Effect of Different Substrate Types on the Growth of Micropropagated Banana Transplants

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Abstract: An experiment was conducted to determine the effect of different substrate types on the growth of banana (*Musa* spp.) transplants derived from *in vitro* propagation. Fully acclimatized plantlets were grown in 1.4L pots filled with (1) sawdust (SD) + vermiculite (v), (2) SD + Cocofiber (Coco), (3) SD + sand (S), (4) S + V, (5) S + Coco, (6) S + rice hulls (RH), (7) Coco + V, (8) 100% Coco, (9) 100% V, (10) 100% SD, and (11) 100% S. Potted plants were arranged on the glasshouse bench in RCB design. Transplant growth (Shoot height, leaf number, corm diameter, leaf area, shoot fresh and dry weights, root fresh and dry weights) were measured, as well as leaf chlorophyll content. Substrate type significantly affected banana transplant growth. The best overall transport growth and chlorophyll content were found with 1: 1 Coco + V, 100% V, or 100% Coco. Transplant grown in 100% sand showed the least performance, but their growth improved in S + V, S + Coco or S + RH substrates.

Keywords: Banana Transplants, vermiculite, Cocofiber.

INTRODUCTION

Micropropagated banana (*Musu* spp.) plants are widely accepted by growers as valuable, pathogen-free stock materials (Buah *et al.,* 1998). Several plant tissue culture labs are engaged in clonal propagation of banana in Egypt, producing at least 1.5 million vitroplants per year. During 2001-2008, more than 100,000 banana plantlets were micro propagated in our plant tissue culture facilities at Suez Canal University and became available to the growers in the region. After a period of *ex vitro* acclimatization, the weaned plants should be raised in pots in the greenhouse to obtain plants large enough to be grown in the field. As in any potted plant, the quality of transplant depends mainly on the substrate utilized. In this regards, Deoe Silva *et al.* (1998) stated that, for the best growth of tissue cultured banana plantlets, 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.

Traditionally, fully acclimatized banana plantlets, are raised in sand plus peatmoss (Murali and Duncan, 1995). However, sand should not be considered as a substrate component in a sustainable system (Morales-Payan and Stall, 2003) and it has the disadvantage of being heavy and difficult to transport when moving transplants to the field (Neal and Wagner, 1983). In addition, peatmoss is not locally available and has shown wide swings in price worldwide (Meerow, 1994). Several locally available organic materials such as sawdust was shown to be acceptable medium, with performance increased by mixing with coco-peat (Boven and Chow, 1984). In Egypt, rice processing (de-hulling) results in large amount of rice hulls (20% of the kernel). However, use of such material as alternative substrate to peatmoss is not well-documented. It was reported by Lee (2007) that a mix of peat, rice hull and sand increased growth rate of banana plantlet, compared to sand only, while sawdust could support their growth for two months. However, pathogen re-infection is possible in such medium. Deoe Silva *et al.* (1998) reported the use of vermiculite mixed with peatmoss and perlite for raising banana plants. Our previous results with transplant production system in strawberry (Mohamed, 1999) and cucurbits (Mohamed *et al.,* 2001) indicated the beneficial effects of vermiculite-amended substrates for better quality transplants. On the other hand, coconut fiber was extensively used as alternative to peatmoss in ornamentals (Linderman and Davis, 2003; Meerow, 1994), papay transplants (Morales-Payan and Stall, 2003) and tomato (Shinohara, *et al.,* 1997). However, use of cocofiber in transplant production of banana is not yet reported.

The objective of this study was to evaluate the growth of micro propagated banana plantlets as affected by selected substrate types under glasshouse conditions.

MATERIALS AND METHODS

This study was conducted during 2008-2009 in the Glasshouse of the Plant Tissue Culture Facilities, College of Agric., Suez Canal University. Banana plantlets cv. Grand Nain were micropropagated in the tissue culture lab during Feb-Sept., 2008. Rooted microplants were acclimatized from mid Sept. to Oct., 2008 under mist in 5 cm pots amended with sand plus peatmoss (1: 1 v/v) in the greenhouse. Fully acclimatized plantlets (approx. 8 cm with 3 leaves) were grown from Nov. 1st 2008 to the end of Apr., 2009 on 1.4L plastic pots filled with the different substrates under test in a single or mixture forms as follow:

(1) Sawdust (SD) + Vermiculite (v); (2) SD + cocofiber (Coco); (3) SD + Sand (5), (4) S + V, (5) S + Coco, (6) S + rice hull (RH), (7) Coco + V, (8) Coco, (9) V, (10) SD, (11) S.

Substrate components were mixed in a 1: 1 volume to volume basis. The coconut fiber used for this study originated in Sri Lanka and imported as compressed bricks 20 x 10 x 5 cm. Sawdust and rice hulls were obtained from local wood factory and rice processing farm, respectively. Grad 2 vermiculite was kindly provided by Green High for Agric. Services, Inc., Ismailia. All substrates we rehydrated before incorporating into media.

Pots were arranged on the glasshouse benches in randomized completed block design and replicated six times. The experimental unit was one pot (one plant). Spacing was 50cm between pots. A drip tube system was installed that allowed uniform application of water and nutrients from a commercial 19-19-19 N-P-K fertilizer plus micronutrients (1.0 g/L solution) applied daily as 100ml/pot.

Physical and chemical properties of each substrate were measured before transplanting. Bulk density (BD) and water holding capacity (WHC) were determined in 3 samples using the protocol from Ingram *et al.* (1990), while pH and EC were determined in a 2:1 saturated extract (Bunt, 1988) using a laboratory pH and EC meters.

Data on transplant growth were taken at two growth stages, after four and five months from transplanting in pots. Growth variables measured included: plant height (from the soil surface to the growing apex), number of leaves/plant, leaf length (L) and width (W) to calculate leaf area (LA) according to the equation: LA = W x L x 0.8 for banana plant (Obiefuna and Ndubizo, 1979), stem base (corm) diameter 2 cm above soil surface, shoot and root fresh weights, shoot and root dry weights following drying at 70°C for 72 hrs. in an oven. All measurements were conducted in three plant samples.

Leaf chlorophyll contents (chl. a, b and total chl.) were estimated in leaf discs homogenized in 80% acetone using spectrophotometer according to Sadasivam and Amanickam (1991).

The obtained data were subjected to analysis of variance (ANOVA) followed by analysis of means, investigating significant differences between substrates with LSD procedure at 5% levels according to Steel and Torrie (1980).

RESULTS

Effect of substrates on banana transplant growth after 4 months.

Banana (cv. Grand Nain) transplants grown in cocofiber plus vermiculite (Coco + V, 1: 1 v/v) were superior in all growth variables measured, including plant height, leaf number/plant, corm diameter, leaf area, shoot and root fresh weights (FW) and root dry weight (Table 2; Fig. 1 and 2). Transplants grown in 100% vermiculite (V) or 100% Coco were not significantly different in their leaf number, corm diameter and shoot FW than those in (Coco + V). However, transplants in 100% vermiculite outperformed those grown in 100% Coco in leaf area, root FW and shoot dry weight (DW).

Banana grown in sand + vermiculite (S + V) were similar to those in (Coco) or (V) in their length, and similar to those in sawdust (SD) in leaf number and corm diameter, showing the fourth best performance after Coco + V, V, and Coco. At this stage, plants in 100% SD significantly showed better growth in terms of plant height, leaf number, corm diameter, leaf area and shoot FW than those in a mixtures of SD + V, SD + Coco or SD + S, as shown in Table (1). On the other hand, the least transplant growth for most growth characters was noticed in plants grown in 100% sand. However, when sand was mixed with vermiculite (S + V), Coco fiber (S + Coco), or rice hull (S + RH), growth characters improved, outperforming those in sawdust – containing substrates.

In this experiment, rice hull could not be assessed as potting medium for banana, being only utilized in one treatment (S + RH). However, compared to S + SD, plants in S + RH had significantly more height, leaf number, leaf area, shoot FW and root DW, indicating better performance of plant in RH over those in SD.

Effect of substrates on banana growth after 5 months.

Except root DW, all growth characters of banana transplants increased after 5 months of growth during April as compared to growth at the 4th months (Table 2 vs. 3). As an average over all tested substrates, the major increases were in shoot FW (97%), leaf area (56.8%) and plant height (43.6%). Corm diameter and root FW increased by 24%. However, 50% reduction in root DW occurred during this period, perhaps due to exhausted biomass diversion to support top growth, especially under root resection in such small pots.

Similar to what was previously noticed after four months, transplant grown in (Coco + V) or 100% vermiculite, followed by those in 100% Coco, continued to perform better than those in most other substrates, especially in plant height, leaf number, leaf area, shoot and root FW (Table 3). At this stage, the quality of banana transplants produced in Coco + V, vermiculite or Coco were ideal for field planting, in terms of their height (30-40cm), leaf number (11-12 leaves/plant), leaf area, stem diameter (4-5cm) and rooting capacity (83-95g FW). Transplants grown in sand mixtures (S + V, S + Coco, and S + RH) had significantly better growth than those in sawdust mixtures (SD +V, SD +Coco, SD +S) especially for plant height, leaf area, corm diameter, and shoot FW (Table 3), indicating the same trend as in stage one (Table 2). However, utilization of sawdust as the main substrate (100% SD) outperformed sand in all growth characters.

Effect of substrates on leaf chlorophyll contents.

Significant differences in chlorophyll (chl.) content were detected in leaves of banana transplant as affected by different substrates (Table 4). Leaves of plants grown on Coco + V or Coco had the highest chl. a, b and total chl. followed by those grown in 100% vermiculite, S + V or SD + V. These results indicate that substrates amended with (V) or (Coco) were superior in their chlorophyll. However, the least chl. a, b, and total chl. were detected in leaves of plants grown in S + SD and S + Coco.

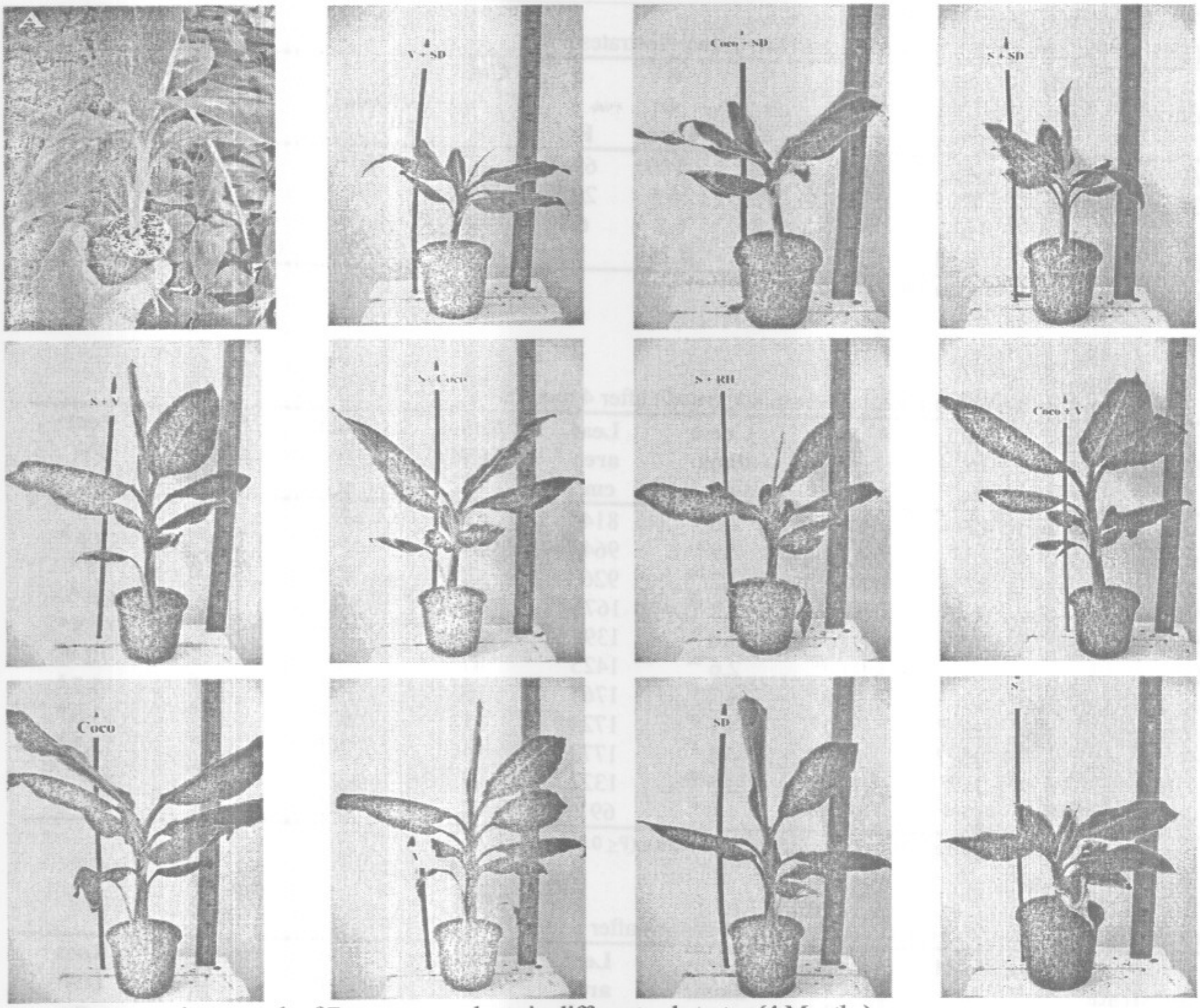

Fig. (1): Vegetative growth of Banana transplants in different substrates (4 Months)
A= Acclimatizes plantlet before transplanting.

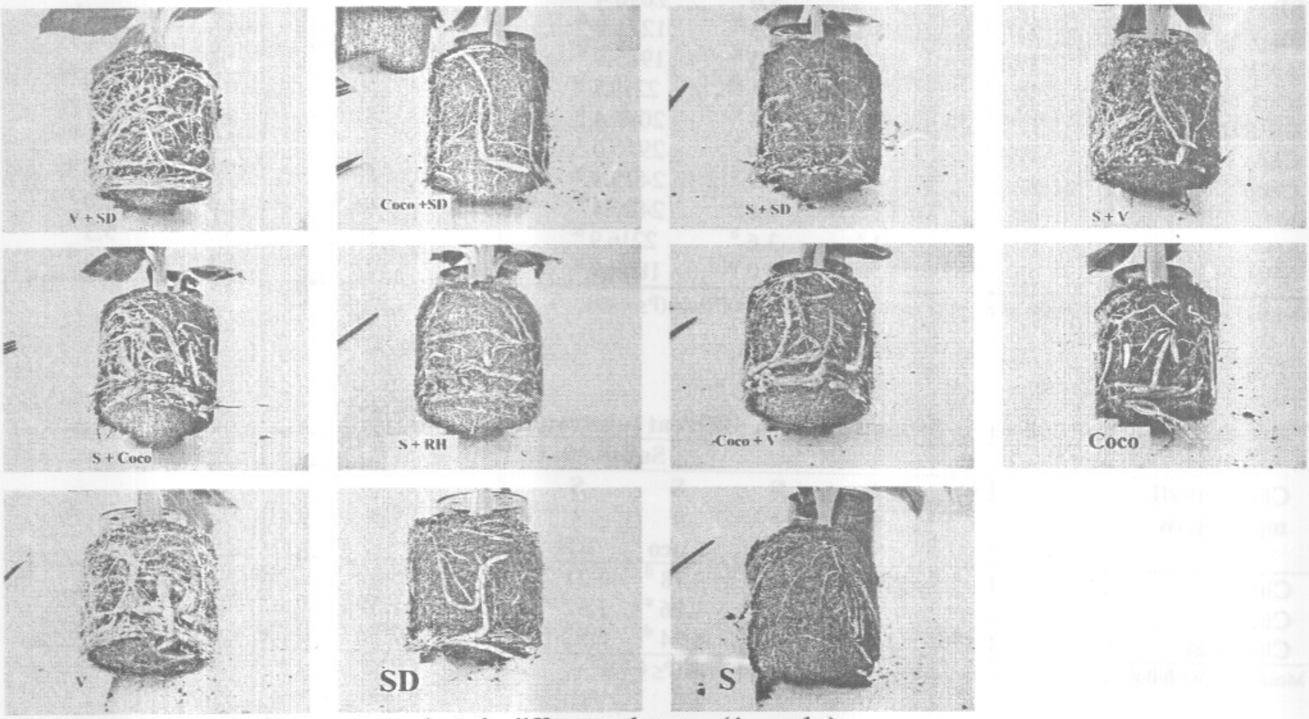

Fig. (2): Root growth of banana transplants in different substrates (4 months).

Table (1): Physical and chemical properties of the tested substrates

Character	SD + V	SD + Coco	SD + S	S + V	S + Coco	S + RH	Coco + V	Coco	V	SD	S
BD g/L	183	402	995	878	1100	69.0	347	530	176	234	1734
WHC %	35	32.5	30	27.5	26.5	29.5	45	37	46	33	20
pH	6.4	6.2	6.9	6.8	6.8	6.3	6.4	6.1	6.9	5.8	7.3
EC $dS.m^{-1}$	0.25	0.64	0.46	0.25	0.75	1.2	0.48	0.79	0.29	0.52	0.78

BD = bulk density, WHC = water-holding capacity

Table (2): Effect of substrates on banana transplant growth after 4 months

Substrate	Plant ht. cm	Leaf No.	Corm diam. cm	Leaf area cm^2	Shoot FW g	Root FW g	Shoot DW g	Root DW g
SD + V	17^{c}	8.0^{b}	2.8^{b}	814^{f}	61^{d}	52^{b}	15^{bc}	14.6^{ab}
SD + Coco	18^{c}	8.0^{b}	2.9^{b}	964^{e}	76^{cd}	57^{b}	17^{bc}	13.6^{b}
SD + S	17^{c}	8.0^{b}	2.7^{bc}	926^{e}	57^{d}	31^{d}	15^{bc}	7.4^{d}
S + V	23^{ab}	9.0^{ab}	3.2^{ab}	1676^{b}	123^{b}	40^{c}	19^{b}	8.6^{d}
S + Coco	21^{b}	8.0^{b}	3.0^{b}	1392^{c}	105^{bc}	54^{b}	18^{b}	11.5^{c}
S + RH	20^{b}	9.0^{ab}	2.6^{bc}	1425^{c}	87^{c}	41^{c}	15^{bc}	9.1^{cd}
Coco + V	30^{a}	10.0^{a}	4.2^{a}	1763^{a}	163^{a}	76^{a}	23^{ab}	15.7^{a}
Coco	23^{ab}	10.0^{a}	4.1^{a}	1723^{ab}	158^{a}	68^{ab}	23^{ab}	13.5^{b}
V	25^{ab}	10.0^{a}	4.1^{a}	1772^{a}	146^{a}	73^{a}	29^{a}	13.3^{b}
SD	21^{b}	9.0^{ab}	3.5^{ab}	1322^{d}	97^{bc}	44^{c}	15^{bc}	8.2^{d}
S	12^{d}	8.0^{b}	1.9^{c}	691^{g}	42^{e}	12^{e}	11^{c}	5.7^{e}

Means in column followed by the same letter are not significantly different ($P \leq 0.05$).

Table (3): Effect of substrates on banana transplant growth after 5 months

Substrate	Plant ht. cm	Leaf No.	Corm diam. cm	Leaf area cm^2	Shoot FW g	Root FW g	Shoot DW g	Root DW g
SD + V	27.0^{b}	10^{b}	3.9^{ab}	1146.7^{d}	142^{c}	58^{bc}	29^{b}	6.6^{b}
SD + Coco	25.0^{bc}	9^{bc}	3.0^{bc}	2096.4^{c}	157^{c}	58^{bc}	26.6^{bc}	5.3^{bc}
SD + S	24.2^{bc}	10^{b}	3.5^{b}	1221.0^{d}	122^{d}	53^{c}	22.7^{c}	5.0^{bc}
S + V	31.3^{ab}	11^{ab}	4.6^{a}	1989.0^{c}	211^{bc}	67^{b}	28.5^{b}	4.3^{c}
S + Coco	30.0^{ab}	11^{ab}	4.1^{ab}	2279.7^{bc}	204^{bc}	59^{bc}	27.1^{b}	6.0^{bc}
S + RH	27.0^{b}	11^{ab}	3.4^{b}	2096.4^{c}	139^{cd}	42^{c}	26.7^{bc}	4.3^{c}
Coco + V	37.4^{a}	12^{a}	4.9^{a}	2917.0^{a}	322^{a}	85^{ab}	38.5^{a}	7.3^{b}
Coco	32.2^{ab}	11^{ab}	4.7^{a}	2425.8^{b}	287^{b}	83^{ab}	37.0^{ab}	7.0^{b}
V	40.3^{a}	12^{a}	4.8^{a}	2426.4^{b}	344^{a}	95^{a}	42.0^{a}	9.0^{a}
SD	27.2^{b}	10^{b}	3.6^{b}	2216.9^{bc}	138^{cd}	59^{bc}	25.7^{bc}	5.3^{bc}
S	25.5^{bc}	9^{bc}	3.0^{bc}	1869.9^{c}	120^{d}	30^{cd}	21.5^{c}	2.0^{d}

Means in column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 4: Chlorophyll content in banana leaves in different substrates

Chlorophyll mg/100g.fw	Substrate Type										
	SD + V	SD + Coco	SD + S	S + V	S + Coco	S + RH	Coco + V	Coco	V	SD	S
Chl. a	7.28^{b}	9.1^{a}	2.13^{e}	7.38^{b}	3.18^{d}	4.21^{cd}	9.08^{a}	5.87^{c}	7.06^{b}	5.24^{c}	3.71^{d}
Chl. b	2.29^{ab}	2.79^{a}	0.77^{e}	2.32^{ab}	1.06^{c}	1.32^{bc}	2.66^{a}	1.89^{b}	2.02^{ab}	1.53^{bc}	1.17^{c}
Chl. total	9.57^{b}	11.87^{a}	2.9^{e}	9.7^{b}	4.24^{d}	5.53^{cd}	11.73^{a}	7.76^{c}	9.08^{b}	6.77^{c}	4.89^{d}

Means in rows followed by the same letter are not significantly different ($P \leq 0.05$).

DISCUSSION

The present study demonstrated the positive effects of vermiculite and cocofiber alone or in combination on all the studied growth characters of banana transplants under glasshouse condition. Their effects were consistent over two tested growth stages until termination of the study. The obtained differences in substrate effects may be due to differences in their physical and chemical characters (Table 1). Vermiculite and cocofiber or their mixture have less bulk density (BD) and more water holding capacity (% WHC) than most other media, especially than sand-amended media. Results of Meerow (1994) indicated that higher substrate WHC was consistently associated with better growth in potted plants. The less BD indicated less substrate compactness and more pore spaces which allowed better root aeration, nutrients and water uptake for subsequent growth enhancement. The beneficial effect of cocofibres was previously noted by other workers (Meerow, 1994; Morales-Payan, and Stall, 2003, and Shinohara, 1997). Vermiculite (inorganic substrate) was shown to have low BD and neutral pH, with high cation exchange capacity (CEC), and thus can hold nutrients in reserve to release them later. It also contains enough Mg (9-12%) and K (5-8%) to support most plants (Hartmann *et al.,* 1990). These properties were reflected in the mixture of coco + vermiculite, the best performing substrate in the present work. Vermiculite-contained media were also effective in strawberry (Mohamed, 1999) and cucurbit (Mohamed *et al.*, 2001) transplants as well as on foliage plants (Poole *et al.*, 1981; Chase and Conover, 1987).

On the other hand, the reduced growth of banana plants grown in sand medium may be due to its low WHC and higher BD than all other tested media (Table 1). Although during this study, irrigation appeared sufficient for growth on sand, more nutrients leaching was likely to have occurred in sand-amended substrates. In addition, sand contains no mineral nutrients, with no buffering capacity or CEC and high pH, which results in deficiency in Fe and B (Hartmann *et al.*, 1990).

Results with sawdust were not as good as we expected, perhaps due to using SD as uncomposted raw material, which may require longer period and additional N application to be composted before use.

In general, banana plants grown in substrate amended with vermiculite or cocofiber had enhanced chlorophyll contents, while in sand or sand mixes, accumulation of chlorophyll in banana leaves was less. Nutrient deficiency, specially N, negatively affect chl. content in banana (Tiwary *et al.*, 1999). Therefore, it is expected that nutrients present in media with vermiculite (Mg) and cocofiber (N), along with their physical properties will contribute to more chl. accumulation in their growing plants. Reduction in chl. was detected in leaves of banana plants under water stress (Thomas and Turner, 2001) which may also explain the less detected chl. in sand – based media characterized by their low WHC. In our result, the issue between Chl. a to b was in the range of 3.0 to 3.4, typical to the finding of Hooks *et al.* (2008) in banana.

In conclusion, economic and practical consideration may help banana nurserymen to determine which substrate is more suitable for their condition. In this case, cocofibres and vermiculite are less costly and commercially available, even in countries that do not produce them. Further studies are needed to re-evaluate composted sawdust and rice hulls, with the possibility for testing other locally available organic residues as substrates.

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تأثير أنواع مختلفة من البيئات على نمو شتلات الموز الناتجة من زراعة الأنسجة

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أجريت تجربة بالصوبة الزجاجية لدراسة تأثير أنواع مختلفة من البيئات على نمو شتلات الموز الناتجة من الإكثار بزراعة الأنسجة حيث زرعت الشتلات تامة الأقلمة فى قصارى سعة ١,٤ لتر فى البيئات: (١) نشارة خشب + فرمكيوليت، (٢) نشارة خشب + ألياف جوز الهند، (٣) نشارة خشب + رمل، (٤) رمل + فرمكيوليت، (٥) رمل + ألياف جوز الهند، (٦) رمل + سرس الأرز، (٧) ألياف جوز الهند + فرمكيوليت، (٨) ١٠٠% ألياف جوز الهند، (٩) ١٠٠% فرمكيوليت، (١٠) ١٠٠% نشارة خشب، (١١) ١٠٠% رمل. وزعت القصارى على بنشات الصوبة فى تصميم قطاعات كاملة العشوائية وتم قياس النمو من حيث طول النبات، عدد الأوراق، قطر الكورمة، المساحة الورقية، الوزن الطازج والجاف لكل من المجموع الخضرى والجذرى وكذلك محتوى الأوراق من الكلوروفيل. أوضحت النتائج وجود تأثير معنوى للبيئات المستخدمة على نمو شتلات الموز حيث أعطت النباتات النامية فى بيئات ألياف جوز الهند المخلوط بالفرمكيوليت بنسبة ١:١ وكذلك بيئة الفرميكوليت أو ألياف جوز الهند المنفردة (١٠٠%) أعلى نمو فى معظم القياسات وكذلك أعلى محتوى من الكلوروفيل. وكان اقل نمو للشتلات فى بيئة الرمل المنفردة (١٠٠%) بينما حفز مخلوط الرمل مع الفرميكلوليت أو ألياف جوز الهند أو سرس الأرز من نمو الشتلات مقارنة بالرمل المنفرد.