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Comparative study of shoot and root development in micropropagated and sucker-derived banana and plantain (*Musa* spp.) plants

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ABSTRACT

Objective: To compare performance of *in vitro* plants and sucker-derived banana seedlings in the field.
Methodology and results: Eight *Musa* genotypes were assessed during the first production cycle. Shoot and root traits were measured during the vegetative and the early reproductive phase. During the mid-vegetative phase, sucker-derived plants produced a larger root system, but leaf area and pseudostem size were similar for both types of propagules. No significant differences were observed at flower emergence between the propagule types for leaf area, corm fresh weight, root traits, height of the tallest sucker and days to flower emergence. Few significant correlations between the same plant growth traits of *in vitro* and sucker-derived plants were observed during the vegetative phase. However, significant correlations between both types of propagule were observed at flower emergence for leaf area, plant height, pseudostem circumference, corm weight and corm size, and root dry weight.
Conclusion and application of findings: Although *in vitro* plants are of higher phytosanitary status, they did not have superior growth than sucker-derived plants. However, the results demonstrate that one major advantage of *in vitro*-derived plants would be their more homogenous growth, which has implications for research and timing of field practices. The larger amount of roots at planting of *in vitro*-derived plants seems not to have a particular advantage during the first cycle and clearly propagules originating from different sources tend to behave similarly at flower emergence.

Key words: *in vitro*-derived plants, root growth, shoot growth, sucker-derived plants

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INTRODUCTION

Plantains and bananas are perennial monocotyledonous tropical herbs that belong to the Eumusa series of the genus *Musa* (Simmonds, 1966). The majority originated in south-east Asia from two wild diploid parent species *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Simmonds & Shepherd, 1955; Simmonds, 1976). Cultivated *Musa* are predominantly triploids (2n = 3x = 33), almost sterile and they develop their fruit parthenocarpically. World production in 2006 was estimated at 104 million tonnes annually, of which about 10 % entered the export trade (FAO, 2007). One third of the global volume is produced in Africa, where plantains and bananas are an important staple food for rural and urban consumers (Vuylsteke et al., 1993).

Plantains and bananas are grown as perennial crops whereby consecutive generations are produced from suckers (i.e. lateral shoots) developing on the main plant (Swennen & Ortiz, 1997). The first cycle after planting is called the plant crop, while the next cycle is called first ratoon and usually originates from the tallest sucker at harvest of the plant crop. The third cycle is the second ratoon crop, and so on. Sucker development consists of three distinct stages: peeper (small sucker appearing just above the ground and bearing scale leaves only), sword sucker (large sucker with lanceolate type leaves), and maiden sucker (large non-fruiting sucker with foliage leaves) (Simmonds, 1966; Swennen, 1984; Swennen & Ortiz, 1997). Sucker vigour shortens the cycle duration, enhances productivity and is required for perennial cultivation of plantains (Wilson *et al.*, 1985; Swennen *et al*., 1988).

Different types of propagules, including sword and maiden suckers, can be used to establish banana and plantain fields (Swennen, 1990). Suckers are most commonly used by farmers, and their preparation consists of removing the upper part of the pseudostem and paring the corm. This type of planting material, however, usually has a poor phytosanitary status, due to contamination with soil-borne pests, is bulky and has a low multiplication rate (Vuylsteke & Ortiz, 1996).

Micropropagation or *in vitro* techniques were established for fast multiplication of plantains and bananas (Vuylsteke, 1989 & 1998). Commercial production of micropropagated bananas is now common in many countries and it is estimated that 25 million plants are produced worldwide each year. In addition, *in vitro*-derived planting material is commonly used in horticultural crop experiments. The morphology of *in vitro* planting material is different from that of suckers since, unlike suckers, *in vitro* plantlets have almost no corm, but many roots and foliage leaves. Advantages of *in vitro* micropropagation include high rates of multiplication, the small amount of space required to multiply large numbers of plants, production of disease-free planting material and the homogeneity of their growth (Vuylsteke & Ortiz, 1996).

In general, micropropagated banana plants establish faster, grow more vigorously, are taller, have a shorter and more uniform production period, and produce higher yields than conventional propagules (Hwang et al., 1984; Israeli et al., 1988; Zamora et al., 1989; Robinson et al., 1993; Robinson, 1996). Furthermore, Drew and Smith (1990) have attributed the superior performance of micropropagated bananas to propagules that already possess an active root and shoot system at planting time.

Field studies carried out in the past have focussed mostly on the above ground plant parts. As the corm and roots are key components for the development of the pseudostem and leaves as well as the next cycle, a detailed study of the root system and corm development was carried out for both types of propagules during the vegetative and the reproductive stage in the first cycle.

MATERIALS AND METHODS

This study was carried out during 1996 and 1997 at the IITA High Rainfall station at Onne (4°42' N, 7°10' E, 5 m asl) in southeastern Nigeria. The soil is derived from coastal sediments and is a deep and freely drained Typic Paleudult/ Haplic Acrisol (Deckers *et al*., 1998; FAO/ISRIC and ISSS, 1998). This soil belongs to the coarse-loamy, siliceous isohyperthermic family, with a pH of < 4 in 1:1 H_2O, and contains 74 % sand, 8 % silt and 18 % clay in the top 15 cm layer. The average annual rainfall is 2,400 mm distributed monomodally from February until November. Details of the site have been described by Ortiz et al. (1997). *In vitro* and sucker-derived plants of eight genotypes, consisting of 5 landraces and 3 hybrids, were assessed in this study (Table 1). *In vitro* plants were obtained through meristem culture using the methods of Vuylsteke (1989, 1998). The plantlets were acclimatized for six weeks in a greenhouse nursery (Vuylsteke, 1998; Vuylsteke & Talengera, 1998), prior to transplantation to the field. Suckers had an average basal pseudostem girth of 32 cm and were thoroughly pared before planting.

Table 1: Name, genome, ploidy level and type of genotypes evaluated.

Name	Genome#	Ploidy level	Type
Yangambi km5	AAA	3	Dessert banana
Valery	AAA	3	Dessert banana
Obino l'Ewai	AAB	3	Plantain
Fougamou	ABB	3	Cooking banana
Cardaba	ABB	3	Cooking banana
TMPx 548-9	AAB x AA	4	Plantain hybrid
TMPx 1658-4	AAB x AA	4	Plantain hybrid
FHIA3	ABB x AA	4	Cooking banana hybrid

#: A: *Musa acuminata*; B: *Musa balbisiana*

The experimental layout was a split plot within a randomized complete block design with two replications of two plants per genotype. Main plot treatments consisted of different times of observation. For the *in vitro*-derived plants, data collection was carried out in the nursery at six weeks after planting (WAP), while field measurements were done during the vegetative stage at 12, 16 and 20 WAP, and at flower emergence. For the sucker-derived plants, measurements were carried out in the field at 6, 12, 16 and 20 WAP, and at flower emergence. Subplot treatments consisted of the genotypes.

The field had been under grass fallow for 8 years. Plant spacing in the field was 2 m x 2 m, except for plants evaluated at flower emergence, which were spaced 4 m x 4 m to avoid overlapping of the roots of neighbouring plants. The soil was only disturbed in the planting hole which measured 40 x 40 x 40 cm. The fields were irrigated during the dry season at a rate of 100 mm per month. Nematodes were controlled with the nematicide Nemacur (a.i. fenamiphos) at a rate of 15 g per plant (3 treatments $year^{-1}$). Plants were fertilized at 300 N and 450 K ($kg \cdot ha^{-1} \cdot year^{-1}$) split over six equal applications during the rainy season, while the fungicide Bayfidan (a.i. triadimenol) was applied three times per year at a rate of 3.6 ml per plant to control the leaf spot disease black sigatoka (*Mycosphaerella fijiensis* Morelet).

The plants were excavated to measure the underground parts. This process started by digging a small trench at about 2 meters from the pseudostem. As roots can reach up to 3 meters, a garden fork was used for the careful removal of the soil. A trench was dug a little deeper than the deepest roots, i.e. 50 cm deep, followed by a small cave under the roots. The upper soil layers were then gradually removed with a garden fork or by hand. First a 45° section of the root system was dug out. This facilitated the removal of the root system in the remaining 315° soil area. The excavation was carefully done to avoid breakage of the roots.

Roots were washed on a large sieve to facilitate the removal of all soil particles. The following characteristics were measured on each plant: leaf area (LA, cm^2) calculated according to Obiefuna and Ndubizu

(1979) and Blomme and Tenkouano (1998), number of leaves (NL), plant height (PH, cm), pseudostem circumference at soil level (PC, cm), corm fresh weight (CW, g), corm height (CH, cm), widest width of the corm (WW, cm), corm width half way between widest width and the apical meristem (CAW, cm), corm width half way between the widest width and the basal point of the corm (CBW, cm), number of suckers on the corm (NS) and height of the tallest sucker (HS, cm). Data collected on roots included root dry weight (DR, g), the number of adventitious or cord roots (NR), cord root length (LR, cm) and the average cord root diameter (AD, mm). The cord root length was measured using the line intersect method (Newman, 1966; Tennant, 1975), while the diameter of the root was measured with a Vernier calliper. Other root characteristics were total root dry weight of the mat (i.e. plant crop and suckers) (TD, g) and total cord root length (TL, cm) of the mat. Dry weight of plant tissue was obtained by drying for one week in a drying room (40°) and subsequently for two days in an oven (80°). In addition, days to flower emergence was recorded (DTFL, days).

Data analysis was carried out using the SAS statistical package (SAS, 1989). Total phenotypic variance was partitioned according to the following sources of variation: propagule type, genotype, genotype by propagule type interaction and replication. Correlation analysis was carried out between the same traits of both propagule types.

RESULTS AND DISCUSSION

Plant height was similar for both types of propagules during the mid-vegetative stage with no significant differences detected at 16 and 20 WAP (Table 2). However, at flower emergence *in vitro*-derived plants were significantly taller (12%) than sucker-derived plants (Table 2). There was also a significant effect of propagule type on plant basal circumference at flower emergence (Table 2) although the corm weight of the *in vitro*-derived plants remained lower than sucker-derived plants (Table 2). The superior height of the *in vitro*-derived plants was associated with a thicker pseudostem. These observations confirms that micropropagated plants grow more vigorously and are taller than those derived from conventional propagules (Drew & Smith, 1990; Robinson et al., 1993), also supporting earlier observations that a high positive correlation exists between plant height and circumference in banana (Swennen & De Langhe, 1985).

Drew and Smith (1990) have attributed the superior performance of micropropagated bananas to the already existing active root and shoot system at planting which is free of most diseases and pests. In addition, for a dessert banana (*Musa* AAA, Cavendish subgroup, Eckstein and Robinson (1995) reported that *in vitro*-derived plants produced 77 % more root dry matter than conventional suckers at 20 WAP. They attributed the rooting vigour of *in vitro*-derived plants to the physiological juvenility of the tissue. In contrast, in this study, involving eight varieties, 20 weeks old sucker-derived plants produced a more robust root system in terms of root dry weight and number of cord roots compared to 20 weeks old *in vitro*-derived plants (Table 2). This is apparently related to the bigger size of the corm in which root formation takes place (Table 2). At flower emergence, corm fresh weight of both propagules was similar, resulting in mostly non-significant differences between identical root traits of both propagule types (Table 2).

Sucker development was retarded on the sucker-derived plants during the vegetative stage as, for example, only small buds were observed on the corms of 20 weeks old plants. This supports observations made by Robinson (1996) who reported faster sucker emergence rate on *in vitro* produced dessert bananas compared with conventional planting material. He postulated that *in vitro* plants have a denser and more vigorous root system, which produces enough phytohormones (cytokinins) to overcome apical dominance and stimulate sucker growth at an earlier stage. However, in this study we observed that sucker-derived plants have a denser root system, which did not result in better sucker development. The tallest plant from the sucker-derived propagules, once established, grew vigorously and no significant differences were detected for sucker height between the two types of propagule at flower emergence (Table 2).

Vuylsteke and Ortiz (1996) reported that *in-vitro*-derived plants flowered earlier than sucker-derived plants for the False Horn plantain (*Musa* spp., AAB group) 'Agbagba'. In this study, no significant differences were detected for days to flower emergence between both propagule types. However, *in vitro*-derived plants

flowered slightly earlier (Table 2). Few significant correlations between the same *in vitro* and sucker-derived plant growth traits were observed during the vegetative growth (Table 3). However, significant correlations were found at flower emergence between both types of propagules, for leaf area, plant height and circumference, corm weight, corm widest width, corm width in between the apical meristem and the widest width, and root dry weight (Table 3). This would indicate that plants originating from different planting material tend to behave dissimilarly during the vegetative stage but similarly at flower emergence.

A higher variability in growth characteristics of sucker-derived plants compared to *in vitro*-derived plants was noted during the vegetative stage (Table 4). However, at flower emergence variability in growth characteristics was quite similar for both types of planting material. The difference in variability during the vegetative stage is attributed to a homogenous growth of *in vitro*-derived plantlets in the greenhouse nursery and the selection of the most similar plants for field planting. In addition, homogenous growth of sucker-derived planting material is uncommon because suckers of similar size could have originated from different places on the plant crop corm (De Langhe, 1961), reflecting differences in ontogeny and physiological status. Lastly, when planting a sucker, a new cycle can start with either the growth of the central growing point or an axillary bud.

Table 2: Overall mean values and t-test for shoot and root system traits of *in vitro*-derived (IV) and sucker-derived (SD) plants at 6, 12, 16 and 20 weeks after planting (WAP) and at flower emergence (FL).

	6 WAP			12 WAP			16 WAP			20 WAP			FL		
Trait#	IV	SD	Sign. Levl	IV	SD	Sign. Level	IV	SD	Sign. Level	IV	SD	Sign. Level	IV	SD	Sign Level
LA	979	1,103		2,799	6,391	***	9,825	8,470		22,120	22,680		89,664	93,667	
NL	7	2	***	7	6		10	7	***	11	10	*	11	13	**
PH	24	23		26	34	*	50	43		80	79		246	220	**
PC	6	11	***	10	16	***	20	19		32	29		65	58	**
CW	na	1,105	na	34	1,043	***	183	1,136	***	507	1,932	***	5,449	5,980	
CH	na	11	na	3	13	***	6	13	***	8	16	***	21	29	***
WW	na	13	na	4	12	***	7	12	***	10	14	***	20	18	**
NS	na	0	na	0.3	0	na	1.1	0	**	3.0	0	***	10	12	*
HS	na	0	na	0.6	0	na	4.0	0	*	10	0	***	119	122	
DR	1	2	***	7	14	**	19	22		54	84	*	322	310	
NR	12	14		27	50	***	50	56		71	98	**	170	176	
LR	276	193	**	542	763		1,143	877	*	2,389	2,950		6,486	7,767	
AD	2.9	3.6	***	3.6	4.0	**	4.5	3.9	**	5.4	4.9	**	5.6	5.2	*
TD	1	2	***	7	14	**	19	22		58	84	*	509	485	
TL	276	193	**	541	763		1,168	877	*	2,607	2,950		11,499	12,591	
DTFL	na	na	na	na	na	na	na	na	na	na	na	na	325	338	

#: LA: leaf area (cm^2), NL: number of leaves, PH: plant height (cm), PC: plant circumference (cm), CW: corm fresh weight (g), CH: corm height (cm), WW: corm widest width (cm), NS: number of suckers, HS: height of the tallest sucker (cm), DR: root dry weight (g), NR: number of adventitious roots or cord roots, LR: cord root length (cm), AD: average basal cord root diameter (mm), TD: total root dry weight of the mat (i.e. plant crop and suckers) (g), TL: total cord root length of the mat (cm), DTFL: days to flowering; *, **, *** Significant at P<0.05, 0.01 and 0.001, respectively.; na: not applicable.

During the mid-vegetative phase, sucker-derived plants produced a larger root system, possibly due to the larger corm, which bears the root initiation zone. However, leaf area and pseudostem size were similar between both types of propagules. No significant differences were observed at flower emergence between the propagule

types for leaf area, corm fresh weight, root traits, height of the tallest sucker and days to flower emergence. Hence the larger amount of roots at planting of *in vitro*-derived plants seems not to have a particular advantage. The higher phytosanitary status of *in vitro* plants did not result in better root growth than for sucker-derived plants. However, since it takes time to build up inoculum (e.g. of nematodes introduced in the corms of sucker planting material) the expected enhanced growth of *in vitro*-derived plants should become clearer during the succeeding ratoon crop.

Table 3: Correlation coefficients between the same plant growth traits of *in vitro* and sucker-derived plants at 6, 12, 16 and 20 weeks after planting (WAP) and at flower emergence (FL).

	WAP				
Trait #	6	12	16	20	FL
LA	0.26	0.12	0.83*	0.49	0.82*
NL	0.66	0.62	0.12	0.73	0.35
PH	0.51	-0.18	0.94**	0.59	0.74*
PC	0.65	0.44	0.77*	0.53	0.92**
CW	na	-0.08	0.48	-0.14	0.77*
CH	na	-0.24	0.60	-0.65	-0.13
WW	na	0.49	-0.30	0.33	0.78*
CAW	na	0.40	0.59	0.08	0.87**
CBW	na	0.33	-0.07	0.10	0.43
NS	na	Na	na	na	0.37
HS	na	Na	na	na	0.41
DR	0.08	0.06	0.74	0.47	0.84**
NR	0.35	-0.06	0.71	0.12	-0.33
LR	0.21	0.37	0.69	0.49	0.20
AD	0.02	0.48	0.65	0.31	-0.09
TD	0.08	0.06	0.66	0.38	0.37
TL	0.21	0.35	0.59	0.34	-0.003
DTFL	na	na	na	na	0.06

#: LA: leaf area (cm^2), NL: number of leaves, PH: plant height (cm), PC: plant circumference (cm), CW: corm fresh weight (g), CH: corm height (cm), WW: corm widest width (cm), CAW: corm apical width (width half way between widest width and the apical meristem) (cm), CBW: corm basal width (width half way between the widest width and the basal point of the corm) (cm), NS: number of suckers, HS: height of the tallest sucker (cm), DR: root dry weight (g), NR: number of adventitious roots or cord roots, LR: cord root length (cm), AD: average basal cord root diameter (mm), TD: total root dry weight of the mat (i.e. plant crop and suckers) (g), TL: total cord root length of the mat (cm), DTFL: days to flower emergence; *, **, *** Significant at P<0.05, 0.01 and 0.001, respectively.; na: not applicable.

Table 4: Coefficient of variation (%) for shoot and root system traits for *in vitro* (IV) and sucker-derived (SD) plants at 6, 12, 16 and 20 weeks after planting (WAP) and at flower emergence (FL).

	Weeks After Planting									
	6		12		16		20		FL	
Trait#	IV	SD	IV	SD	IV	SD	IV	SD	IV	SD
LA	44	89	35	72	31	74	38	67	38	34
NL	16	69	21	26	13	32	17	27	28	18
PH	18	52	17	52	24	47	25	39	12	18
PC	20	39	19	34	16	34	21	32	13	16
CW	na	64	56	51	32	48	47	57	26	45
CH	na	27	13	31	14	21	12	21	13	17
WW	na	23	24	27	12	23	18	25	14	19
CAW	na	27	22	34	13	30	17	23	22	23
CBW	na	23	22	28	11	23	19	26	19	23
NS	na	na	274	na	131	na	78	na	36	26
HS	na	na	287	na	177	na	121	na	56	47
DR	65	75	50	94	38	82	54	68	31	34
NR	25	55	22	58	20	46	30	47	26	37
LR	31	65	27	98	29	59	47	58	38	40
AD	17	21	11	15	11	18	11	11	11	9
TD	65	75	50	94	38	82	50	68	31	35
TL	31	65	27	98	29	59	43	58	50	44
DTFL	na	na	na	na	na	na	na	na	16	18

#: LA: leaf area (cm^2), NL: number of leaves, PH: plant height (cm), PC: plant circumference (cm), CW: corm fresh weight (g), CH: corm height (cm), WW: corm widest width (cm), CAW: corm apical width (width half way between widest width and the apical meristem) (cm), CBW: corm basal width (width half way between the widest width and the basal point of the corm) (cm), NS: number of suckers, HS: height of the tallest sucker (cm), DR: root dry weight (g), NR: number of adventitious roots or cord roots, LR: cord root length (cm), AD: average basal cord root diameter (mm), TD: total root dry weight of the mat (i.e. plant crop and suckers) (g), TL: total cord root length of the mat (cm), DTFL: days to flower emergence; na: not applicable

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