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In vitro propagation of sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars

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ABSTRACT

The study is aimed at establishing a simple protocol for in vitro regeneration of sweet potato with a view to providing planting materials to farmers as well as basis for genetic improvement. Axillary buds were excised and cultured on Murashige and Skoog (MS) basal salts supplemented with 6-benzyl aminopurine (BAP), gibberellic acid (GA_3) and naphthalene acetic acid (NAA) singly or in combination. The shoot height and number of leaves differed significantly among the cultivars. The result also indicated significant difference ($p< 0.01$) among the cultivars with King J recording the highest mean values. Significant differences ($p< 0.05$) was also recorded in the media combination with respect to organogenesis and number of shoots obtained. The results of hardening further revealed 33.33% success in the explants transferred directly to the field, as well as for the plantlets that were gradually weaned in a mixture of 3:1 sand and biochar.

Key words: Gibberellic acid, Murashige and Skoog, Naphthalene acetic acid, Sweet potato, 6-benzyl amino purine.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a dicotyledonous plant belonging to the family, Convolvulaceae. The edible tuberous root is long and tapered, with a smooth skin whose colour ranges between yellow, orange, red, brown and purple. It is typically a smallholder's crop tolerant to adverse growing conditions and often grown on marginal soils with limited inputs (Namanda *et al*., 2011). It is grown worldwide in about 8.1 million hectares with total annual production of 106 – 110 million tons (FAO 2008, 2011). Sweet potato is also source of minerals, vitamins and antioxidants. A number of varieties have been proved to be good sources of beta carotene, the precursor of vitamin A (Islam, 2006; Pfeiffer and McLafferty, 2007; USDA, 2007; Tumwegamire *et al*., 2011).

Sweet potato is highly recalcitrant in generation and response to tissue culture. It is also commonly propagated using vegetative propagation, a technique that favours propagation of viruses infected materials with consequent low yield and high input demand thereby discouraging growers (Aloufa, 2002; El-Afifi *et al*., 2012). The use vegetative planting materials is also hard to come by particularly when dealing with larger fields. . Through plant tissue culture, large number of uniform and healthy plants are produced (Doliñski and Olek, 2013). The use of bud explants was reported to be the best means for regeneration of large planting materials in sweet potato (Doliñski and Olek, 2013). A number of factors including genotypes, nature and doses of different growth regulators are found to determine the rate and nature of regeneration of sweet potato (Shaibu *et al*., 2016). Thorpe (1994) reported plant growth regulators to play a significant role on the possibility for callus formation, organogenesis, or somatic embryogenesis to occur in culture. Plant growth regulators in combination with other nitrogen sources make up the culture medium that supply all the essential mineral ions required for plant growth and development.

Sweet potato productivity in the sub-Saharan Africa is limited due to abiotic and biotic constraints, lack of efficient strategy for multiplication and distribution (Kapinga *et al.*, 2007; ASARECA, 2008; Ogero *et al*., 2011). Recycling sweet potato vine as planting material accumulates systemic pathogens due to exposure to virus vectors such as aphids and whiteflies in the field. Single and complex infection of sweet potato virus diseases can contribute to subsequent yield decline (Adikini *et al*., 2015); a constraint to the crop productivity leading to significant reduction in yields and quality of the crop. This compelled the need to come up with reliable and efficient methods for disease-free sweet potato planting materials and effective genetic improvement of the crop (Sivparsad and Gubba, 2012). An organized protocol for regeneration of planting material will serve in effective improvement of sweet potato. Improved planting materials will motivate growers to cultivate the crops and significantly contribute in poverty alleviation through improved seed quality and through the distribution of improved varieties (Barker *et al.*, 2011). Plant cell and tissue culture provides a promising leads toward achieving these.

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This research was therefore aimed at establishing a simple protocol for in vitro regeneration of sweet potato with a view to providing planting materials to farmers and also to provide a basis for genetic improvement.

MATERIALS AND METHODS

The experiment was conducted at the plant tissue culture laboratory, Centre for Dryland Agriculture, Bayero University Kano, Nigeria (11.97762^{0}N, 008.42567^0 E) 461m a.s.l. Two cultivars (King J and Mothers delight) sourced from National Root Crops Research Institute, Umudike, Nigeria were used. Axillary buds were excised from vigorously growing plants from each of the two cultivars and brought to the laboratory (Fig-1). These were washed under running tap water and later with detergent. Surface sterilization of the explants were carried out using 70% ethanol for 1 minute, washed three times with autoclaved distilled water and then followed by 0.1% Mercury chloride for 15 minutes (Shaibu *et al*., 2016; El-Afifi *et al*., 2012). The explants were rinsed again with autoclaved distilled water, cut to appropriate sizes and inoculated into Murashige and Skoog (MS) media (Murashige and Skoog, 1962) supplemented with various combinations of growth regulators.

The media composed of the MS basal salts supplemented with 6-benzyl aminopurine (BAP), gibberellic acid (GA_3) and naphthalene acetic acid (NAA) singly or in combination. Seven different media combination were prepared for each of the two cultivars. Sucrose (30 g/l) was added to each of the media and pH adjusted to 5.7 before adding agar (8 g/l). The media were autoclaved at 121°C, 15 psi for 20 minutes. The media combinations were:

A. MS + 0.5mg/l BAP + 0.0mg/l GA_3 + 0.05mg/l NAA
B. MS + 0.5mg/l BAP + 0.5mg/l GA_3 + 0.05mg/l NAA
C. MS + 0.5mg/l BAP + 1.0mg/l GA_3 + 0.05mg/l NAA
D. MS + 0.5mg/l BAP + 2.0mg/l GA_3 + 0.05mg/l NAA
E. MS + 5.0mg/l BAP
F. MS + 0.1mg/l BAP + 0.1mg/l GA_3 + 0.01mg/l NAA
G.. MS + 0.25mg/l BAP + 0.01mg/l GA_3 + 0.2mg/l NAA

Giberrellic acid (GA_3) was sterilized by membrane filtration and added to the autoclaved media when the latter has cooled to around 40^{0}C just before the setting of agar. 50 ml of the media were dispensed into autoclaved bottle jars. The media combination served as the experimental



Fig-1: Explant before surface sterilization

treatments and was laid out in completely randomized design with seven replicates. Three buds explant were inoculated in each media. The inoculated media was kept in the incubation chamber at 25 ± 2^{0}C under white fluorescent light (16 hour photoperiod) with an intensity of 25–30 µmol m^{-2} s^{-1}. In hardening (weaning) sets of plantlets were taken directly from the laboratory to the field and transplanted in a net tunnel. Another set of plantlets were transferred to a plastic pot of top soil, while the third set of the plantlets were transferred to a mixture of 3:1 sand and biochar. Top soil was collected in plastic pots, watered and after two days sweet potato plantlets were transferred to the pots.

Data were recorded on number of shoots, height of shoots, number of callus, degree of callus, number of roots, length of roots, number of leaves and survival rate. This was analysed using mixed procedure of SAS (SAS, 2015). Trend analysis was also carried out to determine the pattern of response of sweet potato cultivars to GA_3 concentrations.

RESULTS AND DISCUSSION

Significant ($p< 0.05$) differences was observed between the media used for number of explant that transformed into shoot, number of shoots produced and survival rate of the inoculated explants (Table 1). The shoot height and number of leaves also differed significantly among the cultivars. Mean performance of sweet potato cultivars and the effect of media on parameters recorded are presented in Table 2. This showed that King J produced the longest shoot (1.5 cm) as compared to Mothers Delight (0.25 cm). Similarly, more leaves was produced by King J. Response of sweet potato may be variety dependent and this could likely be a reason for the variation observed in the two cultivars.

Table 1: Effects of media, cultivar and their interactions on some growth parameters of sweet potato.

Source	DF	Shoot height (cm)	Root length (cm)	†Shoot number	†Callus number	Degree of callus	Number of root	Number of leaves	Number of shoot%	Survival
Rep	6	4.36*	104.38*	6.81	6.55	0.16	0.13	0.48*	0.25*	0.93
Media (M)	6	2.88	27.19	31.66*	24.70	0.13	0.08	0.23	0.20*	4.13
Cultivar (C)	1	19.21**	0.13	0.16	2.63	0.17	0.37	1.65**	0.24	2.97
M*C	5	0.57	37.34	16.27	8.52	0.00	0.00	0.02	0.13	1.61
Error	31	1.62	36.30	12.34	12.09	0.08	0.11	0.19	0.06	1.75

† = percent numbers of explant transformed into shoot or callus

Table 2: Media and cultivar use effect on some growth parameters on sweet potato.

Treatment	Shoot height (cm)	Root † length (cm)	Shoot number	†Callus number	Degree of callus	Number of root	Number of leaves	Number of shoot %	Survival
Cultivar (C)									
King J	1.50	3.66	36.72	63.28	1.38	0.86	1.24	1.05	83.10
Mothers Delight	0.25	3.76	32.02	67.98	1.16	0.42	0.05	0.58	77.22
p-level	0.0017	0.9533	0.9089	0.6439	0.1548	0.0693	0.0063	0.0599	0.2019
Media (M)									
A	1.72	6.15	49.83	50.17	1.40	0.90	1.40	1.30	84.00
B	1.54	1.57	42.86	57.14	1.29	0.43	1.29	0.86	85.71
C	0.81	3.26	18.75	81.25	1.63	0.75	0.75	0.75	85.00
D	1.09	4.79	40.63	59.38	1.25	0.75	0.75	1.25	94.64
E	0.13	0.00	0.00	100.00	1.00	0.00	0.00	0.00	50.00
F	0.74	4.57	57.14	42.86	0.71	0.86	0.29	0.86	71.43
G	0.00	2.29	0.00	100.00	1.43	0.43	0.00	0.00	64.29
p-level	0.1373	0.6149	0.039	0.0895	0.1607	0.6088	0.3359	0.0148	0.0535
Interaction									
C × M	NS	NS	NS	NS	NS	NS	NS	NS	NS

† = percent numbers of explant transformed first into shoot or callus

The results further revealed media A (MS + 0.5 mg/l BAP + 0.0 mg/l GA_3 + 0.05 mg/l NAA) as having the highest mean values for shoot height (1.72 cm) and number of leaves (1.40). This was followed by media B (MS + 0.5 mg/l BAP + 0.5 mg/l GA_3 + 0.05 mg/l NAA) (1.54 cm) and (1.29) respectively. It equally recorded the highest mean values of number of shoots (1.30) followed by B and F (MS + 0.1 mg/l BAP + 0.1 mg/l GA_3 + 0.01 mg/l NAA) with mean value of both 0.86; number of root (0.90) and root length (6. 15 cm) followed by F and D respectively. This corroborates with the works of Dugassa and Feyissa (2011) who reported that 1 mg/l BAP + 1 mg/l GA_3 + 0.01mg/l NAA and 1 mg/l BAP + 2mg/l GA_3 + 0.01 mg/l NAA gave the highest shoot induction rate in some sweet potato cultivars (Awassa-83 and Awassa local) in Ethiopia.

Furthermore, media E (MS + 5.0 mg/l BAP) and G (MS + 0.25 mg/l BAP + 0.01 mg/l GA_3 + 0.2 mg/l NAA) showed 100% number of explants that first transformed to calluses before shooting (Fig. 2). This recorded the least mean value (0.00) in number of leaves. This is because all the explants inoculated in such media produced callus initially and will take time before it shoots and produced leaves as compared to others that had to go through direct organogenesis. This media also recorded the least mean value in all the measured parameters except for shoot height and degree of callusing. Addae-Frimpomaah *et al* (2014) obtained callus in media supplemented with low concentration of BAP or kinetin (KIN). They reported 0.25 mg/l BAP to have given the highest result of shoot induction (80%). Media F (MS + 0.1 mg/l BAP + 0.1 mg/l GA_3 + 0.01 mg/l NAA) also gave the highest number of number of explants (57.14) that first transformed into shoots. This was followed by media A (49.83). Genotypes, nature and concentration of growth hormones in a media were reported to affect the rate of propagation as well as nature of growth of different plant species including sweet potato (Shaibu *et al*., 2016). This also corroborated with the findings of several authors; Thorpe (1994); Sihachakr *et al* (1997); Gubba and Sivparsad (2002) and Gaba (2005) who reported that right hormonal combination also determines what obtained from the inoculated sweet potato explants. Varied responses to different cytokinins (BAP; Thidiazuron, TDZ; KIN; N6-(2-isopentenyl) adenine, 2ip and Phloroglycenol, PG) was

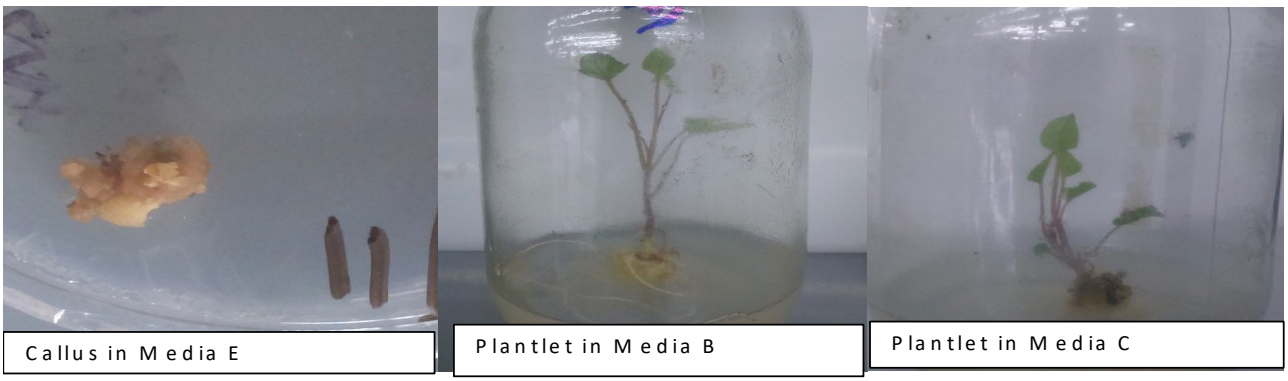


Fig-2: Callus and plantlets from some of the media combinations

Table 3: Trend analysis of media use effect on some growth parameters of sweet potato cultivars.

Source of variation	DF	Shoot height (cm)	Root length (cm)	†Shoot number	†Callus number	Degree of callus	Number of root	Number of leaves	Number of shoot	%Survival
Media	6	28.78	0.15	2.13	0.14	0.09	0.18	21.52	32.89	4.57
Lin	1	25.35	0.01	6.13	0.36	0.03	0.21*	27.69	48.79*	6.88
Quad	1	3.68	0.00	0.53	0.07	0.00	0.28	21.96	13.01	0.71
Cub	1	107.69**	0.72**	0.84	0.19	0.03	0.14	0.64	107.01	5.35
Quart	1	7.37	0.00	3.19	0.14	0.00	0.00	8.77	5.76	0.26
Deviations	2	14.3	0.10	1.06	0.04	0.23	0.21	35.03	11.38	7.12
Cultivar	1	1.71	0.00	12.38	0.91	0.25	0.11	19.75	5.80	0.52
Media.cult	6	14.35	0.05	2.52	0.16	0.04	0.12	50.30	20.04	1.63
Lin.cult	1	26.4	0.07	3.18	0.17	0.01	0.31	60.48	26.88	3.48
Quad.cult	1	2.92	0.00	2.31	0.19	0.02	0.02	5.08	16.42	1.51
Cub.cult	1	17.94	0.01	5.37	0.34	0.20	0.32	169.87	55.30	1.80
Quart.cult	1	30.73	0.15	3.10	0.21	0.02	0.01	17.68	13.99	1.47
Deviations	2	4.05	0.02	0.58	0.04	0.00	0.02	24.35	3.82	0.75
Residual	26	11.82	0.09	1.67	0.19	0.10	0.07	28.10	10.88	1.79

† = percent numbers of explant transformed first into shoot or callus
*, ** = significant at 5% and 1% level of probability, respectively



Fig-3: Hardened plants

recorded with TDZ (1mg/l) thus giving the highest shoot multiplication rate (El-Afifi *et al.*, 2012). Successful regeneration of sweet potato was also reported by Mukherjee (2002) and Onwubiko *et al.* (2015) in media supplemented with BAP, GA_3 and NAA.

The trend pattern of the media combinations for GA_3 are presented in Table 3. This showed that shoot height and root length had significant cubic effects. This is an indication that increasing the GA_3 concentration will lead to increase in shoot height and root length up to a certain level after which they tend to decrease. Any further increase in the concentration will lead to increase in these parameters. The effect of GA_3 on number of roots and shoot was linear. Increasing GA_3 concentration will lead to a proportionate increase in number of roots and shoots. As such there is no optimum concentration of GA_3 for induction of high number of roots and shoots in sweet potato. The result of hardening revealed 33.33% success in both weaning in a mixture of 3:1 sand and biochar (Figure 3B) and under net tunnel (Figure 3A)

CONCLUSION

The present study affirmed *in vitro* culture to be an important method of regeneration of healthy sweet potato planting materials. King J cultivar recorded the best response. It was also established that media passage was not necessary as the induction of shoots and roots took place on the same media. Since our target was planting materials, further study is recommended with a view to meet farmers' changing preferences. Basis for genetic manipulation was also achieved with the establishment of calluses and high amount of 6-benzyl amino purine in the media singly or in combination with other growth regulators.

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