

Plantain Acclimatisation in Relation to Substrate Type

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Abstract – Acclimatization is an important step in the micro propagation of plantain. During *in vitro* culture, plantlets grow under very special conditions in relatively air tight culture vessels such as high humidity and lower irradiance than in conventional culture. The aseptic environment *in vitro* also reduces the stress of pathogenic organisms. After a period of growth in *in vitro* conditions (rooting stage), plantain plantlets were transferred to the screen house for acclimatization using soil and river sand (3:1 v/v) which is the traditional substrate as control, soil alone, sand alone, biochar alone and soil: sand: biochar (1:1:1 v/v) as treatments. This was replicated three times in a randomized complete block design. Data was collected on number of leaves, leaf diameter, stem length, root number and root length. These data were analyzed by ANOVA. From results obtained in terms of number of leaves, stem length, leaf diameter, root number and root length, the treatment (substrate) biochar:soil:sand proves to be a better substrate for acclimatization of the two plantain varieties (Ebanga and FHIA21).

Keywords – Acclimatization, Biochar, Ebanga, FHIA21, Plantain Plantlets.

I. INTRODUCTION

Plantain (*Musa spp AAB.*) is a major staple and the most important food cash crop in many countries of humid West and Central Africa. It closely resembles bananas but the fruits are consumed cooked as the starch component of a dish [6], [12]. *Musa spp* are referred to by their groups which indicate their ploidy and putative genomic constitution with respect to the parents [12]. Plantain (AAB) is thus a triploid, has two genomes from *Musa acuminata* (A) and one from *Musa balbisiana* (B). Plantains are further divided into four groups based on the morphological features of the inflorescence: French, French horn, False horn and True horn [17], [13].

In Cameroon, plantain is next to yam (*Dioscorea spp.*), the most preferred staple, and has a huge potential for rural income generation [6]. However, productivity is threatened by sucker transmitted pests and diseases. To safeguard sustainable production, the need for high quality planting material is crucial. Banana and plantain can now be propagated aseptically in the laboratory through tissue culture techniques. *In vitro* micro-propagation eliminates all sucker-transmitted pests and diseases, with the exception of viruses [11].

Micro propagation has been extensively used as a method for the rapid and large multiplication of planting material. However, its more widespread use is restricted by the often high percentage of plants lost or damaged during transfer from the laboratory to the green house or field [8], [14].

Acclimatization is an important step in micro propagation [16]. During *in vitro* culture plantlets grow under very special conditions in relatively air tight culture vessels, e.g. high humidity and lower irradiance, than in conventional culture. The aseptic environment *in vitro* also reduces the stress of pathogenic organisms. Shoots and plants in culture are also grown in conditions that provide little physiological stress since the culture media are often supplemented with sucrose as a source of carbon and energy, as well as plant growth regulators [14], [15]. These chemical (culture medium) and physical (incubation) conditions combine to produce plants uniquely unsuited for survival in green house and field conditions. In the green house, and especially in the field, irradiance is much higher and air humidity much lower than in the vessels [15], [14].

After a period of growth in *in vitro* conditions (rooting stage), plantain plantlets are transferred to the screen house and transplanted in pots to obtain plants large enough to be grown in the field. As in any potted plant, the quality of the transplants depends mainly on the type of potting substrate used [4], [2]. As stated by [4], for the best growth of tissue cultured *Musa spp*, a potting substrate should have a good balance between its water holding capacity and drainage characteristics, which allows the roots to obtain sufficient water without drowning. The traditional potting medium used by nurseries is top soil dug from farm land and amended with poultry manure. Traditionally, plantain plantlets are transplanted in a potting substrate of top black forest soil and sand (3:1 v/v). Some locally available organic materials like saw dust has also shown to be an acceptable substrate. It was reported by [9], that a potting medium of peat moss, rice husk and sand (1:1:1 v/v), increased the growth rate of banana (*Musa sp*) plantlets, compared to sand only. Potting substrates can also be amended with organic waste like fowl droppings to enhance the growth of the transplants. However the use of biochar (a solid material obtained from the carbonization of biomass), which is a valuable soil amendment [7] as potting mix component, has not been reported.

The objective of the present work is to evaluate the growth of micro propagated plantain plantlets in different potting substrates, singly and in mixture forms, under screen house conditions.

II. MATERIALS AND METHODS

The experiment was carried out in the plant tissue culture laboratory and the screen house of IRAD Bambui.

Plant material

Micro propagated plantlets of two *Musa sp* FHIA 21 (a hybrid, AAAB, French) and Ebanga (a local variety, AAB, False Horn) were obtained from the Tissue Culture laboratory of IRAD Bambui. Explants for *in vitro* culture were apical shoot tips extracted from suckers grown in the field collection. The suckers were washed thoroughly under running tap water and the roots and outer leaves carefully removed with a sharp knife. The corm and outer leaf sheaths were trimmed to obtain a shoot tip measuring about 1-2 cm long and 1 cm at the base.

Surface sterilization of explants

The explants were brought to the laboratory in containers for surface sterilization as described by [1].

- Disinfection solutions were prepared as follows: sterile distilled water, alcohol solution (ethanol 70% v/v), sodium hypochlorite solution (2.6 % active agent NaOCl) at 10 to 20% containing a few droplets of a wetting agent, e.g., Tween 80.
- Explants were rinsed quickly with tap water once or twice to wash off soil particles and thereafter soaked in several solutions in succession (occasionally shaken for better contact) as follows:
 - 1) 15 min in 20% sodium hypochlorite solution.
 - 2) 5 min in alcohol solution (Ethanol 70% v/v).
 - 3) 20-25 min in 10% sodium hypochlorite solution.
 - 4) Rinsed three times in sterile distilled water.

N/B: Step 2 to 3 are done under the laminar flow hood and the disinfection solutions prepared with sterile distilled water.

In vitro culture

The surface sterilized explants were further trimmed using sterile scalpel blade (size 10 or 23) by removing the leaf sheaths and the corm to a size of about 50mm³. These were planted in baby food jars containing semi solid medium made of Murashige and Skoog (MS) basal salts [10] supplemented with BAP (2.25mg/l), IAA (.018mg/l), sucrose (30.0g/l), gelrite (2.3g/l) and pH adjusted to 5.8. An anti-oxidant (ascorbic acid 1mg/l) was also added to the medium to reduce blackening. The culture vessels with explants were sealed with Para film and transferred to the growth chamber and kept under optimum growth conditions (T °25 ± 1 °C, 12h light/12h dark and light intensity of 38 μ mol/m²/s provided by white fluorescent bulbs).

The cultures were sub cultured after every 4 weeks unto fresh proliferation medium and at the 8th sub culture level,

shoots were rooted in semi-solid rooting medium in Magenta vessels containing MS basal salts [10] supplemented with 30.0g/l sucrose, 1.0mg/l NAA, 2.3g/l gelrite and the pH adjusted to 5.8. The shoots were kept in the growth chamber for 4 weeks to elongate and develop roots. Rooted plantlets with an average height of 7cm and 3 to 4 leaves were taken to the screen house for the acclimatization.

Potting substrates

Top black soil and sand were obtained from IRAD Bambui research farm and river Menchum respectively. Biochar was produced from the pyrolysis of rice husk. Prior to use, the substrates were pasteurized in metal drums.

The substrates were used in single or mixture forms and five treatments generated as follows:

- Soil and river sand (3:1 v/v), control
- Soil only
- Sand only
- Soil: sand: biochar (1:1:1 v/v)
- Biochar only

Preparation of plant material and Acclimatization

The rooted micro propagated plantlets were taken to the screen house, deflasked, and the gelrite thoroughly washed off with tap water. They were transplanted in 200ml pots containing the various substrates/mixtures and each cup enclosed in a transparent plastic bag. These were kept to grow under screen house conditions for 4 weeks and watered using a spray bottle twice per week. The experimental layout was a completely randomized design with 3 replicates per treatment.

Data collection

Plant height, number of leaves and leaf diameter were measured weekly and plant mortality recorded. Plant height (cm) was measured from the substrate surface to the V junction of the last two fully expanded leaves [3] while diameter (cm) was taken on the 3rd fully expanded leaf. After 4 weeks root length and the number of roots were recorded. The plants were carefully off rooted and substrate attached to roots carefully washed off under running water; thereafter the number of live roots was counted and the average length (cm) of the well-developed roots taken.

Statistical analysis

Data collected was based on mean values per treatment and these were analyzed using ANOVA through JMP software.

III. RESULTS AND DISCUSSION

Table I: Influence of Substrate on Formation of Number of Leaves

Substrate	After 7 Days		After 14 Days		After 21 Days		After 28 Days	
	FHIA 21	EBANGA	FHIA 21	EBANGA	FHIA 21	EBANGA	FHIA 21	EBANGA
biochar	3.6 ^a	4.3 ^a	3.6 ^a	4.3 ^a	3.6 ^a	4.3 ^a	3.6 ^a	5.0 ^a
sand	2.3 ^a	4.3 ^a	2.3 ^a	4.0 ^a	2.6 ^a	4.3 ^a	2.6 ^a	4.6 ^a
biochar:soil:sand	3.6 ^a	4.0 ^a	3.6 ^a	4.0 ^a	4.3 ^a	4.3 ^a	4.3 ^a	5.3 ^a
sand : soil	3.0 ^a	4.0 ^a	3.0 ^a	4.0 ^a	3.3 ^a	5.0 ^a	3.3 ^a	5.0 ^a
Soil	3.6 ^a	3.6 ^a	3.6 ^a	3.6 ^a	3.6 ^a	4.6 ^a	3.6 ^a	4.6 ^a

Levels not connected by same letter in same column are significantly different.

After 28 days of acclimatization, it was noticed that the number of leaves formed is not significantly affected by the substrate type in both plantain varieties (Table I). However, it is realized that, the treatment with sand alone

gave the least number of leaves (2.3) after 14 days for the FHIA 21 hybrid while the treatment soil alone gave the least number of leaves (3.6) after 14 days for the local variety Ebanga.

Table II: Influence of Substrate on Leaf Diameter (CM)

Substrate	After 7 Days		After 14 Days		After 21 Days		After 28 Days	
	FHIA 21	EBANGA	FHIA 21	EBANGA	FHIA 21	EBANGA	FHIA 21	EBANGA
biochar	1.03 ^a	1.26 ^a	1.03	1.3 ^a	1.13 ^{ab}	1.63 ^a	1.3 ^{ab}	1.63 ^{ab}
sand	1.16 ^a	1.23 ^a	0.7	1.4 ^a	0.83 ^b	1.53 ^a	0.9 ^b	1.53 ^b
biochar:soil:sand	1.3 ^a	2.03 ^a	1.46	2.66 ^a	1.63 ^a	2.83 ^a	1.8 ^a	2.93 ^a
sand : soil	1.0 ^a	1.3 ^a	1.0	1.33 ^a	1.2 ^{ab}	1.33 ^a	1.43 ^{ab}	1.53 ^b
Soil	1.23 ^a	1.26 ^a	1.23	1.26 ^a	1.3 ^{ab}	1.43 ^a	1.5 ^{ab}	1.5 ^b

Levels not connected by same letter in same column are significantly different.

With regards to influence of substrate on leaf diameter (table II), it is noticed that the treatment biochar: soil: sand, gave the largest diameter (1.8cm) after 28 days for the local variety Ebanga which is significantly different from the treatment of sand alone (0.9cm). Similar results

were recorded with the Ebanga variety with the treatment biochar: soil: sand recording 2.93cm in diameter which is significantly higher than that of soil alone (1.5cm), sand: soil (1.53cm) and sand alone (1.53cm).

Table III: Influence of Substrate on Stem Length (CM)

Substrate	After 7 Days		After 14 Days		After 21 Days		After 28 Days	
	FHIA 21	EBANGA	FHIA 21	EBANGA	FHIA 21	EBANGA	FHIA 21	EBANGA
biochar	2.46 ^a	4.23 ^a	3.3 ^a	4.43 ^a	3.73 ^a	4.7 ^a	4.43 ^a	6.73 ^{ab}
sand	2.96 ^a	4.36 ^a	2.9 ^a	4.46 ^a	2.4 ^a	4.6 ^a	2.5 ^a	5.6 ^{ab}
biochar:soil:sand	3.03 ^a	4.36 ^a	3.3 ^a	4.8 ^a	3.6 ^a	5.53 ^a	5.3 ^a	7.06 ^a
sand : soil	2.16 ^a	4.3 ^a	2.46 ^a	4.53 ^a	3.3 ^a	4.66 ^a	3.96 ^a	4.96 ^b
Soil	3.3 ^a	4.1 ^a	3.43 ^a	4.53 ^a	3.63 ^a	4.6 ^a	4.7 ^a	5.7 ^{ab}

Levels not connected by same letter in same column are significantly different.

Table III shows that there is a significant difference in stem length after 28 days in the Ebanga variety with regards to biochar: soil: sand in comparison to Sand: soil treatments. Biochar: soil: sand gave a stem length of 7.06cm significantly higher than the treatment sand: soil

(4.96cm).similarly the hybrid FHIA21, recorded the highest stem length of 5.3cm from the treatment biochar: soil: sand after 28 days though it was not significantly different from the other treatments.

Table IV: Influence of Substrate on Root Number and Length (CM)

SUBSTRATE	ROOT NUMBER		ROOT LENGTH	
	FHIA 21	EBANGA	FHIA 21	EBANGA
biochar	0.66 ^b	2.66 ^a	2.63 ^{ab}	6.7 ^a
sand	1.0 ^{ab}	2.0 ^a	1.93 ^b	4.26 ^a
biochar:soil:sand	2.0 ^a	1.66 ^a	3.86 ^{ab}	4.23 ^a
sand : soil	1.33 ^{ab}	1.66 ^a	2.56 ^{ab}	4.3 ^a
Soil	2.0 ^a	2.0 ^a	4.4 ^a	4.1 ^a

Levels not connected by same letter in same column are significantly different.

Results obtained from table IV show that there was no significant difference in root numbers from all treatments with the Ebanga variety. On the other hand, there was a significant difference in root number based on treatments with the hybrid (FHIA 21). Biochar alone produced the least significant number of roots (0.66) compared to biochar: sand: soil that produced 2 roots. In terms of root length of FHIA 21, soil alone treatment produced roots that were significantly longer (4.4cm) than sand alone treatment that had roots of 1.93cm long. The other treatments were not significantly different in terms of root length. With the Ebanga variety, biochar alone treatment

gave the longest root (6.7cm) though it was not significantly different from other treatments.

IV. CONCLUSION

From results obtained in terms of number of leaves, stem length, leaf diameter, root number and root length, the following conclusions can be drawn:

1. The plantlets of the local variety, Ebanga look more vigorous than the hybrid FHIA 21 during acclimatization in pots.

2. There is no significant difference in number of leaves formed for both varieties independent of substrate type.
3. The treatment biochar: soil: sand positively influenced the leaf diameter and stem length of both varieties of plantain than any other treatment.
4. While biochar positively influenced root development in Ebanga variety than any other treatment, soil alone positively influenced root development in the hybrid FHIA 21.
5. The treatment (substrate) biochar: soil: sand proves to be a better substrate for acclimatization of the two plantain varieties (Ebanga and FHIA21) in terms of leaf, stem and root development.

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