

Micropropagation and postflask management of sweet potato using locally available materials as substrates for hardening

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Abstract

The suitability of using locally available substrates (river sand, saw dust and rice mill waste) as an alternative to conventional substrate (jiffy peat) for hardening was investigated. Ninety seven percent (97%) of shoots were induced from stem cuttings of two months old mother plants of twenty sweet potato cultivars (*cv. A5* and *cv. 50*) grown on (Murashige and Skoog (MS)) medium after surface sterilization in 70% ethanol and 5% bleach. Axillary shoot-bud explants were excised from the proliferating shoots of the cultures and re-cultured in the same medium for multiplication. The *in vitro* developed plants were subculture two more times to produce enough plants in culture. Acclimatization of the *in vitro*-produced propagules was conducted on substrates containing a mixture of 2:1 river sand and saw dust, saw dust and rice mill waste, river sand and rice mill waste, river sand, rice mill waste, saw dust and jiffy peat for 4 weeks. Competitive results (17.6±6.4 cm plant height, 6.8±1.3 leaves and 7.2±1.4 nodes) was achieved in four weeks compared to 20.3±5.2 cm plant height, 9.2±3.1 leaves and 8.6±1.8 nodes) were obtained when the plants were grown on river sand/saw dust substrate and on jiffy peat respectively. The survival rate of the plants was 100% irrespective of the substrates or substrate combinations used. In summary the best locally available substrate for the acclimatization of the *in vitro*-grown sweet potato plants is the combination of river sand and saw dust. However, the superiority of jiffy peat substrate over the 2:1 combination of river sand and saw dust was outstanding but not significant.

Keywords: Acclimatization; plantlets; jiffy peat; local substrates, sweet potato.

Abbreviation: RMW-Rice mill waste; SD-Saw dust; RS-River sand; MS-Murashige and Skoog.

Introduction

Sweet potato (*Ipomoea batatas*) is a high yielding crop and ranked second in the world after potatoes (Deng et al., 2012). It is a major food, feed and industrial raw material in China whose total output in the world is estimated to be more than 80% (Islam 2006; Farmer et al., 2007; Liu et al., 2011). Sweet potato is characterized by its fast growth, active root formation and development which confers greater survival rate of the seedlings and the expected output (Alam et al., 2010). Consequently its formation of a large root system in the early stage promotes robust growth of the seedlings in early development, flourishing stems and leaves that are conducive to overall yield (Deng et al., 2012). It is an important food crop after cassava, rice and yam in the tropics. It is cultivated mainly for its storage roots and is probably the food crop that produces the most energy per unit area. Its high-energy value exceeds even that of potato, cassava and other known tubers. The orange flesh varieties have sufficient carotene that serves as a good source of vitamin A. Recent studies by Islam (2006) have revealed that sweet potato leaf extracts contains radical scavenging, antimutagenic, anticancer and antibacterial activities. Hence, sweet potato leaves is a veritable source of natural antioxidant. Presently, the total production land mass and yield is approximately 6.6 million ha and 106 million tons respectively (Zhang et al., 2009). Recent explosive growth of fast food restaurants and snacks in the sub-Saharan Africa has given sweet potato a new status, new prominence and new potential and in Nigeria, one-third of the population still depends on sweet

potato for food security. Profitable production of sweet potato through vine cuttings has declined due to its susceptibility to biotic stress. The crop thrives well in marginal soils producing good yield with little demand for either fertilizer or water. Although sweet potato is relatively drought tolerant and can provide good ground cover and often cultivated without pesticides (Ewell, 1990), its productivity is however, limited by a number of both biotic and abiotic constrains (Alam et al., 2010). Amongst the 20 viruses that have been identified to infect sweet potato in the world, sweet potato feathery mottle virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) is more prevalent in all sweet potato growing areas while the others are localized to one or more geographic areas (Moyer and Salazar, 1989; Kreuze et al., 2000). The most severe sweet potato virus disease (SPVD), usually caused by the dual infection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV), can result to 56-98% yield loss (Gibson et al., 1998). Thus, SPVD epidemics have been associated with low on farm productivity and with the disappearance of the once elite cultivars (Gibson et al., 1997). A regular supply of clean materials for planting is therefore necessary for sustainable production (Wang and Valkonen, 2008). These intractable problems associated with virus infestation of the planting materials demands for alternative approach. Interestingly, tissue culture has been identified as a powerful tool to overcome these limitations. One of the tissue culture techniques termed micropropagation aims at rapid multiply-

Table 1. Mean plant height of the two sweet potato cultivars hardened on local and conventional substrates.

Substrates	Mean plant height					
	A5			CIP 50		
	1 st 2 weeks	2 nd 2 weeks	Mean	1 st 2 weeks	2 nd 2 weeks	Mean
RS	17.2±6.1 ^a	19.8±3.6 ^a	18.5±4.6 ^a	10.3±4.6 ^b	10.8±3.4 ^b	10.6±3.4 ^b
SD	13.6±4.3 ^b	15.8±4.4 ^a	14.7±3.1 ^b	8.6±2.2 ^b	8.6±1.2 ^c	8.6±0.7 ^c
RMW	6.2±1.4 ^c	7.4±1.7 ^c	6.8±2.6 ^c	3.1±1.3 ^d	3.3±1.0 ^d	3.2±1.2 ^d
RS/RMW	10.5±0.8 ^b	12.6±2.8 ^b	11.6±3.1 ^b	5.2±1.4 ^c	5.9±2.3 ^c	5.6±1.4 ^c
RS/SD	16.8±4.2 ^a	18.4±7.2 ^a	17.6±4.3 ^a	11.8±1.8 ^b	12.6±3.2 ^b	12.2±3.3 ^b
SD/RMW	2.5±0.4 ^d	3.1±0.7 ^d	2.8±0.3 ^d	3.4±0.8 ^d	3.8±0.3 ^d	3.6±0.8 ^d
Jiffy peat	20.4±5.2 ^a	20.3±5.3 ^a	20.3±5.2 ^a	13.7±4.6 ^b	13.5±4.6 ^b	13.6±3.0 ^b

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at $p \leq 0.05$.
RMW-Rice mill waste; SD-Saw dust; RS-River sand.

ing clonal superior genotypes of disease-free and pest free plants (Smith and Drew, 1990). Strategies for postflask management of *in vitro* cultured sweet potato plantlets have been reported. Cassana et al. (2010) conducted an extensive research on the photochemical efficiency of leaves of sweet potato grown in an *in vitro* and *ex vitro* conditions. Semoradova et al. (2002) reported on responses of tobacco plantlets during transfer from an *in vitro* to an *ex vitro* conditions. Seelye et al. (2003) examined some of the major physiological events that occurs during *in vitro* transition of plantlets to *ex vitro* environment while Hazarika (2003) reviewed some current and developing methods for acclimatization of *in vitro*-produced plantlets. Despite the successes and the benefits of using jiffy peat for acclimatization, the prohibitive cost of postflask management of tissue-cultured plantlets has been a source of concern in the sub-Saharan Africa. The present study therefore aims at addressing this problem through the use of locally available materials as an alternative hardening substrate to jiffy peat a conventional substrate.

Results

Figure 1a depicts the development of apical shoots from the primary explants (wild-type) used to generate the *in vitro* plantlets. Development of the axillary buds and roots were observed after 4 and 5 days growth in culture respectively and within 16 weeks of shoot multiplication 100 healthy plantlets of both cultivars were generated (Fig. 1b).

Determination of the mean plant height of the two sweet potato cultivars hardened on local and conventional substrates

Table 1 shows the effects of substrates and substrate combinations on hardening on plant height. Mean heights of 17.6±4.3 to 20.3±5.2 cm were obtained after 4 weeks of acclimatization on RS/SD, RS and jiffy peat substrates in a humidity chamber for cultivar A5 while mean heights of 10.6±3.4 to 13.6±3.0 cm was obtained for cultivar 50. Hardening on substrate RS/SD was observed to be optimum for cultivars A5 and 50 with values of 17.6±4.3 and 12.2±3.3 cm respectively although jiffy peat a conventional substrate gave the best result with values of 20.3±5.2 and 13.6±3.0 cm for cultivars A5 and 50 (Table 1).

Determination of the mean number of leaves produced by the two sweet potato cultivars hardened on local and conventional substrates

The mean number of leaves produced by the two sweet potato cultivars hardened on local and conventional substrates is as shown in Table 2. While hardening on RMW

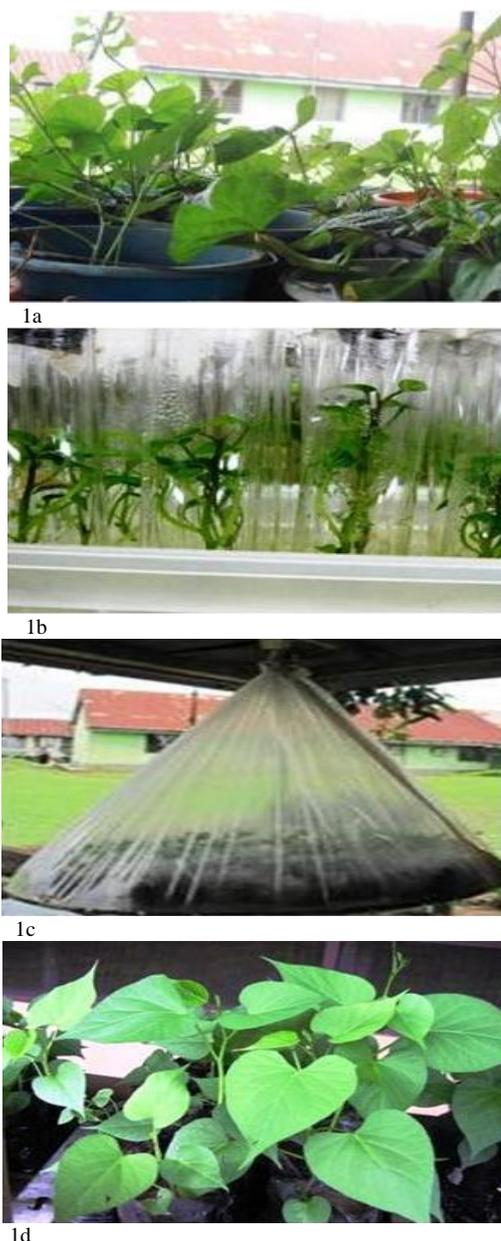


Fig 1. The developmental stages of explants used to generate the *in vitro* plantlets. 1a (Wild type); 1b (In vitro plantlets in MS medium); 1c (Acclimatization of the *in vitro* plantlets in the substrates contained in transparent polybags in a humidity chamber); 1d (Acclimatized *in vitro* plantlets in top soil ready to be transplanted to the field).

Table 2. Mean number of leaves produced by the two sweet potato cultivars hardened on local and conventional substrates

Substrates	Mean number of leaves					
	A5			50		
	1 st 2 weeks	2 nd 2 weeks	Mean	1 st 2 weeks	2 nd 2 weeks	Mean
RS	5.8±0.6 ^b	6.2±1.8 ^b	6.0±0.7 ^b	3.8±1.4 ^c	5.4±0.9 ^b	4.6±0.5 ^b
SD	5.5±1.2 ^b	6.8±0.7 ^a	6.2±1.3 ^b	3.5±0.3 ^c	3.9±0.6 ^c	3.7±0.6 ^c
RMW	4.2±0.3 ^b	4.4±1.4 ^c	4.3±0.6 ^c	2.2±0.4 ^d	2.8±0.1 ^d	2.5±0.3 ^d
RS/RMW	5.8±1.4 ^b	6.5±1.3 ^b	6.2±1.4 ^b	3.6±0.5 ^c	3.9±1.2 ^c	3.8±0.4 ^c
RS/SD	6.6±2.1 ^a	6.9±2.4 ^a	6.8±1.3 ^a	6.0±1.8 ^b	7.2±0.8 ^a	6.6±2.1 ^a
SD/RMW	3.3±1.3 ^c	3.2±0.1 ^c	3.3±0.5 ^c	2.9±0.4 ^d	3.2±1.2 ^c	3.1±0.2 ^c
Jiffy peat	9.3±2.4 ^a	9.1±1.6 ^a	9.2±3.1 ^a	8.2±1.7 ^a	8.6±0.5 ^a	8.4±2.2 ^a

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at $p \leq 0.05$. RMW-Rice mill waste; SD-Saw dust; RS-River sand.

substrate gave the least mean values of 4.3 ± 0.6 and 2.5 ± 0.3 for cultivars A5 and 50 respectively, jiffy peat substrate recorded the highest values of 9.2 ± 3.1 and 8.4 ± 2.2 for the two cultivars A5 and 50 respectively, followed by substrate RS/SD with values of 6.8 ± 1.3 and 6.6 ± 2.1 for cultivars A5 and 50 respectively (Table 2).

Determination of the mean number of nodes produced by the two sweet potato cultivars hardened on local and conventional substrates

Table 3 depicts the mean number of nodes produced by the two sweet potato cultivars hardened on local and conventional substrates. Although hardening on the substrate jiffy peat gave the best result of 8.6 ± 1.8 and 6.6 ± 2.1 for the two cultivars A5 and 50 respectively, it was closely followed by substrate RS/SD that recorded an average number of nodes of 7.2 ± 1.4 and 5.2 ± 1.3 for both cultivars A5 and 50 respectively. However, hardening on RMW gave the least result of 5.1 ± 0.4 and 3.2 ± 1.2 for the two cultivars A5 and 50 respectively (Table 3).

Assessment of the overall performance of the two sweet potato cultivars on the local and the conventional substrates within the 4 weeks hardening period

The overall performance of the two sweet potato cultivars on the local and the conventional substrates within the 4 weeks hardening period is as captured in table 4. Comparing the effects of hardening of the *in vitro* plants on the substrates, jiffy peat a conventional substrate out-competed the rest of the local substrates both in combination and singly for the two cultivars for all the parameters investigated. Thus, for the parameters: plant height, number of leaves and number of nodes, jiffy peat substrate recorded the highest values of 20.3 ± 5.2 and 13.6 ± 3.0 cm, 9.2 ± 3.1 and 8.4 ± 2.2 and 8.6 ± 1.8 and 6.6 ± 2.1 for the two cultivars A5 and 50 respectively. This was closely followed by hardening on substrate RS/SD with values of 17.6 ± 4.3 and 12.2 ± 3.3 cm (plant height), 6.8 ± 1.3 and 6.6 ± 2.1 (number of leaves) and 7.2 ± 1.4 and 5.2 ± 1.3 (number of nodes) for the two cultivars A5 and 50 respectively. However, hardening on substrate SD/RMW produced the least result with values of 2.8 ± 0.3 and 3.6 ± 0.8 cm (plant height), 3.3 ± 0.5 and 3.1 ± 0.2 (number of leaves) and 4.2 ± 0.1 and 3.7 ± 0.6 (number of nodes) for the two cultivars A5 and 50 respectively.

Discussion

Culture establishment

The concept of growing wild-type plants in screen house exclusive of their normal field condition prior to surface sterilization was to reduce microbial load such as yeasts,

Pseudomonas aeruginosa, *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus fumigatus*. Sixty eight (68) percent of the apical shoots that developed from the primary explants (Fig. 1a) were free from bacterial and fungal contamination after the application of surface sterilization procedures described in this study. Axillary buds and roots of the two cultivars started developing after 4 and 5 days growth in culture respectively. Sub-cultured nodal segments continued to develop new axillary shoots and roots and within 16 weeks of shoot multiplication 100 healthy plantlets of both cultivars were generated (Fig. 1b).

Acclimatization/Hardening

Acclimatization of *in vitro* produced plantlets requires that *ex vitro* environmental factors be gradually modified for successful survival and establishment of the transferred plantlets. Such is to help improve the internal structure of the plantlets and to give a more successful establishment. The gradual reduction of the relative humidity in the humidity chamber may have enhanced the survival and the establishment of the *in vitro* plantlets in this study. A fact that was supported by the reports of Cassels and Walsh, (1994) and Seelye et al. (2003) that a reduction in relative humidity leads to increases in plants nutrient uptake and transpiration with associated development of functional stomata for controlling plant water loss. The effects of the substrates and substrates combinations on plant height are as presented in Table 1. Substrates RS, jiffy peat and the RS/SD were significant for both cultivars (Table 1). Hence after 4 weeks of adaptation on substrates RS/SD, RS and jiffy peat, the mean plant height ranged from 17.6 ± 4.3 to 20.3 ± 5.2 cm for cultivar A5 in contrast to 10.6 ± 3.4 to 13.6 ± 3.0 cm for cultivar 50. A significant growth of 17.6 ± 4.3 and 12.2 ± 3.3 in height was observed on substrate RS/SD for cultivars A5 and 50 respectively. However, the best result in terms of plant height was obtained from the conventional substrate (jiffy peat), although the effectiveness of this treatment did not differ significantly from that of substrates RS and RS/SD. However, substrates SD/RMW and RMW were not significant for both cultivars in contrast to RS, RS/SD and jiffy peat. Interestingly, the *ex vitro* survival and development of the *in vitro* plantlets into well-developed plants was 100% irrespective of the substrates or substrate combinations used except for three incidences of caterpillar attacks. This is in contrast to the *in vitro* plantlets grown on untreated control (unsterilized substrates) that produced a comparatively low survival rate of 58%. Certainly *in vitro* grown plantlets are sterile and are also characterized by some anatomical deficiencies such as low deposition of surface wax, stomatal abnormalities and a non-continuous cuticle (Hazarika, 2003). In view of these factors, the substrates were sterilized to reduce possible biotic stress and the humidity inside the

Table 3. Mean number of nodes produced by the two sweet potato cultivars hardened on local and conventional substrates.

Mean number of nodes						
Substrates	A5			CIP 50		
	1 st 2 weeks	2 nd 2 weeks	Mean	1 st 2 weeks	2 nd 2 weeks	Mean
RS	5.7±0.8 ^b	6.8±1.2 ^a	6.3±0.5 ^b	3.8±0.2 ^b	5.6±1.3 ^b	4.7±0.5 ^a
SD	6.4±1.3 ^b	6.7±2.3 ^a	6.6±2.0 ^a	4.0±0.2 ^b	4.7±1.4 ^b	4.4±0.6 ^b
RMW	4.6±1.5 ^c	5.5±0.7 ^b	5.1±0.4 ^b	2.9±0.3 ^c	3.4±0.6 ^c	3.2±1.2 ^c
RS/RMW	5.0±0.4 ^b	5.6±1.4 ^b	5.3±0.5 ^b	3.5±1.2 ^b	4.6±0.7 ^b	4.1±0.8 ^b
RS/SD	6.6±2.3 ^a	7.8±1.6 ^a	7.2±1.4 ^a	4.5±1.4 ^a	5.8±2.4 ^b	5.2±1.3 ^a
SD/RMW	3.8±1.2 ^c	4.5±0.6 ^c	4.2±0.1 ^c	3.3±0.7 ^c	4.1±1.5 ^b	3.7±0.6 ^b
Jiffy peat	8.8±2.2 ^a	8.4±3.1 ^a	8.6±1.8 ^a	6.5±0.4 ^a	6.6±1.5 ^a	6.6±2.1 ^a

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05. RMW-Rice mill waste; SD-Saw dust; RS-River sand.

Table 4. Overall performance of the two sweet potato cultivars on the local and the conventional substrates within the 4 weeks hardening period.

Substrates	Plant height (cm)		Number of leaves		Number of nodes	
	A5	CIP 50	A5	CIP 50	A5	CIP 50
RS	18.5±4.6 ^a	10.6±3.4 ^a	6.0±0.7 ^b	4.6±0.5 ^b	6.3±0.5 ^b	4.7±0.5 ^b
SD	14.7±3.1 ^b	8.6±0.7 ^b	6.2±1.3 ^b	3.7±0.6 ^b	6.6±2.0 ^a	4.4±0.6 ^b
RMW	6.8±2.6 ^c	3.2±1.2 ^c	4.3±0.6 ^c	2.5±0.3 ^c	5.1±0.4 ^b	3.2±1.2 ^c
RS/RMW	11.6±3.1 ^b	5.6±1.4 ^b	6.2±1.4 ^b	3.8±0.4 ^b	5.3±0.5 ^b	4.1±0.8 ^b
RS/SD	17.6±4.3 ^a	12.2±3.3 ^a	6.8±1.3 ^a	6.6±2.1 ^a	7.2±1.4 ^a	5.2±1.3 ^a
SD/RMW	2.8±0.3 ^d	3.6±0.8 ^c	3.3±0.5 ^c	3.1±0.2 ^c	4.2±0.1 ^c	3.7±0.6 ^c
Jiffy peat	20.3±5.2 ^a	13.6±3.0 ^a	9.2±3.1 ^a	8.4±2.2 ^a	8.6±1.8 ^a	6.6±2.1 ^a

Key: Values represents mean ± standard errors for three replications for each treatment. Same letters are not significantly different at p≤0.05. RMW-Rice mill waste; SD-Saw dust; RS-River sand.

chamber (Fig. 1c) was gradually reduced in order to mimic *ex vitro* conditions and improve the internal structure of the *in vitro* plantlets in order to give a more successful establishment. Thus, Short et al. (1987), reported 80% relative humidity for optimum growth and *in vitro* hardening of cultured cauliflower and chrysanthemum. Therefore the survival of the *in vitro* produced plantlets in the first 2 weeks of acclimatization, may suggest that the *in vitro*-formed roots may have enhanced the continued growth and establishment of the plants during the initial acclimatization period. Thus, Leclerc et al. (1994) reported a 2 week period of sustained growth by the *in vitro*-formed roots of potato. They further stated that *in vitro*-formed roots not only survived transfer to the substrate but elongated and formed secondary and tertiary root systems with root hairs, implying that *in vitro* formed roots are functional during acclimatization and contributes significantly to the early growth of transplants *ex vitro*. This is in complete contrast to the *in vitro*-produced leaves that were observed not to have significant influence on the growth and establishment of the transplanted plantlets. The effects of the substrates were evaluated on leaf production by the two sweet potato cultivars (Table 2). After 4 weeks of growth in the substrates, leaf development was poor on substrates RMW and SD/RMW for the two cultivars compared to the other treatments (Table 2). The number of leaves produced after 4 weeks using jiffy peat substrate was significantly higher than that obtained from substrates RMW and SD/RMW but comparable to substrates RS and RS/SD. Mean number of leaves produced on jiffy peat substrate were 9.2±3.1 and 8.4±2.2 while 6.8±1.3 and 6.6±2.1 leaves were produced on substrate RS/SD (Table 2). Number of leaves produced on substrates RS, SD, RMW, RS/RMW and SD/RMW differed significantly from that produced on substrates RS/SD and the conventional substrate. Although, the number of leaves produced on the conventional substrate was slightly higher, they were not significantly different from that produced on RS/SD substrate. *In vitro*-raised plantlets are usually grown in an environmental condition that is

different from the natural field conditions and *in vitro*-produced leaves are associated with a number of anatomical and physiological changes compared to greenhouse and field-grown plants. Thus plantlets produced under conventional photomixotrophic conditions, such as high relative humidity has reduced epicuticular wax deposition, poor cuticle development and malfunctional stomata (Zobayed et al., 2000). Although *in vitro*-formed leaves may be photosynthetically competent, they are often replaced shortly after transfer to the green house by leaves with higher photosynthetic activity (Huylensbroeck et al., 1996). Nodal increase of plants is associated with increases in growth. Table 3 shows the mean number of nodes produced by the two sweet potato cultivars hardened on local and conventional substrates after 4 weeks growth period. The highest numbers of nodes (8.6±1.8 and 6.6±2.1) were obtained on the conventional substrate followed by 7.2±1.4 and 5.2±1.4 on RS/SD substrate for cultivars A5 and 50 respectively (Table 3). Substrates (RS, RMW, RS/RMW and SD/RMW) were not significant when the number of nodes produced for both cultivars per substrate was considered (Table 3). Differences in *in vitro* grown plant have been reported. Dessai et al. (1995) reported differences in nodal production of 27 cultivars of sweet potato during *in vitro* propagation. Ogero et al. (2012) evaluated the responses of two sweet potato cultivars when cultured *in vitro* with low cost and conventional media. Possible reasons for variability could be due to the balanced nutrient composition of the conventional substrate and differences in the genetic make-up of the crop species. The overall response of the two sweet potato cultivars on the seven substrates considered in this study is as captured in Table 4. Generally, the conventional substrate (jiffy peat) out-competed the rest of the substrates (RS, SD, RMW, RS/RMW, RS/SD and SD/RMW), though comparable to RS/SD substrate. Considering plant height, cv. A5 responded well to the conventional substrate and substrate RS/SD in contrast to the performance of cultivar 50 on these substrates. Thus, the obtained plant height on RS/SD

substrate and on the conventional substrate for cultivar A5 are significantly different from the values obtained for cultivar 50 on the same substrates (Table 4). Similarly, number of leaves and nodes produced on the same substrates followed the same pattern for both cultivars, suggesting that the conventional and the RS/SD substrates are better substrates compared to the other substrates. Conclusively, although jiffy peat substrate is superior to RS/SD which is a locally available substrate, RS/SD substrate is therefore a considerable alternative when economics and availability is considered.

Materials and methods

Establishment of explants from mother plants

Two sweet potato cultivars (A5 and 50) were used for the research. The cultivars were obtained from National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Abia State, Nigeria farm. Apical cuttings (1.5 cm) were excised from the two sweet potato cultivars (wild-type) grown in plastic buckets for two months. The folia leaves were removed and the cuttings washed with mild detergent (Teepol) and subsequently under running water for 20 minutes. The explants were further rinsed with sterile distilled water and transferred to the aseptic room.

Surface sterilization

The explants were first immersed in 70% ethanol and 5% bleach for 5 min and 20 min respectively. They were further rinsed three times (3x) with sterile distilled water and kept in sterile water to prevent the buds from desiccation.

Establishment of cultures

Apical shoot-bud (4 mm stem single-node) explants were aseptically excised in a laminar airflow cabinet from proliferating shoot cultures of the two sweet potato cultivars after eight weeks of growth in Murashige and Skoog (MS) medium. The pH of the medium was previously adjusted to 5.8 before sterilizing by autoclaving at 121°C for 20 min. The tender leaves were aseptically removed with sterile forceps and knives in sterile petri dishes in a laminar air-flow cabinet. The nodal cuttings were seeded singly into sterile test tubes containing 10 ml sterile MS medium solidified with 8 g/l of agar and supplemented with 20 g/l of sucrose. The test tubes were sealed properly with parafilm and clearly labeled before growing the culture at a temperature of 28±2°C for 16 h photoperiod and 8 h darkness at light intensity of 2000-3000 lux. They were sub-cultured every 2 months in order to generate enough plantlets for weaning.

Acclimatization

The plantlets were gently washed with tap water to remove the adhering media on the roots before planting with care onto sterile local weaning substrates (river sand (RS), rice mill waste (RMW) and saw dust (SD)) in various combinations in a 5 x 5 cm transparent polybags alongside the conventional substrate (Jiffy peat). The combinations were: RS/SD (2:1), SD/RMW (2:1) and RS/RMW (2:1). The humidity chamber was generously sprayed with water and kept under a shade. The polybags containing the substrates and the plantlets were watered, labeled and kept in the humidity chamber. On the third day morning after transplanting, three holes of about 1 cm in diameter were

made at the sides of the humidity chamber while on the fourth day the humidity in the chamber was reduced by cutting an opening (window) at the lower side of the chamber. A wash bottle containing six grains of fertilizer and water were mixed properly before adequate spraying of the substrates and the humidity chamber as well. The substrates and the humidity chamber were again sprayed with water towards evening time before closing the window. On the morning of the fifth day, the previous window was re-opened and a second window on the opposite side of the chamber was opened. The plants and the chamber were again generously sprayed with water. After 2 and 4 weeks, the following parameters were assessed: plant height, number of leaves and nodes before transplanting into top soil in a 5 x 5 cm black polybags. They plants were watered once a day and occasionally with dilute fertilizer and kept under a shade for another 4 weeks before transplanting to the field.

Experimental design and data analysis

Completely randomized design was used for the experiments. Each treatment was repeated three times and data recorded two and four weeks after the beginning of the treatments. Analysis of variance (ANOVA) was used to compare the number of leaves, nodes and plant height in the conventional and the local substrates. Means were separated using Tukey's test at the level of 5%.

Conclusions

There exist a number of recognizable differences between *in vitro* and greenhouse environment/field environment. *In vitro*-developed roots can be of immense importance in enhancing the initial growth and establishment of plantlets during acclimatization. Development of new leaves and roots during acclimatization are of prime importance in the survival and establishment of hardened plantlets. But it is important to mention that acclimatization protocol varies from species to species which may probably be due to their inherent genetic constitution. Thus, the described postflask management protocol will help to solve the associated financial handicap involved in the *in vitro* hardening of sweet potato plants in the sub-Saharan Africa.

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