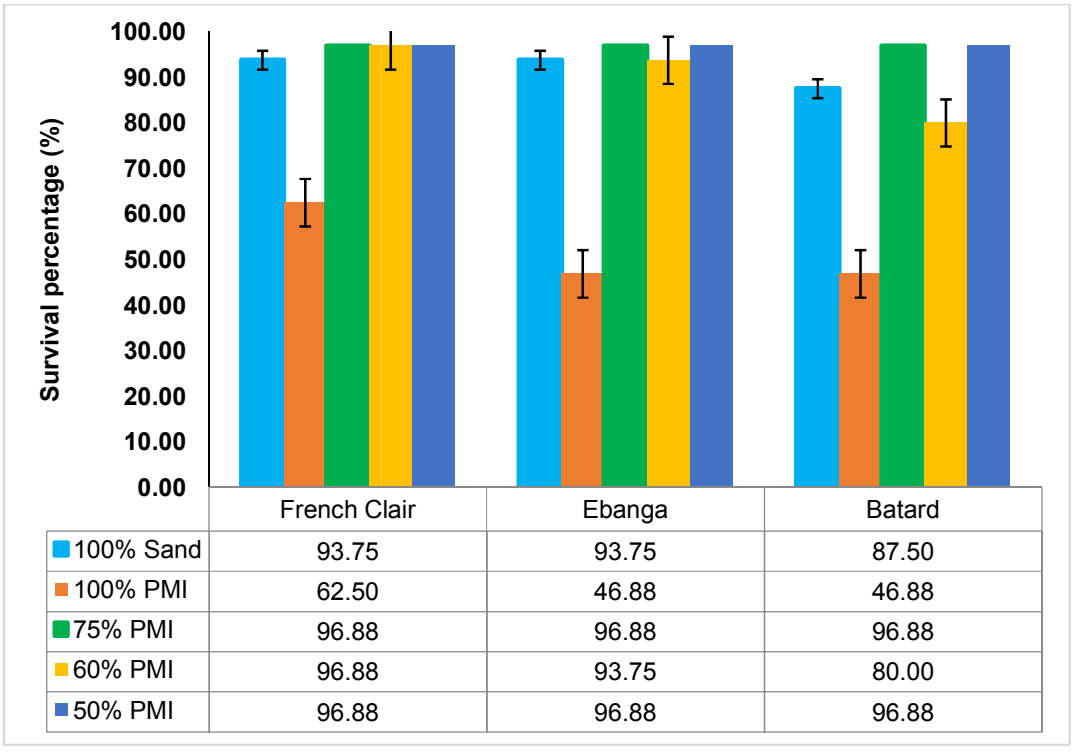
3.2 The Effect of Substrate Concentration on Growth and Development of Plantlets

Other parameters that were used to evaluate the substrate were the growth and development of plantlets. The following measurements were made every two weeks during the period of the study; plantlet height, plantlet diameter, number of leaves, leaf area, and leaf emergence rate. The plantlets growth and development were significantly (P = 0.001) influenced by substrate concentration methods in all the cultivars (Table 2). The three cultivars - Batard, French Clair and Ebanga - showed best result with 50, 60 and

75% PMI substrate, respectively. All the cultivars in 50, 60 and 75% PMI substrate showed that they were no significant difference (P = 0.05) in their plantlet height, plantlet diameter, number of leaves, and leaf area ranging from 2.03 to 6.2 cm, 0.39 to 0.62 cm, 3.00 to 5.00 leaves, 3.75 to 20.48 cm^2, respectively. Generally, in all the cultivars, it was observed that the plantlets produce averagely one leaf every two weeks. The growth and development characteristics during weaning were consistent in all three cultivars in 50%, 60%, and 75% PMI substrates. This may be due to less bulk density and more water holding capacity. The less bulk density indicates less substrate compactness and greater pore spaces (air filled porosity) which allowed better root aeration, nutrient and water uptake for subsequent growth enhancement [27,28].

Robinson and Sáuco [27], Abul-Soad et al. [28], and Mirani et al. [29] reported that acclimatization medium with air-filled porosity (AFP) as close as possible to 20%, is considered optimal. Robinson and Sáuco [27], concluded that AFP of a medium should not be less than 10% and should not be greater than 25%. They also recommended that the water-holding capacity (WHC) of hardening medium should be between 40% and 50%. These results were similar with earlier studies that showed that higher substrate water holding capacity was consistently associated with better growth in potted banana plantlets [13,10,7]. With Batard plantlet, growth in 50% PMI substrate was not significantly different (P = 0.05) from plantlet growth in 100% Sand. The sand may have relatively good size particles [28] for good aeration which support plantlets growth. Mirani et al. [29] reported that hill sand have relatively big size soil particles which made good aeration and mechanical support to orchids plantlets growth and development.

The roots grow through the substrate, they come in direct contact with and intercept nutrients associated with substrate particles that are displaced by roots [14]. The quantity of nutrients absorbed by plant roots through root interception depends on the substrate volume occupied by the roots, the concentration of nutrients in the soil, and the root morphology [14,15]. Plantlets grown in all the five substrate concentration showed significant difference (P = 0.001) with root length and root numbers (Table 3).



	French Clair	Ebanga	Batard
100% Sand	93.75	93.75	87.50
100% PMI	62.50	46.88	46.88
75% PMI	96.88	96.88	96.88
60% PMI	96.88	93.75	80.00
50% PMI	96.88	96.88	96.88

Fig. 1. Average survival percentage of plantlets of three plantain cultivars with respect to the different substrate used

PMI= palms male inflorescence

Table 2. The effects of substrate concentration on the growth and development of plantain plantlets

Treatment	French clair					Ebanga					Batard				
	PH (cm)	PD (cm)	NL	LA (cm^2)	LE	PH (cm)	PD (cm)	NL	LA (cm^2)	LE	PH (cm)	PD (cm)	NL	LA (cm^2)	LE
100% Sand	4.23^{bc}	0.48^{bc}	3.76^{ab}	12.08^{ab}	1.21^{bc}	4.23^{b}	0.47^{b}	3.76^{ab}	10.04^{b}	1.27^{b}	4.29^{b}	0.52^{a}	4.52^{a}	10.81^{ab}	1.06^{b}
100% PMI	3.16^{c}	0.44^{c}	2.83^{c}	6.85^{b}	1.13^{c}	3.01^{b}	0.43^{b}	3.10^{b}	7.52^{ab}	1.22^{b}	2.03^{c}	0.39^{b}	2.91^{c}	3.75^{b}	1.00^{b}
75% PMI	5.34^{ab}	0.54^{abc}	3.39^{bc}	16.55^{ab}	1.49^{abc}	6.18^{a}	0.62^{a}	4.39^{a}	20.48^{a}	1.76^{a}	4.18^{ab}	0.52^{a}	3.82^{ab}	11.83^{ab}	1.42^{a}
60% PMI	6.03^{a}	0.60^{a}	4.42^{a}	20.23^{a}	1.64^{a}	4.87^{ab}	0.53^{ab}	4.27^{a}	13.91^{ab}	1.33^{b}	3.01^{bc}	0.43^{b}	3.23^{bc}	5.45^{ab}	1.35^{ab}
50% PMI	5.33^{ab}	0.58^{ab}	3.64^{abc}	14.15^{ab}	1.55^{ab}	4.78^{ab}	0.61^{a}	3.88^{ab}	14.32^{ab}	1.61^{ab}	4.41^{a}	0.55^{a}	4.55^{a}	12.96^{a}	1.55^{a}

Values represent Means were separated using Tukey HSD = 0.05.
Mean with similar letters within column indicate no significant differences among treatments
PMI= palm male inflorescence, S = Sand, PH = Plant Height, PD = Plant Diameter, NL = Number of Leaves, LA = Leaf Area, LE = Leaf Emergence rate

Table 3. The effects of substrate concentration on the root development response to acclimatization

Treatment	French clair		Ebanga		Batard	
	No of Root	Root Length (cm)	No of Root	Root Length (cm)	No of Root	Root Length (cm)
100% Sand	6.36^{bc}	13.50^{b}	6.24^{b}	15.94^{c}	4.85^{a}	14.96^{b}
100% PMI	3.27^{d}	13.84^{b}	1.61^{c}	16.75^{bc}	1.52^{c}	18.10^{ab}
75% PMI	7.18^{ab}	18.49^{a}	6.97^{b}	19.93^{ab}	5.94^{c}	18.64^{a}
60% PMI	7.70^{a}	18.86^{a}	6.52^{b}	22.16^{a}	3.00^{b}	13.62^{b}
50% PMI	5.67^{c}	16.16^{ab}	8.09^{a}	17.67^{bc}	5.73^{a}	16.86^{ab}

Values represent Means were separated using Tukey HSD = 0.05.
Mean with similar letters within column indicate no significant differences among treatments
PMI= palm male inflorescence, S = Sand

Table 4.The effects of substrate concentration on the fresh matter

Treatment	French clair			Ebanga			Batard		
	RFW (g)	AFW (g)	TFW (g)	RFW (g)	AFW (g)	TFW (g)	RFW (g)	AFW (g)	TFW (g)
100% Sand	2.57^{bc}	2.68^{c}	5.00^{c}	3.38^{a}	3.47^{c}	6.81^{b}	1.21^{c}	1.60^{c}	2.75^{c}
100% PMI	1.28^{d}	3.06^{c}	4.33^{d}	1.53^{a}	3.71^{bc}	5.18^{c}	0.41^{d}	1.26^{c}	1.68^{d}
75% PMI	3.20^{a}	9.72^{a}	14.09^{a}	3.49^{a}	6.60^{a}	10.05^{a}	3.00^{a}	5.81^{a}	8.79^{a}
60% PMI	2.34^{c}	5.44^{b}	7.52^{b}	3.81^{a}	6.34^{a}	10.15^{a}	0.39^{d}	1.84^{c}	2.26^{cd}
50% PMI	2.65^{b}	4.95^{b}	7.76^{b}	1.42^{b}	4.91^{b}	6.27^{bc}	1.75^{b}	4.77^{b}	6.57^{b}

Values represent Means were separated using Tukey HSD = 0.05.
Mean with similar letters within column indicate no significant differences among treatments
RFW= Root fresh weight, AFW= Aerial fresh weight, TFW = Total fresh weight and PMI= palm male inflorescence, S = Sand

Table 5. The effects of substrate concentration and cultivar on the dry matter response to acclimatization

Treatment	French clair			Ebanga			Batard		
	RDW(g)	ADW (g)	TDW (g)	RDW (g)	ADW (g)	TDW (g)	RDW (g)	ADW (g)	TDW (g)
100% Sand	0.23^{a}	0.16^{d}	0.42^{b}	0.29^{a}	0.28^{bc}	0.59^{a}	0.20^{a}	0.12^{c}	0.32^{b}
100% PMI	0.06^{c}	0.19^{d}	0.23^{c}	0.07^{c}	0.19^{c}	0.27^{b}	0.02^{c}	0.07^{c}	0.09^{c}
75% PMI	0.24^{a}	0.56^{a}	0.81^{a}	0.21^{ab}	0.74^{a}	0.56^{a}	0.01^{c}	0.09^{c}	0.10^{c}
60% PMI	0.14^{b}	0.29^{c}	0.44^{b}	0.19^{b}	0.34^{b}	0.54^{a}	0.22^{a}	0.37^{a}	0.59^{a}
50% PMI	0.19^{ab}	0.35^{c}	0.52^{b}	0.08^{c}	0.22^{bc}	0.29^{b}	0.08^{b}	0.24^{b}	0.33^{b}

Values represent Means were separated using Tukey HSD = 0.05.

Mean with similar letters within column indicate no significant differences among treatments

RDW= Root dry weight, ADW= Aerial dry weight, TDW = Total dry weight, PMI= palm male inflorescence, S = Sand

Cultivars grown in 100% Sand showed very poor performance in root number and root length except for Batard where root length (18.10 cm) in 100% Sand was better than all other substrate concentrations. The poor result obtained with 100% PMI may be explained by its structure which becomes muddy and compact [30]. This also indicates that the air-filled porosity may be less than 10%, thus having high risk of compaction, water logging and inhibited root growth [27].

3.3 The Effect of Substrate Concentration on Fresh and Dry Weight

The fresh and dry weight of the plantlet were used to assess the total biomass of the plantlets. Fresh weight of plantlet was also affected by substrate concentration. Plantlets grown in the different substrate were significantly different (P = 0.001) in their fresh weight (Table 4). The results showed that French Clair and Batard cultivars grown in 75% PMI substrate had more fresh weight in all variables measured with a total fresh weight of 14.09 and 8.79 g, respectively. Though not significant difference was observed between 75% PMI substrate and 60% PMI substrate, Ebanga grown in 60% PMI substrate recorded the highest total fresh weight (10.15 g). It may be possible that plantlets with greater leaf area and more number of functional leaves per unit of foliar fresh weight would achieve more net photosynthesis and will contribute to the positive growth and development of a plant. Marschner [31], reported that cells of green leaves contain more than 75% of the total organic nitrogen in the chloroplasts.

The plantlets dry weight was significantly (P = 0.001) influenced by different substrate concentration in all three cultivars (Table 5). Root dry weight of plantlets grown in 75% PMI and 100% sand substrates showed no significant difference (P = 0.05) in French Clair (0.24 and 0.23 g) and Ebanga (0.21 and 0.29 g), respectively. For Batard cultivar, 60% PMI and 100% sand substrates had no significant difference (P = 0.05) with root dry weight (0.22 and 0.20 g), respectively. Arial dry weight was significantly higher in 75% PMI, 0.56 g and 0.74 g for French Clair and Ebanga respectively while for Batard it was significantly higher (0.37 g) in 60% PMI substrate. This data indicates that PMI during the acclimatization of micropropagated plantlets of *Musa* improved the further development of leaf area, stems, and roots, hence, increased the accumulation of dry matter. The accumulation of dry matter, especially during the vegetative growth phase, likely depend on the amount of intercepted solar radiation and the CO_2 fixation. Factors such as nutrition and water condition of the plant likely had an effect on the plant performance, thereby altering the leaf area index, the interception of light, and net photosynthesis [32].

Although 100% sand substrate did not have the worst result as expected, the hypothesis would have been true if the study was longer than 24 weeks. The results would have been very poor after 24 weeks due to lack of nutrients to sustain long term growth, insufficient buffering capacity or cation exchange capacity (CEC), and high pH. The plantlets would have shown signs of iron and boron deficiencies.

4. CONCLUSION

The study showed that the type of substrate affects survival and growth parameters of plantains plantlets during acclimatization. A combination of sand and PMI substrates greatly improved the performance of plantain plantlets. The improvements from these substrates were likely due to the combined benefit to the root system and the flow of air and water. Using 50% or 75% PMI substrate was not significantly different, hence, the availability and cost of PMI or sand should be a determining factor on the proportion of the substrate to use. A further research is needed to evaluate the performance of plantain and banana plantlet during secondary hardening.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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