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Regeneration of Three Sweet Potato (*Ipomoea batatas* (L.)) Accessions in Ghana via, Meristem and Nodal Culture

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ABSTRACT

In vitro regeneration of three sweet potato accessions UE007, UK-BNARI and SA-BNARI using meristem or nodal cuttings was studied. Meristematic explants cultured on (Murashige and Skoog, 1962) basal medium supplemented with low concentration of benzylaminopurine (BAP) or kinetin resulted in callus with or without shoot development which delayed shoot emergence. The degree of callus development increased as the concentration of the cytokinin in the culture medium increased. Although, callus development was comparatively lower on kinetin amended medium than BAP amended medium (Murashige and Skoog, 1962) medium supplemented with 0.25 mg L^{-1} BAP had the highest shoot induction (80%). For further differentiation of callus or shoots into distinct stem and leaves, the cultures were transferred into fresh MS medium supplemented with $0.25 \text{ mg L}^{-1} \text{ BAP}$, $0.1 \text{ mg L}^{-1} \text{ NAA}$ and 0.1 mg L^{-1} gibberellic acid. To overcome the delay in shoot initiation using meristem culture, nodal cuttings of sweet potato were used as explants and cultured on MS medium amended with 0.3-0.9 mg L^{-1} BAP. All explants cultured on 0.3 or 0.6 mg L^{-1} BAP developed shoots. Post flask acclimatisation of meristem or nodal cutting derived plantlets showed that meristem derived plantlets were better acclimatised than nodal cutting plants due to vigorous root development leading to higher percentage survival in pots and subsequent tuber production as well as other yield parameters. The successful regeneration of sweet potato plantlets in vitro using meristem and nodal cutting explants could be used to complement conventional propagation methods and integration into plant breeding programmes for sweet potato.

Key words: Meristematic, cytokinin, gibberellic acid, nodal

INTRODUCTION

Taxonomically, sweet potato (*Ipomoea batatas* (L.)) is a dicotyledonous plant that belongs to the family Convolvulaceae. The crop is a major starch staple in Africa with production estimated at 14,000,000 mt, about 6% of the world's production (FAO, 2010). It ranks the third largest cultivated root crop (7.9 million ha) after potato and cassava (FAO, 2008) and seventh among all food crops cultivated worldwide. The International Potato Centre has reported that more than 130,000,000 t of sweet potatoes are produced annually (CIP, 2010). Although, sweet potato is

widely consumed in Ghana, its production is low, about 130,300 mt (FAO, 2010). Thus, Ghana is not really recognized as a sweet potato producing country in the world.

The crop has received increased attention because it can adapt to a wide range of environmental conditions and fits into the low input agriculture system practiced by subsistence farmers. Besides, it is relatively drought tolerant, can grow in marginal areas with poor soils of limited fertility and inadequate moisture and under adverse farming conditions (Prakash and Hinata, 1980), making it a good food security crop especially in the developing countries. According to Woolfe (1992), sweet potato gives good yield per unit area per unit time, making it attractive to subsistence farmers.

Sweet potato has diverse usage both domestically and industrially. The leaves and roots are eaten in many countries while the vines are used as animal feed. The tubers of sweet potato are good source of starch, rich in complex carbohydrates, dietary fibre, β -carotene, vitamin C and B₆ (Purseglove, 1991). Also, the leaves are nutritionally valuable in protein and minerals and therefore can improve the nutritional status of its consumer (Lin *et al.*, 2007). Industrially, the crop is used in the production of confectionaries such as biscuits, noodles. In some developed countries, it is used for the production of alcohol, starch, spirit and flour for manufacturing of industrial products.

In spite of its high economic potential as a source of food, the yield of the crop has been declining over the years due to several factors (Oggema et al., 2007). The major constraint to high yield is the susceptibility of sweet potato to viral diseases. According to Kapinga et al. (2007), sweet potato viral diseases cause up to 98% yield reduction. Sweet potato viruses are systemic, therefore, they are harboured within the vines. Since the crop is propagated vegetatively from vines from previous season, these systemic viruses are transmitted to the next generation and consequently, the yield of the crop is dramatically reduced. Yield reduction caused by these viruses can be overcome by the use of virus-free planting materials for propagation. The limitations to conventional method of sweet potato propagation have compelled researchers to consider in vitro techniques as an alternate method to overcome viral transmission in the cultivation of the crop.

Meristem culture is one of the most important *in vitro* culture techniques used for the elimination of viruses from vegetatively propagated crops (Badoni and Chauhan, 2010). According to Pruski (2001), meristem culture, does not only result in disease elimination but also in rapid multiplication of elite clones.

Although, several studies have shown that *in vitro* propagation techniques can be used as a means to produce clones that increase sweet potato yield, however, there is no documentation on which of the techniques provides the utmost yield from sweet potato in Ghana. Therefore, this study was aimed at assessing the post flask tuberisation of three accessions of sweet potato regenerated using meristem culture and nodal culture. Specifically, the study sought to determine the effect of different concentrations of BAP and kinetin on plantlet regenerated from meristem culture and determine the effect of different concentrations of BAP on shoot multiplication from nodal culture.

MATERIALS AND METHODS

Study site and planting material: This study was conducted at Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra-Ghana. Three sweet potato accessions obtained from Ghana, South Africa and United

Kingdom were used for the study. The accession UE007 was from the upper east region of Ghana while SA-BNARI and UK-BNARI were from South Africa and the United Kingdom, respectively. SA-BNARI and UK-BNARI were used because they are rich in β -carotene which confers high export potential while UE007 has high acceptability among the indigenes. Fresh vine cuttings, (12-15 cm) long with 5 nodes were planted in black polyethylene bags containing a mixture of loamy soil and saw dust in a ratio of 2:1. The pots were kept under the plant barn and irrigated with tap water thrice a week. Regenerated shoots were harvested as when required for the study.

Culture media: The culture media consisted of (Murashige and Skoog, 1962) basal salt (MS) powder or stock solutions. The medium was supplemented with growth regulators according to the experimental requirement. The MS media were supplemented with 30 g L⁻¹ sucrose, 1.0 mg L⁻¹ thiamine-HCl, 1.0 mg L⁻¹ nicotinic acid, 1.0 mg L⁻¹ pyridoxine-HCl, 2.0 mg L⁻¹ glycine and 100 mg L⁻¹ myoinisitol. The pH of the media was adjusted to 5.8 using either 1 M NaOH or 1 M HCl. The media were gelled with 3.5 g L⁻¹ phytagel prior to autoclaving at a temperature of 121°C and 15 psi pressure for 15 min. Twenty milliliter of the medium was dispensed into test tube or honey jars (50 mL) depending on the experiment and stored at room temperature or in a refrigerator.

Culture conditions: The cultures were incubated in a growth room at a temperature of 27±2°C, with a photoperiod of 16 h light and 8 h darkness and a light intensity of 2700 lux provided by white fluorescent tube.

Regeneration of shoots from meristem: The protocol for meristem culture was adopted from International Potato Centre (Panta et al., 2006) with slight modification where about 1-2 cm long shoot tip were harvested from two weeks old vines. Explants were washed under tap water for 15 min and quickly transferred into a sterilized honey jar bottle on the laminar airflow cabinet (Nuaire Biological Safety Cabinet, UK). The explants were immersed in 70% ethanol with two drops of tween-20 for 5 min and then rinsed three times with sterile distilled water for 3 min each to remove traces of the sterilant. Sterilized shoots were placed in a sterile Petri dish under a dissecting stereomicroscope (Leica Zoom 2000, China). Using the forceps and scalpel, the young leaves and leaf primordial were removed. The removal was accomplished by scraping off the leaf primordial followed by dissecting of the meristematic tissues using a new scalpel with care being taken not to cause wounding of tissues. Approximately 0.5 mm diameter meristem was excised and cultured in a test tube containing 20 mL of MS basal salts (initiation medium) supplemented with 30 g $\rm L^{-1}$ sucrose, 1.0 mg L⁻¹ thiamine-HCl, 1.0 mg L⁻¹ nicotinic acid, 1.0 mg L⁻¹ pyridoxine-HCl, 2.0 mg L⁻¹ glycine and 100 mg L^{-1} myo-inisitol and varying concentrations (0.0, 0.25 and 0.50 mg L^{-1}) of Benzylaminopurine (BAP) or kinetin $(0.0, 0.50 \text{ and } 1.00 \text{ mg L}^{-1})$. The test tubes were sealed with parafilm and labeled accordingly. The number of explants that developed callus, shoots, roots and number of leaves per shoot were counted after six weeks of culture. Thereafter, all responding meristems were transferred to fresh MS medium supplemented with 0.25 mg L⁻¹ BAP (optimal concentration for shoot initiation) and 0.1 mg $L^{-1}\alpha$ -Naphthaline Acetic Acid (NAA) and 0.1 mg L^{-1} gibberellic acid (GA_o) for subsequent shoot development. The percentage shoot survival, shoot height, root number, root length and number of leaves per shoot after three months of culture were again recorded.

Regeneration of shoots from nodal cuttings: Two week-old shoot tips (1-2 cm long) were harvested from pot-grown sweet potato vines. The shoot tips were washed under running tap water for 30 min. After washing, the explants were sterilized by immersion in 70% ethanol with two drops of tween-20 for 5 min as previously described. Sterilized explants were cultured on 20 mL of MS basal salt medium supplemented with 0.3, 0.6 or 0.9 mg L⁻¹ BAP, sealed with parafilm and labelled accordingly. Cultures were incubated in a growth chamber for four weeks under the same conditions as previously described earlier. Data on duration for shoot emergence, percentage shoot formation, number of roots and leaves, percentage root formation, shoot height, root length were recorded when cultures were four weeks old.

Acclimatization of *in vitro* regenerated plantlets: Twelve-week old *in vitro* plantlets regenerated from meristem or nodal explants were transferred to loamy soil: Saw dust mixture in a ratio of 2:1 in black polythene bags. Prior to transfer into pots, the roots of plantlets were gently washed in tap water to remove all adhering phytagel and then dipped in a fungicide solution to prevent fungal growth. The plantlets were then covered with transparent plastic cups to create a humidity chamber and kept under the plant barn with 70% of sunlight. After one week of transfer, the plastic cups were partly removed to allow for further acclimatization and then watered with 1000 mL super master (NPK 20-20-20) fertilizer (Agri-Mat Ltd., Ghana) solution at a concentration of 3.3 g L⁻¹ twice a week. The plastic cups were finally removed two weeks after transplanting. The number of plantlets which survived was counted. Plants were transferred to bigger black polythene bags (20×20 inches) after three weeks under full sunlight conditions in a pot experiment.

Meristem or nodal cutting derived plantlets and post flask tuber development: Three week old post acclimatised plantlets regenerated from meristem or nodal cuttings of SA-BNARI, UK-BNARI and UE007 were transferred singly to pots filled with loamy soil. The pots were watered immediately after transplanting and then placed in the open to allow direct exposure to sunlight and rainfall. They were watered twice a week with tap water in the absence of rain. The plantlets were fertilized with 6.6 g L⁻¹ super master NPK 20-20-20 fertiliser (Agri-Mat Ltd., Ghana) one week after transplanting and subsequently fertilised every fortnight until 60 days. Weeds were controlled by hand removal from the pots as and when necessary. Data on plantlet survival were taken 30 Days After Planting (DAP). Also, the numbers of branches, leaves (green and fully opened) per plant as well as vine height (the main vine) were recorded fortnightly up to the tenth week. The leaf area as well as leaf weight of five expanded randomly selected leaves was measured using area meter AM300 (ABC BioScientific Ltd., England) and weighing balance (Mettler-Toledo Limited, United Kingdom) after 30, 60 and 90 days of transplanting. The number of storage roots per plant was counted 30 days after planting and subsequently after every 30 days using destructive sampling. Also, the tuber weight per plant was measured using weighing balance (Mettler-Toledo Limited, United Kingdom). The skin and flesh colour of storage roots from the three sweet potato accessions grown in pots were assessed 90 days after planting using CIP (1991) descriptor list for sweet potato.

Determination of dry matter content of tubers: The dry matter content was determined 90 DAP using the method described by Carey and Reynoso (1999) with slight modification. Storage roots (200 g) were randomly sampled from each meristem or nodal cutting derived plants, washed, peeled and chopped into pieces or cubes. A total fresh weight of two hundred gram was weighed

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from the chopped pieces from each treatment (accession×source of explant) and put into envelopes, labelled and dried in an oven (Statim Electric Oven, Australia) at 60°C. The weights of the tubers were measured after every 24 h until a constant weight was achieved three days. The Dry Matter (DM) content was calculated using the equation:

DM (%) =
$$\frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Statistical analysis: GenStat (2007) was used for the statistical analysis. A factorial experiment was used for the regeneration of plantlets *in vitro* while split-plot design in RCBD with three replicates with accessions as main plots and sources of explants as subplots (meristem and nodal cutting derived plantlets) was used for the post flask tuber development and analysed separately using ANOVA. The significance of differences between treatments was determined, using the Least Significant Difference (LSD) at 5% probability.

RESULTS

Effect of BAP and kinetin on shoot induction from meristem explants: Meristems of all the *Ipomoea batatas* accessions cultured on MS medium supplemented with BAP or kinetin developed callus between six and ten days after culture except in the control (Fig. 1). The number of

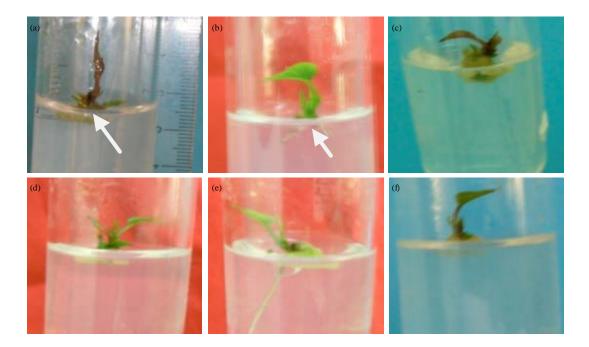


Fig. 1(a-f): Effect of BAP and kinetin on shoot regeneration in three accessions of sweet potato SA-BNARI accession on (a) 0.25 mg L⁻¹ BAP, (b) 0.50 mg L⁻¹ kinetin, UK-BNARI accession on (c) 0.5 mg L⁻¹ BAP, (d) 0.5 mg L⁻¹ kinetin, UE007 accession on (e) 0.25 mg L⁻¹ BAP and (f) 0.50 mg L⁻¹ kinetin. Arrows showing the degree of callusing from the different concentrations of the two cytokinins (Bar: c = 2 mm, b, d, e and f = 5 mm)

Table 1: Effect of BAP or kinetin on degree of callus, shoot initiation and height of shoot six weeks after culture

			Shoot formation (%)			Height of shoot (cm)			
Growth	Concentration	Degree of callus							
regulators	(mgL^{-1})	${\it development}$	SA-BNARI	UK-BNARI	UE007	SA-BNARI	UK-BNARI	UE007	
BAP	0.00	-	0.0ª	0.0ª	0.0ª	0.0ª	0.0ª	0.0ª	
	0.25	++	80.0 ^b	50.0^{b}	80.0^{b}	$1.45{\pm}0.4^{\rm bc}$	1.35 ± 0.4^{bc}	$1.69{\pm}0.4^{\rm bc}$	
	0.50	+++	50.0^{b}	60.0 ^b	40.0ab	1.00 ± 0.4^{b}	0.70 ± 0.4^{ab}	$0.71{\pm}0.4^{\text{ab}}$	
Kinetin	0.00	-	0.0^a	0.0^a	0.0^a	0.0^a	0.0^{a}	0.0^a	
	0.50	+	60.0^{b}	50.0^{b}	50.0^{b}	0.90 ± 0.4^{b}	0.81 ± 0.4^{ab}	$0.70{\pm}0.4^{\text{ab}}$	
	1.00	++	50.0^{b}	50.0^{b}	40.0^{ab}	$0.70{\pm}0.4^{ab}$	$0.60 {\pm} 0.4^{\rm ab}$	$0.65{\pm}0.4^{\text{ab}}$	

Values in the same column followed by same superscripts are not significantly different at $p \le 0.05$ according to Tukey's pair wise comparisons, Ten meristem explants were cultured per treatment, -: No callus formed, +,++,+++: Low, moderate and high callus formation, respectively

meristems which developed callus as well as the size was significantly (p<0.05) affected by the type and concentration of cytokinin in the culture medium (Table 1). Generally, the size of callus formed or degree of callusing increased with increasing cytokinin concentration in the growth medium (Table 1). Meristems cultured on MS medium supplemented with BAP developed comparatively or had higher degree of callus formation than those cultured on MS medium supplemented with kinetin (Fig. 1). The degree of callus formation from a $0.25 \text{ mg L}^{-1} \text{ BAP}$ supplemented medium was twice that of a 0.50 mg L^{-1} kinetin supplemented medium indicating that BAP enhanced better callus development than kinetin (Table 1). Doubling the concentrations of both BAP and kinetin correspondingly resulted in the doubling of callus development. The percentage shoot developed ranged from 40-80% in BAP supplemented medium while in kinetin it ranged from 40-60%. The highest shoot induction (80%) was observed in SA-BNARI and UE007 accessions cultured on 0.25 mg L⁻¹ BAP while the lowest (40%) was observed in UE007 on a medium supplemented with 0.50 mg L⁻¹ BAP and 1.0 mg L⁻¹ kinetin, respectively. Factorial analysis did not show any significant difference (p<0.05) between the accessions and cytokinins on shoot initiation. Shoot development in accession UE007 cultured on 0.25 mg L⁻¹ BAP amended MS medium differed from those cultured on 0.50 mg L^{-1} medium (Table 1) suggesting that the concentration of BAP in the medium affected shoot induction. In the kinetin amended medium, doubling the concentration from $0.50-1.0 \text{ mg L}^{-1}$ marginally decreased the percentage of shoots developed in SA-BNARI and UE007 while shoot development in UK-BNARI remained the same. Shoot development from the meristem explants were hindered by the excessive callus development from the two cytokinins used in the culture medium. The height of shoots ranged from (0.7-1.69 cm) for medium supplemented with BAP while on medium modified with kinetin it ranged from 0.60-0.90 cm (Table 1). The effect of the two cytokinins (BAP and kinetin) on height of shoot declined when concentration was doubled (Table 1). Shoots of UE007 grew faster reaching a height of 1.69 cm after six weeks followed by SA-BNARI (1.45 cm) then UK-BNARI (1.35 cm) in that order. In general, meristem explants from the accessions responded better by way of shoot growth to BAP supplemented medium than those on MS medium supplemented with kinetin.

Effect of BAP, NAA and GA₃ on shoot elongation: Shoot development on the fresh medium ranged from 57.5-75% (Table 2) with the highest survival (75%) observed on accession SA-BNARI and UE007 while the lowest (57.5%) was observed in UK-BNARI. The percentage development of SA-BNARI and UE007 differed from accession UK-BNARI (Table 2). There was no multiple shoot

Table 2: Effect of 0.25 mg L⁻¹ BAP, 0.1 mg L⁻¹ NAA and 0.1 mg L⁻¹ GA₃ on growth and shoot development of three accessions of *Ipomoea batatas*

-2							
	No. of shoot or	Shoot development	Shoot height	Root development	No. of roots per	Root length	No. of
Accessions	calli transferred	(%)	(cm)	(%)	${ m shoot}$	(cm)	leaves
Control	0	0.00^{a}	0.00ª	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
SA-BNARI	24	75.0⁴	1.73 ± 0.77^{b}	65.0^{b}	4.50 ± 0.52^{d}	2.78±0.69°	4.60±0.91°
UE007	21	75.0°	2.05 ± 0.77^{b}	50.0^{b}	3.10±0.52°	1.90 ± 0.69^{bc}	$2.45\pm0.91^{\rm b}$
UK-BNARI	21	57.5^{bc}	$1.53\pm0.77^{\rm b}$	60.0 ^b	1.05 ± 0.52^{b}	1.38±0.69 ^b	3.55 ± 0.91^{bc}

Values in the same column followed by same superscripts are not significantly different at p≤0.05 according to Tukey's pair wise comparisons

development from the meristem. In general, survival rate was 50% or above in all the accessions (Table 2). The presence of GA₃ enhanced shoot elongation. The height of shoot ranged from 1.53-2.05 cm with accession UE007 showing faster growth in height (2.05 cm) than the remaining accessions. Although, the height of the plantlets after three months of culture varied according to the accessions, there were no statistical differences between the heights of the accessions. On transfer to the lower concentration of BAP, GA₃ and NAA amended medium, all the cultures developed roots. However, the number of cultures that developed roots as well as the mean number of roots developed varied. Accessions SA-BNARI had the highest percentage (65%) root development followed by UK-BNARI (60%) and UE007 (50%) in that order with no statistical difference between the root developments. However, the mean root length differed significantly (p<0.05) between some accessions. Accession SA-BNARI had the longest root (2.78 cm) and this was significantly different (p<0.05) from the rest of the accessions. Even though accessions UK-BNARI had the second highest percentage root formation, the mean root length was shorter (1.38) compared to UE007 that had the least percentage root formation. The number of leaves developed by the accessions varied significantly (p<0.05) ranging from 2.45-4.60 indicating that the number of leaves dependent on the accession (Table 2). The accession SA-BNARI had significantly (p<0.05) higher number of leaves (4.60) than the other two accessions followed by UK-BNARI (3.55) and UE007 (2.45) in that order. In general, accession UE007 produced the lowest (2.45) mean number of leaves.

Effect of different BAP concentration on shoot regeneration using nodal cuttings:

Almost all nodal cutting explants of the accessions developed shoots (Table 3) within two weeks of culture ranging from 4-13 days. Of the three accessions, UE007 developed shoots earlier (4-8 days) almost independent of the concentration of the BAP in the culture medium followed by UK-BNARI (5-13 days) and SA-BNARI (6-11 days) in that order indicating that shoot emergence is genotypic dependent (Table 3). In general, days to shoot emergence on 0.6 mg L⁻¹ BAP supplemented medium was much faster (4-8 days for UE007, 5-13 for UK-BNARI and 6-11 days for SA-BNARI) in all the three accessions studied than those on the other concentrations (Table 3). The accessions UK-BNARI and SA-BNARI developed 100% shoots after two weeks of culture on MS medium independent of BAP concentration while in UE007 83.3% developed shoot on medium supplemented with 0.9 mg L⁻¹ BAP (Table 3). Factorial analysis showed that BAP concentration significantly affected shoot development.

Similarly, there was significant interaction between the concentrations of BAP and the accessions on shoot development. Growth of the shoots (height) varied between accessions as well as the concentrations of BAP in the culture medium. The height of shoots varied significantly (p<0.05) as it decreased from lowest concentration to highest in all the accessions. Nodal cuttings

Table 3: Effect of different BAP concentrations on days to shoot emergence, shoot height, number of leaves per shoot and root length from nodal explants after 4 weeks of culture

			Shoot		Roots		
Accessions	BAP (mg L ⁻¹)	Days to shoot emergence	Shoot formation (%)	Height (cm)	Formation (%)	Length (cm)	No. of leaves
SA-BNARI	0.0	0	0.0ª	0.00ª	0.0ª	0.00ª	0.00ª
	0.3	7-11	100.0°	3.30 ± 0.28^{d}	71.4^{bc}	2.01 ± 0.58^{b}	$2.86{\pm}1.79^{ab}$
	0.6	6-11	100.0°	$2.23\pm0.28^{\circ}$	$85.7^{\rm bc}$	1.89 ± 0.58^{b}	3.00 ± 1.79^{ab}
	0.9	10-11	100.0°	1.47 ± 0.28^{b}	$85.7^{\rm bc}$	$1.76 \pm 0.58^{\rm b}$	3.43 ± 1.79^{ab}
UK-BNARI	0.0	0	0.0^{a}	0.00ª	0.0ª	0.00^{a}	0.00ª
	0.3	6-13	100.0°	3.99±0.28°	100.0°	$2.84 \pm 0.58^{\circ}$	4.00 ± 1.79^{b}
	0.6	5-13	100.0°	2.34±0.28°	100.0°	2.73 ± 0.58^{b}	$7.86{\pm}1.79^{\rm cd}$
	0.9	8-13	100.0°	$1.24{\pm}0.28^{b}$	57.1 ^b	2.83 ± 0.58^{b}	10.71 ± 1.79^{cd}
UE007	0.0	0	0.0^a	0.00^a	0.0^a	0.00^{a}	0.00ª
	0.3	5-8	100.0°	4.21 ± 0.28^{e}	100.0°	2.71 ± 0.58^{b}	$4.86{\pm}1.79^{bc}$
	0.6	4-8	100.0°	$2.23\pm0.28^{\circ}$	$85.7^{\rm bc}$	$1.97 \pm 0.58^{\rm b}$	5.29 ± 1.79^{bc}
	0.9	5-8	83.3 ^b	1.44 ± 0.28^{b}	85.7^{bc}	1.86 ± 0.58^{b}	6.29 ± 1.79 bc

Values in the same column followed by same superscripts are not significantly different at p≤0.05 according to Tukey's pair wise comparisons. Eleven nodal cuttings explants cultured per treatment

of UE007 cultured on 0.3 mg L⁻¹ amended MS medium developed faster attaining a height of 4.21 cm after four weeks of culture while UK-BNARI cultured on 0.9 mg L⁻¹ had the least growth in height (1.24 cm). The percentage root formation varied, ranging from 57.1% in accession UK-BNARI cultured on MS medium supplemented with 0.9 mg L⁻¹ to 100% on MS medium supplemented with 0.9 mg L⁻¹ to 100% on MS medium supplemented with 0.6 and 0.9 mg L⁻¹ BAP while 100% was achieved on MS amended with 0.3 mg L⁻¹ BAP. Generally, more than 50% root development was observed in all the BAP concentrations tested. The length of root per shoot varied from 1.76-2.84 cm with accession UK-BNARI developing the longest roots (2.84 cm) on medium with 0.3 mg L⁻¹ BAP while nodal cuttings of accession SA-BNARI cultured on 0.9 mg L⁻¹ BAP had the shortest root (1.76 cm) (Table 3).

However, they were not statistically different. Although root length decreased as the concentration of the BAP in the MS medium increased from 0.3-0.9 mg L⁻¹ in accession SA-BNARI and UE007, in accession UK-BNARI, there was a slight increase in root length when the concentration was increased from 0.6-0.9 mg L⁻¹. In general, the longest root length in all the accessions studied was obtained from nodal explants cultured on 0.3 mg L⁻¹ BAP. The mean number of leaves also varied but the trend differed from that of the height. The number of leaves was significantly (p<0.01) affected by the BAP in the culture medium. In accession SA-BNARI, the number of leaves increased from 0.3 mg L⁻¹ BAP and reached an optimum when the concentration of the growth regulator was doubled. However, in UK-BNARI and UE007, there was an increase in the number of leaves when BAP concentration was increased. Accession UK-BNARI had the highest (10.71) leaves per shoot on a medium supplemented with 0.9 mg L⁻¹ BAP and this declined to less than 5 on a medium with 0.3 mg L⁻¹ BAP (Table 3).

Post flask acclimatization: All plantlets with well developed roots from both meristem and nodal cutting explants were transferred into loamy: saw dust mixture in a ratio of 2:1. Generally, post flask survival was comparatively higher in meristem derived explants than nodal cuttings

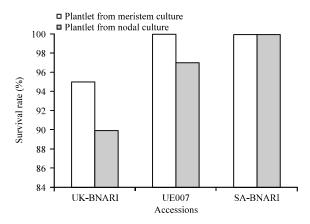


Fig. 2: Effect of plant source on post flask acclimatization and survival rate of sweet potato plants, three weeks after acclimatization



Fig. 3(a-b): (a) Well established plantlets of nodal cuttings and (b) Meristem derived plantlets of UK-BNARI

(Fig. 2 and 3). In accession SA-BNARI both meristem and nodal cuttings derived-plantlets had 100% post flask survival. In UK-BNARI and UE007, meristem derived plants had higher percentage post flask survival than nodal cutting derived explants. Although, post flask survival was high in all the accessions (more than 90%), plantlets of accession UK-BNARI had the least.

Post flask tuberisation of plantlets regenerated from meristem and nodal cultures Effect of sources of explants on survival of plants: Plantlets from both meristem and nodal cuttings were well established 30 days after planting. Generally, meristem derived plantlets significantly had the highest percentage survival percentage in the pots (96.3%) compared to nodal cutting derived plantlets (77.8%) (Table 4). The percentage survival of meristem derived plants ranged from 88.9-100% while for nodal cutting derived plants it ranged from 66.7-88.9%. Among

Table 4: Effect of source of explant on plant stand of three accessions of sweet potato grown in pots at 30 DAP

Accessions	Source of explant	Survival (%)
SA-BNARI	Meristem	100.0ª
	Nodal cutting	77.8 ^{ab}
UK-BNARI	Meristem	100.0ª
	Nodal cutting	66.7 ^b
UE007	Meristem	88.9 ^{ab}
	Nodal cutting	88.9 ^{ab}

Values in the same column followed by same superscripts are not significantly (p<0.05) different

the three accessions, SA-BNARI and UK-BNARI had the highest (100%) while the lowest stand count (88.9%) was recorded in UE007 (Table 4) for meristem derived plants. With nodal cutting plants, the highest (88.9%) was attained in accession UE007 and the lowest (66.7%) in UK-BNARI.

Effect source of explants on the morphological characters of three accessions of *Ipomoea batatas* (L.)

Effect of source of explant on leaf area, mean weight of leaf and mean weight of fresh vine: The leaf area increased as the plant grew from 30-60 days and then decreased at 90 days. Hence, the leaf area at 60 days was significantly higher in both meristem and nodal cutting derived plants than at 90 days. In all the accessions, there was slightly significant difference between meristem and nodal cutting derived plants except at 30 days where a significant difference was observed only in accessions SA-BNARI. The highest leaf area was observed at 60 days after planting in the pots with accession SA-BNARI followed by UK-BNARI the UE007 for the meristem derived plants. Similar trend was observed for nodal derived plants (Table 5). At 90 DAP, the leaf area decreased in both meristem-and nodal derived plants for all the tested accessions. Accession UE007 recorded the highest (1052.5 cm²) at 90 days of planting whereas, SA-BNARI (540.7 cm²) obtained the least leaf area for the meristem derived plants. With the nodal cuttings derived plants, the highest (839.4 cm²) was obtained from SA-BNARI while UE007 gave the lowest (760.3 cm²).

The fresh weight of the leaf decreased from 30-90 days in all the three accessions. Generally, meristem derived plants had the highest fresh weight compared to nodal cutting derived plants at all the harvest periods except at 90 days where in most of the accessions they were almost the same. The fresh weight at 30 days was twice heavier than at 90 days in all the accessions. With the exception of UK-BNARI at 30 days, there was no statistical difference in the weight of the leaves derived from meristem and nodal cuttings. Contrary to the weight of leaves, the weight of fresh vines increased from 30-90 days. The weight of vines at all the harvest periods was comparatively higher in meristem derived plants than nodal cutting explants in all the accessions studied (Table 5). However, at 90 days, the weight of vines from meristem derived plants was significantly higher than nodal cutting plants with accession SA-BNARI recording the highest (506.7 g) followed by UE007 (420 g) and UK-BNARI (296.7 g), respectively. Likewise, nodal cuttings derived plants also followed similar trend in the tested accessions (Table 5).

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Table 5: Effect of sources of explant and days after planting on leaf area, leaf weight and vine weight of 3 sweet potato accessions grown in pots

in pots					
		DAP			
Accession	Sources of explants	30	60	90	
Mean leaf area (cn	n²)				
SA-BNARI	Meristem	866.7 ± 1125.2^{b}	$1186.7{\pm}1125.2^{ab}$	54.7±1125.2°	
	Nodal cutting	$1082.1{\pm}1125.2^{\rm ab}$	1233.1 ± 1125.2^{a}	839.4±1125.2b	
UK-BNARI	Meristem	985.7 ± 1125.2^{ab}	$1186.1{\pm}1125.2^{ab}$	945.1±1125.2ªb	
	Nodal cutting	1066.2 ± 1125.2^{ab}	1225.3 ± 1125.2^a	749.0 ± 1125.2^{b}	
UE007	Meristem	1107.9±1125.2ab	$1170.7{\pm}1125.2^{ab}$	1052.5±1125.2ab	
	Nodal cutting	990.7 ± 1125.2^{ab}	1219.8 ± 1125.2^a	760.3 ± 1125.2^{b}	
Mean leaf weight ((g)				
SA-BNARI	Meristem	$1.5{\pm}0.2^{\rm b}$	$0.9\pm0.2^{\circ}$	$0.5{\pm}0.2^{\circ}$	
	Nodal cutting	1.3 ± 0.2^{b}	$0.8 \pm 0.2^{\circ}$	$0.5{\pm}0.2^{\circ}$	
UK-BNARI	Meristem	$1.7{\pm}0.2^{\rm b}$	$0.8 \pm 0.2^{\circ}$	$0.6\pm0.2^{\circ}$	
	Nodal cutting	2.4 ± 0.2^{a}	$0.9\pm0.2^{\circ}$	$0.6\pm0.2^{\circ}$	
UE007	Meristem	1.2 ± 0.2^{b}	$0.9\pm0.2^{\circ}$	$0.5{\pm}0.2^{\circ}$	
	Nodal cutting	1.2 ± 0.2^{b}	$0.7{\pm}0.2^{\circ}$	$0.6\pm0.2^{\circ}$	
Mean vine weight	(g)				
SA-BNARI	Meristem	$145.5{\pm}12.1^{\circ}$	149.5±12.1°	506.7±12.1ª	
	Nodal cutting	$117.7{\pm}12.1^{\circ}$	$132.5 \pm 12.1^{\circ}$	$246.7{\pm}12.1^{\rm bc}$	
UK-BNARI	Meristem	$116.6 \pm 12.1^{\circ}$	121.5±12.1°	296.7 ± 12.1^{b}	
	Nodal cutting	65.6±12.1°	83.3±12.1°	103.0±12.1°	
UE007	Meristem	116.0±12.1°	132.9±12.1°	420.0±12.1ª	
	Nodal cutting	102.4±12.1°	132.4±12.1°	134.2±12.1°	

DAP: Days after planting. Values in the same column followed by same superscripts are not significantly p<0.05 different based on Least Significant Differences (LSD)

Plant height, number of branches and leaves: Both meristem and nodal cutting derived plantlets were well established in the pots, two weeks after transplanting. Plant height at ten weeks after transplanting (10 WAP) varied significantly depending on the accession and the source of the explants. With the exception of UE007, meristem derived plants grew faster in height than nodal cutting derived plants (Fig. 4a). Of the three accessions, UK-BNARI had the highest height from both meristem and nodal cutting derived plants (307.9 and 217.5 cm, respectively) than the remaining accessions indicating that it grew faster than the remaining accessions. The accession UE007 had the least growth in height (121.7 and 156.3 cm, respectively). There were highly significant differences among the accessions and the sources of explants. For number of branches produced, it was significantly (p<0.05) affected by the accession. However, no significant difference was established between the sources of explants after 10 WAP of transplanting. The highest number of branches per plant was obtained from nodal derived plants compared to meristem derived plants (Fig. 4b). Generally, the number of branches developed was low in UK-BNARI (10.7 and 14.1 meristem and nodal cuttings, respectively) while SA-BNARI developed the highest branches (Fig. 4b).

The number of leaves developed after the ten weeks of transplanting (10 WAP) followed a similar trend as that shown by the branches. The number of leaves per plant ranged from 246.7-468 for meristem derived plants while for nodal derived plant it ranged from 302.5-479.6. The highest (468) number of leaves per plant for meristem derived plant was obtained from UE007 whereas the least (246.7) was obtained from UK-BNARI. Also, for nodal derived plants, the highest

(479.6) was observed from SA-BNARI and the least (302.5) from UK-BNARI (Fig. 4c). In this particular parameter, nodal cutting derived plants significantly developed more leaves than meristem derived plants in all the tested accessions.

Effect of sources of explants on tuber development and morphology: The effect of source of explants (meristem or nodal cutting) on number of tubers developed at 30, 60 or 90 days after transplanting in pots is shown in Table 6. The number of tubers produced by meristem derived plants increased from 30-90 days in almost all the three accessions while for nodal cutting derived plants the trend of tuber production was not consistent as the number of tubers produced was comparatively lower at 90 days after transplanting. Generally, meristem derived plants significantly (p<0.05) produced more tubers (Fig. 5) than nodal cutting derived plants. The highest

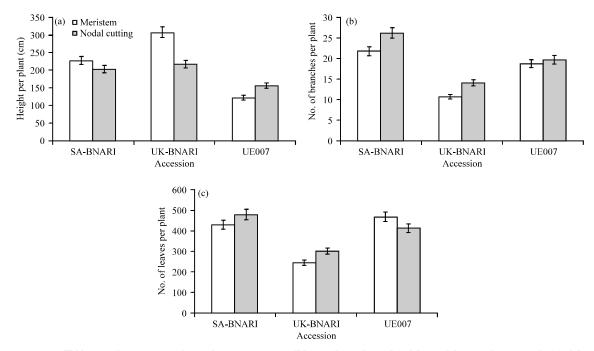


Fig. 4(a-c): Effect of source of explants on (a) Plant height, (b) No. of branches and (c) No. of leaves ten weeks after planting

Table 6: Effect of source of explants and days to harvest on number of tubers per plant, tuber weight per plant after 30, 60 and 90 and percentage tuber dry matter

		No. of tubers per plant (DAP)			Tuber weight per plant (g) (DAP)			Tuber dry matter(%)(DAP)	
Accessions	Sources of explant	30	60	90	30	60	90	90	
SA-BNARI	Meristem	5.3 ^{bc}	5.7 ^{bc}	5.8 ^{bc}	45.1 ^d	139.5°	277.3 ^b	28.2 ^{ab}	
	Nodal cutting	3.8°	5.5^{bc}	3.3°	20.3^{de}	95.8 ^{cd}	183.3°	$27.1^{\rm b}$	
UK-BNARI	Meristem	2.6°	6.8^{b}	9.8ª	68.2^{cd}	115.0°	406.7ª	25.9 ^b	
	Nodal cutting	$4.3^{ m bc}$	3.8°	4.0^{bc}	$42.1^{\rm d}$	$83.7^{ m cd}$	170.0°	23.4°	
UE007	Meristem	$4.7^{ m bc}$	3.5°	4.9^{bc}	21.3^{d}	59.2^{d}	340.0ª	29.5ª	
	Nodal cutting	3.7°	$4.2^{ m bc}$	3.2°	6.1°	50.5 ^d	127.5°	29.6ª	

Values in the same column followed by same superscripts are not significantly (p<0.05) different based on Least Significant Differences (LSD)



Fig. 5(a-c): No. of storage roots harvested from meristem derived plants per stand at 90 DAP (a) SA-BNARI, (b) UK-BNARI and (c) UE007

number of tubers (9.8) was produced by meristem derived plants of UK-BNARI after 90 days of transplanting (Fig. 5) while the lowest was produced by UE007 nodal cuttings harvested at 90 days.

The weight of tubers in both meristem and nodal cutting derived plants increased from 30-90 days after transplanting. At 90 days after transplanting, the weight of tubers was at least more than four times that at 30 days and three times that at 90 days after transplanting. The difference in weight of tubers between meristem and nodal cutting derived explants was highly significant (p<0.05) at 90 days after planting in all the accessions. At 30 and 60 days after transplanting, the difference between the meristem and nodal cuttings was slightly significant only in UK-BNARI. Again the tuber weight was higher (406.7 g) in meristem derived plants of UK-BNARI and lower (127.5 g) in nodal cutting derived plants of UE007.

Table 6 presents the percentage dry matter content of tubers of three accessions of sweet potato examined. The statistical analysis revealed highly significant (p<0.01) differences among the accessions. Again, the percentage dry matter was independent of the source of explants used to derive the plants. The local accession (UE007) produced the highest percentage dry matter for plants regenerated from meristem (29.5%) and nodal cuttings (29.6%) followed by SA-BNARI

(28.2 and 27.1% for meristem and nodal cuttings derived plants, respectively) and UK-BNARI (25.9 and 23.4% for meristem and nodal cuttings derived plants, respectively), respectively.

DISCUSSION

Shoot initiation on BAP or kinetin amended medium: The deployment of in vitro regeneration techniques can play a tremendous role in the sweet potato production system. The technique has the advantages of producing disease-free planting materials at a faster rate for both commercial and subsistence farmers involved in the production of the crop. In vitro regeneration is based on totipotency of plant cell which in many cases are enhanced by addition of exogenous growth regulators which play a critical role in shoot initiation from meristematic tissues. Cytokinins have the ability to initiate cell division in meristematic cells (George et al., 2008). Thus, the presence of BAP or kinetin in the MS basal medium induced calli in the meristem explants of sweet potato accessions prior to shoot development. However, the intensity of the callus development depended on the concentration of the cytokinin in the culture medium as relatively higher concentration of either BAP or kinetin led to higher degree of callus formation. Also, it was observed that calli formation was comparatively lower in kinetin amended medium than BAP medium. In micropropagation, regeneration of plantlets without calli formation is critical to the maintenance of genetic fidelity of the clones being produced (Chalageri and Babu, 2012; Liu et al., 2011). It has been shown that genetic instability or infidelity is caused by dedifferentiation of tissues which occur via calli formation (Kaeppler et al., 2000). While calli derived instability, a phenomenon known as somaclonal variation is not good for producing in vitro plants or micropropagation, in plant breeding, it has often been used for producing useful traits in plants (Larkin and Scowcroft, 1981).

Additionally, calli formation has been reported to retard shoot development in many cultured tissues (Karthikeyan et al., 2009; Ozturk et al., 2004). Thus, it took about 3-5 weeks for shoot development from the calli of the sweet potato accessions in this study. The number of shoots developed also depended on the type of cytokinin (BAP or kinetin) as well as their concentration in the culture medium. BAP amended medium developed more shoots than kinetin modified medium indicating the potency of BAP over kinetin. Independent reports by Yang (2010), Barka (2009) and Wondimu (2009) have shown that BAP is a useful growth regulator for shoot development from meristematic explants of sweet potato.

BAP and GA_3 on shoot elongation and development: The presence of excessive callus formation from the meristem delayed shoot initiation and development. Thus, callus induced from meristematic explants with or without shoots were transferred to a fresh MS medium containing low concentration of BAP (0.25 mg L⁻¹), 0.1 mg L⁻¹ NAA and 0.1 mg L⁻¹ GA₃ for further differentiation and/or elongation and root development. NAA is an auxin known to influence adventitious root formation (Pop et al., 2011) while GA_3 enhances shoot elongation (Alam et al., 2010). The presence of these two growth regulators led to proper shoot differentiation of the plantlets. The effects of GA_3 on shoot elongation are well documented in several plants cultured in vitro including Ipomoea batatas (Alam et al., 2010; Barka, 2009; Wondimu, 2009), potato (Nagib et al., 2003), Cephaelis ipecacuanha (Isogai et al., 2008) and Acacia sinuate (Vengadesan et al., 2002). The positive effect of GA_3 may be attributed to the intensification of soluble carbohydrates which provide a source of energy for shoot development and other metabolic processes (Sexton and Roberts, 1982). However, the extent of shoot development was influenced

by the accessions suggesting genotypic influence. The shoots produced developed roots compared to those initiated on the initiation medium where there was no root development suggesting that the presence of as low as 0.1 mg L⁻¹ NAA was effective in adventitious root development. NAA has been reported to induce root formation in sweet potato (Yang, 2010). According to Alam *et al.* (2010) rooting in sweet potato can be achieved with low or no auxin at all in the culture medium since sweet potato is associated with rooting even without exogenous addition of auxin. Even though the number of plantlets that developed roots did not differ significantly among the accessions, root length in the accessions differed and this might be due to differences in genotypic response of the sweet potato accessions to growth and development.

Shoot regeneration from nodal cuttings: In micropropagation, different explants are used as a source of explants. Thus, to overcome the long delay in shoot initiation from meristematic explants, nodal cuttings were used to regenerate shoots from the sweet potato accessions. Using the same cytokinin, BAP, the nodal cuttings developed shoots in a shorter time without callus formation compared to their initiation from meristematic explants. Rapid shoot production from nodal cuttings and subsequent multiplication are prerequisite step for in vitro clonal propagation of elite cultivars to be released for farmers. Nodal cuttings therefore provide a reliable source of explants for rapid micropropagation of sweet potato for farmers. However, to provide disease free planting materials, meristem culture should be used for initial elimination of any systematic diseases before subsequent use of nodal cuttings for rapid multiplication for farmers. The height of shoots of the developing plantlets was influenced by the concentration of BAP in the culture medium. Lower concentration of BAP led to increased height while an increased concentration decreased the height of the plantlets. The reduction in the shoot height with the increased in BAP concentration in the culture medium may be as a result of inhibitory effect of BAP at higher concentration on shoot elongation. According to George et al. (2008) higher concentration of BAP inhibits shoot elongation. Most of the shoots developed roots which enhanced rapid growth and leaf development.

Post flask acclimatisation and establishment of plantlets: Post flask survival rates of meristem regenerated plantlets were comparatively higher than those of nodal cutting plantlets. Prerequisite for post flask survival is vigorously growing plantlets with well developed rooting system for absorption of nutrients and establishment. Meristem derived plantlets had well developed roots than nodal cuttings. Thus, they were able to establish better under post flask acclimatisation conditions than nodal cutting derived plantlets. The longer root length observed in meristem derived plantlets may be attributed to NAA in the culture initiation medium. El Far et al. (2009) and Ozturk et al. (2004) also observed that meristem derived plantlets on NAA amended medium had longer root length and this may explain the comparatively higher post flask acclimatisation.

Sources of explants on tuber development: Both meristem and nodal cutting derived plantlets acclimatised before transplanting to pots for tuber evaluation were well established after 30 days of transplanting. Due to well root development aforementioned, meristem derived plant were better established than nodal cutting derived plants. Subsequently, meristem derived plants grew vigorously and developed more leaves compared to nodal cutting derived plants. Again, leaf area development was affected by the sources of explants used. Higher leaf area was observed in nodal

derived plants at 30 and 60 DAP than meristem derived plants. There was a steady increase in the leaf area from 30 DAP, reaching a maximum at the 60 DAP then decreased at the 90 DAP irrespective of the sources of explants. The observations may be due to the high production of assimilates in the leaves which might have caused cell division and expansion in the leaf during the 30 and 60 DAP then reduces during its accumulation in the storage organs. Similar observations have been made by Bourke (1985) and Li and Kao (1985) in sweet potato.

To determine the best source of explants that will produce more tuber number and yield for its deployment into sweet potato production system, plants derived from meristem explants and nodal cutting explants were assessed on post flask tuber development. It was observed that the number of tubers produced per plant increased from 30-60 DAP for both meristem and nodal derived plants. The maximum numbers of tubers per plant for meristem derived plants were observed at 90 DAP. On the other hand, nodal derived plants reached the maximum tuber number per plant at 60 DAP, though, the number produced was lower than those obtained from meristem derived plants at the same time and then decreased at 90 DAP. These observations may be attributed to the higher number of leaves observed in meristem derived plants than nodal derived plants. This confirms the earlier report by Hahn (1977) that the yield and number of tubers of sweet potato depend on the number of leaves and the ability of the leaves to produce assimilates and the extent to which they can be accumulated by the sink signified by the organs that are harvested.

Percentage dry matter content was generally higher for the cream flesh (UE007) compared to the orange fleshed accessions SA-BNARI and UK-BNARI which were all introduced. These results are consistent with of Brabet *et al.* (1998) who reported that orange flesh sweet potato genotypes have lower percentage dry matter than the white/cream or yellow fleshed genotypes.

CONCLUSION

It could be concluded from the present study that, the regeneration of sweet potato *in vitro* was highly influenced by the type of cytokinin in the culture medium. Meristem explants reacted to the culture media growth regulators by forming callus which delayed shoot emergence. Among the two cytokinins used kinetin amended medium produced shoots with minimal callus formation compared to BAP amended medium.

While meristem culture delayed shoot initiation, nodal cutting explants enhanced shoot development and thus could be deployed in the sweet potato production system to enhance rapid multiplication of the plant for farmers. However, post flask survival was high among meristem derived plants than nodal cutting plants.

Meristem derived plants produced higher number of tubers than nodal cutting derived plants which is an index for total root yield indicating meristem culture should be used to produce sweet potato propagules for farmers. This will increase yield in sweet potato production system in the country.

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