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IN VITRO PROPAGATION OF SWEET POTATO *(Ipomoea batatas (L.) Lam.)* THROUGH LATERAL BUD CULTURE

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Abstract

Sweet potato is economically important root crop that belongs to the family Convolvulaceae. The production of sweet potato has been generally low mainly due to a multitude of biotic and a biotic stresses. For callus inductions, lateral bud of two varieties, Kullufo and Tulla sweet potato were cultured on MS basal medium contained 30 g/l sucrose, 7 g/l agar, and vitamins supplemented with 1, 2, 3 and 4 mg/l of NAA combined with 2 mg/l of Kn adjusted pH to5.8.The parameters measured were: Percentage of callus formation, Bud forming explants, Length of shoot and root (Cm), fresh and dry weight of Shoot and root (g), rooting response and Survival rate. In shoot multiplication, MS media supplemented with 0.5, 1, 1.5 and 2 mg/l of BAP or 0.5, 1, 1.5 and 2 mg/l of Kn, were used. In root inductions, MS media supplemented with 0.25, 0.5, 0.75 and 1 mg/l of IBA or 0.25, 0.5, 0.75 and 1 mg/l of NAA, were used. In acclimatization, the effect red soil, sand soil and compost culture substrates on the survival of in vitro propagated sweet potato were studied. The results showed that the best callus formation, 97.7 & 95.5%, was observed when MS medium was supplemented with 2 mg/l NAA in combination with 2 mg/l of kinetin. In shoot formation experiment, best shooting was observed at 0.5 and 1 mg/l concentrations of both cytokinins (BAP and Kn). The two PGRs used for rooting (IBA and NAA) had significant rooting effect at 0.25 and 0.5 mg/l. The mixture of red soil, sand soil and compost (1:2:1) cultural substrates showed 81.25% and 70.59% plantlet survival for Kullufo and Tulla varieties, respectively. The optimal protocol for micro-propagation of sweet potato varieties through lateral bud culturing on MS basal medium supplemented with appropriate concentrations of different Plant growth regulators.

Keywords: Auxin, Cytokinin, Kullufo, MS medium and Tulla.

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INTRODUCTION

Most small-scale farmers in Africa and Asia use sweet potato as source of carbohydrate and vitamin for human food and livestock feed [3]. In Ethiopia, sweet potato is cultivated as an important root crop mostly for human consumption, which ranks third after Enset (*Ensete ventricosum*) and potato (*Solanum tuberosum*). Moreover, conventional breeding efforts and genetic improvement have been seriously limited by difficulties in sexual crosses mainly due to self-incompatibility and male sterility, hexaploid nature, dichogamy, seed dormancy, abnormal seed and seedling development within species [12].

Tissue culture techniques have opened a new frontier in agricultural science by addressing food security and emphasis on the use of biotechnological methods for genetic improvement of exacting traits in sweet potato. Little work has been achieved, particularly the exploitation of some clonal variation and genetic transformation [9]. Some plant regeneration procedures through organogenesis and somatic embryogenesis have been described for sweet potato using different explants such as meristem, leaf, stems, petioles, storage roots, anthers and ovaries supplemented with MS medium, sucrose, agar and various plant growth regulators has been successfully used to establish various explants culture of sweet potato cultivars [5].

Shoot initiation from meristem requires 0.5-2.5mg/l of BAP or Kn concentration and combination of BAP and Kn plant growth regulators. Despite of initiation [13], MS medium supplemented with NAA as an auxin source, BAP as a cytokinin and GA3 as gibbrellins to induce shoots from sweet potato bud explants and stated that these substances are critical for shoot initiation in sweet potato varieties. On the other hand, [8] reported that, the lower concentrations of NAA and BAP in initiation medium resulted in higher shoot production in sweet potato.

The present study aimed to evaluate *in vitro* development and the effect of plant growth regulators in culture media interacted with plant variety for initiation of shoot, root and acclimatization of sweet potato varieties through lateral bud explants.

MATERIALS AND METHODS

Lateral bud explants were obtained from the mother plants of Kullufo and Tulla orange flesh sweet potato varieties from Mekelle Agricultural Research Centre (MARC) plant propagation laboratory. Lateral buds (1 cm) were excised using sterile scissor from green house mother plant and put in tap water in 50 ml glass bottle. The explants were then washed with largo soap and rinsed for one hour with tap water and quickly dipped in 70% alcohol for one minute in a

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sterilized bottle. They were then sterilized with 10% (w/v) sodium hypochlorite with three drops of Tween-20 for 15 minutes and rinsed 4 times with sterile double distilled water.

The explants were transferred to callus induction media consisting MS basal medium supplemented with 100 mg/l Myo-inositol, 2 mg/l Thiamin-HCl, 30 g/l Sucrose, 10 m/l Vitamin stock solution and 1, 2, 3 and 4 mg/l of NAA and 2 mg/l of Kinetin at a rate of 1 ml/l were filter sterilised and added to the medium under aseptic laminar air hood [2]. Totally 45 lateral buds from most recently expanded *in vitro* plantlets were cut and placed on medium. Therefore, the total treatment in this experiment was five with three replications arranged randomly on the bench in the laboratory. The laboratory had average temperature of 25±2^{o}C and 16/8 h of photoperiod. The light condition was maintained using white florescent light (20 µ mol $m^{-2}s^{-1}$).

For shoot initiation, MS medium supplemented with 0.5, 1, 1.5, 2 mg/l of BAP or 0.5, 1, 1.5, 2 mg/l of Kn, and combination of the two PGR in a ratio of 0.5 mg/l BAP: 2 mg/l Kn or 2 mg/l BAP: 0.5 mg/l Kn were used (Beyl, 2005). For root initiation about 10 cm long shoots regenerated from lateral bud calli of Kullufo and Tulla orange flesh sweet potato varieties were cultured on MS medium supplemented with 0.25, 0.5, 0.75, 1 mg/l of IBA or 0.25, 0.5, 0.75, 1 mg/l of NAA and combination of 0.25 mg/l IBA: 1 mg/l NAA or 1 mg/l IBA: 0.25 mg/l NAA for root induction. Therefore, the total number of treatments used in this experiment had 11 with 3 replications arranged randomly on the culture media. The culture was maintained in a growth chamber at a temperature of 25±2^{0}C and 16/8 h photoperiod provided by white florescent light of (20 µ mol $m^{-2}s^{-1}$) intensity.

After three weeks, the well rooted *in vitro* propagated sweet potato plantlets were taken out gently from each PGR treatment of the culture media flasks and washed under running tap water to remove traces of agar that prevent the absorption of nutrients from the acclimatization culture substrates by roots. The plantlets were transferred to pots containing soil: red soil, sand soil and compost in the ratio of 1:2:1 and placed in pots, covered with transparent plastic bags (in order to keep humidity) at 25^{o}C with random holes for air circulation and the underside of the pots were drilled for drainage.

Growth Parameters Measured: To select the most suitable plant regeneration method for the local sweet potato cultivars were Percent of Callus forming, Bud forming explants, Average number of days to shoot emergence, mean shoot number, Length of shoots and roots, Shoot and

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root fresh weight, Shoot and root dry weight, Rooting response, mean root number and survival rate.

Data Analysis: Treatment effects in all experiments were determined by using the linear models analysis of variance (ANOVA) and significant differences among treatments were determined by Fisher's Least Significance Difference (LSD) using the SAS (version 9.2). For all the data analysis, $P \leq 0.05$ was considered.

RESULTS AND DISCUSSION

Effect of NAA and kinetin on Callus Induction

Callus was induced from lateral bud explants of both verities of sweet potato, i.e., Kullufo and Tulla. Compared to the control, callus formation was significantly higher when MS medium was supplemented with all concentration levels of NAA mixed with 2 mg/l of kn. However, the best callus formation was observed when MS medium was supplemented with 2mg/l NAA in combination with 2mg/l kinetin. Callus formation did not highly vary with varieties (Table 1). Rise in the concentration of NAA beyond 2mg/l was found to reduce callus formation. The result of this study is also in accordance with that of [10] on callus induction of sweet potato from petiole and leaf explants.

Table 1: Effect of different concentrations of NAA combined with 2 mg/l Kn on the percent of callus formation from lateral bud explants of Kullufo and Tulla sweet potato varieties. Value are mean and n=3

Concentrations of PGRs (mg/l)		Percent of Callus formation	
NAA	**Kn**	**Kullufo**	**Tulla**
0.00	0.00	0.00	0.00
1	2	93.3^{b}	92.2^{b}
2	2	97.7^{a}	95.5^{a}
3	2	92.2^{c}	88.8^{c}
4	2	81.1^{d}	74.4^{d}
Significance		**	**
LSD		4.12	3.25
CV %		3.11	2.52

Mean with same letter within the column are not significantly different at p<0.01 ANOVA, Fisher's least significance difference (LSD). CV = coefficient variation (%),

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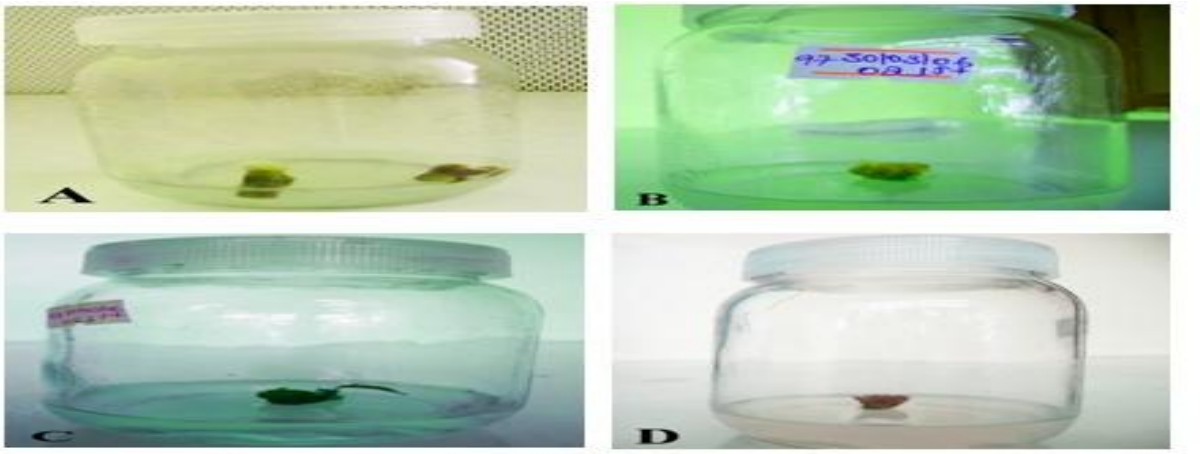

Figure 1: *In vitro* Calli induction of sweet potato explants after two weeks in Culture (A-D). Where, (A) MS medium without PGRs; (B) 1mg/l of NAA and 2 mg/l of Kn; (C) equal concentration of NAA and Kn and (D) 3 mg/l of NAA and 2 mg/l of Kn

Effect of Cytokinins on *In Vitro* Shoot Initiation

The morphogenetic response of explants to BAP and Kn are summarized in Table 2. Compared to the control, both BAP and Kn showed significant positive effect on shoot formation of both varieties of sweet potato at all concentration levels.

Table 2: Effect of supplementation of MS basal medium with different concentrations of cytokinins (BAP and Kn) in sole or combination on the morphogenetic responses of Kullufo and Tulla sweet potato varieties. Values are mean and n=3.

Sweet potato varieties	Cytokinins (mg/l)		Bud forming explants %	No. of days to shoot emergence (n)	No. of shoots per expt. (n)	Shoot length per expt. (cm)	Shoot fresh wt. per expt. (g)	Shoot dry wt. per expt. (g)
	BAP	Kn						
Kullufo	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00
	0.5	0.0	97.76^{ab}	10.666^{k}	19.666^{a}	3.966^{a}	0.946^{a}	0.436^{bc}
	1	0.0	98.87^{a}	12.333^{j}	19.00^{ab}	3.933^{ab}	0.956^{a}	0.453^{a}
	1.5	0.0	85.50^{d}	13.666^{ghi}	16.334^{def}	3.503^{ef}	0.726^{ef}	0.250^{j}
	2	0.0	81.10^{ef}	14.667^{fg}	15.667^{efg}	3.433^{f}	0.706^{fg}	0.233^{kl}
	0.0	0.5	97.73^{ab}	12.333^{j}	16.334^{def}	3.766^{bcd}	0.866^{b}	0.436^{bc}
	0.0	1	95.50^{abc}	13.334^{hij}	17.33^{cd}	3.70^{cd}	0.853^{b}	0.410^{d}
	0.0	1.5	87.73^{d}	17.667^{bc}	14.333^{hij}	3.10^{gh}	0.730^{ef}	0.336^{i}
	0.0	2	84.40^{de}	17.666^{bc}	14.00^{ij}	2.933^{h}	0.493^{j}	0.333^{i}
	0.5	2	93.33^{c}	15.333^{ef}	16.333^{def}	3.666^{de}	0.743^{de}	0.393^{e}
	2	0.5	87.73^{d}	16.667^{cd}	14.666^{ghij}	3.634^{de}	0.593^{h}	0.423^{cd}
Tulla	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00
	0.5	0.0	93.33^{c}	14.333^{fgh}	18.00^{bc}	3.733^{cd}	0.856^{b}	0.440^{ab}
	1	0.0	94.40^{c}	14.666^{fg}	16.331^{def}	3.866^{abc}	0.770^{cd}	0.346^{hi}
	1.5	0.0	87.77^{d}	16.00^{de}	15.333^{efg}	3.434^{f}	0.690^{g}	0.243^{jk}
	2	0.0	81.10^{ef}	16.666^{cd}	15.00^{ghi}	3.366^{f}	0.533^{i}	0.226^{l}
	0.0	0.5	93.33^{c}	13.00^{ij}	16.667^{de}	3.633^{de}	0.710^{efg}	0.373^{f}
	0.0	1	93.30^{c}	14.666^{fg}	15.666^{efg}	3.40^{f}	0.70^{fg}	0.363^{fg}

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	0.0	1.5	77.73^{fg}	18.666^{a}	13.667^{jk}	3.133^{g}	0.330^{k}	0.210^{m}
	0.0	2	74.40^{g}	20.00^{a}	12.667^{k}	3.033^{gh}	0.296^{k}	0.150^{n}
	0.5	2	85.50^{d}	13.333^{hij}	16.332^{def}	3.633^{de}	0.786^{c}	0.340^{hi}
	2	0.5	81.10^{ef}	15.334^{ef}	17.332^{cd}	3.50^{ef}	0.713^{efg}	0.353^{gh}
	Significance		**	**	**	**	**	**
	LSD		4.32	1.1105	1.0624	0.1986	0.0342	0.0165
	CV%		3.558	5.387	4.838	4.12	3.565	3.578

Means with same letter within a column are not significantly different at $p<0.01$ ANOVA, Fisher's least significance difference (LSD). CV = coefficient variation (%), No. = number, expt. = explants and wt = weight.

Percent bud formation of both varieties is significantly higher under both BAP and Kn at all concentration levels when compared with the control ($P<0.01$). Though no significant difference was observed between 0.5 and 1mg/l of BAP on percent bud formation, the effect of BAP at 0.5 and 1mg/l was significantly higher than its effect at 1.5 and 2mg/l on percent bud formation of Kullufo and Tulla varieties. Previously, [11] reported that lateral buds of *I.batatas* explants cultured on MS basal medium supplemented with 1.5 mg/l of BAP produced a maximum of 11.6 shoot buds per explant in contrast to the current finding where 10.98 and 10.85 shoot buds were obtained on full strength MS media supplemented with 1 mg/l of BAP or 0.5mg/l of Kn for the variety Kullufo. This shows that different hormones may have different ability to induce bud formation. Combination of the two PGR in a ratio of 0.5mg/l BAP to 2mg/l Kn enhanced more bud formation in both varieties than their combination in a ratio of 2mg/l BAP to 0.5mg/l Kn.

Number of days to shoot emergence increased with increased in concentration of BAP and Kn supplemented in sole in both sweet potato varieties. This finding is in line with that of [6] who reported the effectiveness of low concentration of BAP to result in rapid shoot multiplication of *I.batatas* cv. Nepali and NARC-IV due to the activation of tRNA cytokinins resulting in rapid proliferation of shoot primordial. Relatively, Kullufo variety had faster response to both PGRs applied in sole than Tulla variety in terms of shoot emergency (Table 2). Combination of the two PGR in a ratio of 0.5mg/l BAP to 2mg/l Kn enhanced more number of shoot per explants in both varieties than their combination in a ratio of 2mg/l BAP to 0.5mg/l Kn. [4] reported that, BAP at the concentration of 5mg/l gives low number of shoot per explant and they concluded shoot number decreases as increase of BAP concentrations.

Though no significant difference was observed between 0.5 and 1mg/l of BAP, the effect of BAP at 0.5 and 1mg/l was significantly higher than its effect at 1.5 and 2mg/l on shoot length shoot fresh weight and shoot dry weight per explant of Kullufo and Tulla varieties. Combination of the

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two PGR in a ratio of 0.5mg/l BAP to 2mg/l Kn enhanced shoot length in both varieties more than their combination in a ratio of 2mg/l BAP to 0.5mg/l Kn. [1] reported that, the effectiveness of cytokinin for shoot induction from shoot tip explants in *I.batatas* L.cv. Possible justification for the concentration of cytokinin increased beyond the optimal need of the plant, they inhibit the release of endogenous cytokinins and assimilation of the given nutrients by inhibiting the activities of enzymes.

Figure 2: *In vitro* shoot multiplication from sweet potato callus after two weeks in culture (A-D). Where, (A) MS medium without PGRs; (B) BAP 0.5 mg/l; (C) BAP 1 mg/l; and (D) 2 mg/l Kn + 0.5 mg/l BAP

Effect of Auxins on In Vitro Root Induction

Compared to the control, morphological response of both IBA and NAA showed significant positive effect on root formation of both varieties of sweet potato at all concentration levels.

Table 3: Effect of supplementation of MS basal medium with different concentrations of auxins (IBA and NAA) in sole or combination on rooting responses of Kullufo and Tulla sweet potato varieties. Values are mean & n = 3.

Sweet potato varieties	Auxins (mg/l)		Rooting response (%)	Mean root no. per expt.(cm)	Root length per expt. (cm)	Root fresh wt. per expt. (g)	Root dry wt. per expt. (g)
	IBA	NAA					
Kullufo	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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	0.25	0.00	100^{a}	9.12^{d}	10.96^{a}	1.73^{cde}	0.15^{b}
	0.5	0.00	95.53^{abc}	9.90^{ab}	10.83^{ab}	1.95^{b}	0.17^{a}
	0.75	0.00	78.83^{h}	8.0^{ef}	7.30^{f}	1.76^{cd}	0.110^{h}
	1	0.00	79.96^{gh}	7.83^{f}	6.83^{fg}	1.60^{ef}	0.053^{j}
	0.00	0.25	95.53^{abc}	9.30^{cd}	10.63^{ab}	2.1^{a}	0.144^{bc}
	0.00	0.5	96.63^{ab}	7.00^{ghi}	9.93^{cd}	2.0^{ab}	0.14^{cd}
	0.00	0.75	87.73^{de}	6.633^{j}	6.73^{g}	1.63^{def}	0.023^{l}
	0.00	1	84.40^{efg}	6.267^{k}	4.73^{h}	1.53^{fg}	0.0333^{kl}
	0.25	1	93.33^{bc}	7.20^{gh}	9.93^{cd}	1.73^{cde}	0.126^{ef}
	1	0.25	92.20^{bcd}	6.867^{hij}	8.83^{e}	1.63^{def}	0.116^{fgh}
Tulla	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.25	0.00	96.63^{ab}	10.10^{a}	9.73^{d}	1.86^{bc}	0.146^{bc}
	0.5	0.00	94.43^{bc}	9.267^{cd}	8.53^{e}	1.73^{cde}	0.123^{efg}
	0.75	0.00	85.50^{ef}	5.967^{kl}	6.63^{g}	1.43^{hg}	0.090^{i}
	1	0.00	83.20^{efgh}	5.767^{l}	4.93^{h}	1.33^{hi}	0.040^{k}
	0.00	0.25	91.10^{cd}	9.567^{bc}	9.86^{b}	1.76^{cd}	$0.13d^{e}$
	0.00	0.5	87.73^{de}	9.20^{d}	8.84^{e}	1.37^{hi}	0.120^{ef}
	0.00	0.75	82.2^{fgh}	7.967^{ef}	4.03^{i}	1.36^{hi}	0.043^{jk}
	0.00	1	82.16^{fgh}	7.33^{g}	3.16^{j}	1.26^{i}	0.033^{kl}
	0.25	1	92.20^{bcd}	6.733^{ij}	10.3^{cb}	1.77^{cd}	0.133^{de}
	1	0.25	91.07^{cd}	5.80^{l}	9.73^{d}	1.53^{fg}	0.113^{gh}
	Significance		**	**	**	**	**
	LSD		5.48	0.35	0.54	0.14	0.012
	CV%		4.46	3.27	4.87	6.03	8.69

Mean followed by the same letter within a column are not significantly different at $P<0.01$ ANOVA, Fisher's least significance difference (LSD). CV = coefficient variation (%), no. = number, expt. = explants and wt = weight.

Percent root responses, number of main root, root length, fresh and dry weight of root of both varieties was significantly higher under both IBA and NAA at all concentration levels when compared with the control ($P<0.01$). Though no significant difference was observed between 0.25 and 0.5 mg/l of IBA on percent root formation, the effect of IBA at 0.25 and 0.5 mg/l was significantly higher than its effect at 0.75 and 1 mg/l on Kullufo and Tulla varieties. The impact of NAA on percent root formation of both varieties showed similar trend to IBA, i.e., higher bud formation at 0.25 and 0.5 mg/l and lower at 0.75 and 1 mg/l (Table 3). Previously, [15] reported that apical meristem of *I.batatas* explants cultured on MS basal medium supplemented with 0.5 mg/l of IBA produced a maximum of 91.6 root formed per explants in contrast to the current finding where 100 and 96.63 root formation were obtained on full strength MS media supplemented with 0.25 mg/l of IBA or 0.5 mg/l of NAA for the variety Kullufo. Moreover, the

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number of root formation obtained was lower for Tulla than Kullufo variety under both auxins used, suggesting variation in plant species or varietal responses to the same PGRs. Combination of the two PGR in a ratio of 0.25mg/l IBA to 1mg/l NAA enhanced more roots formation in both varieties than their combination in a ratio of 1mg/l IBA to 0.25mg/l NAA.

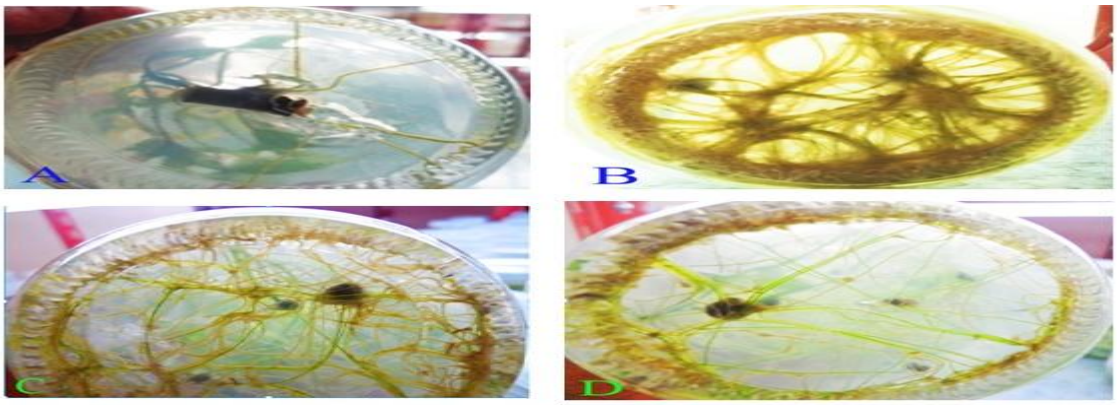

Figure 3: *In vitro* root induction of sweet potato per shootlet explants after two weeks in culture (A-D). Where, (A) MS medium without PGRs; (B) IBA 0.25 mg/l; (C) IBA 0.5 mg/l; and (D) 1 mg/l IBA + 0.25 mg/l NAA

Acclimatization

In vitro induced shoots are very delicate and prone to sudden environmental changes that may damage the plantlets unless it is gradually adapted to the new environment. Thus acclimatization is essential to enable the rooted plantlets to adapt the natural environment in *ex vitro* conditions at temperature of 27^{o}c and 21humidity of green house conditions. In the acclimatization stage of this experiment, a total of 112 well rooted shootlets (56 shootlets from each sweet potato variety) from each treatment of plant growth regulators were transferred to pots containing either moist red soil alone, sand alone, compost alone or a mixture of the three culture substrates at ratio of 1:2:1 and acclimatized for one week by irrigating with water and aeration daily to maintain the humidity of the plantlets. At this mixture culture substrates, it showed significantly maximum (81.25%) and (70.59%) plantlet survival for Kullufo and Tulla sweet potato varieties, respectively. These results are supported by the finding of [7] who reported that the plantlets regenerated from leaf and stem cutting of *S.commersolani* shoots readily rooted when cultured in half strength MS medium that lacks growth regulators and rooted plantlets growth were vigorous and their survival percentage was good in soils. Although the report of [14] showed that plantlets obtained from cytokinins containing media were readily established in green house conditions with a survival frequency of 68-84%.

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Table 5: Effect of different culture substrates on the survival of *in vitro* regenerated plantlets of Kullufo and Tulla sweet potato varieties without PGRs during acclimatization

Types of sweet potato varieties	Types of culture substrates	Total No. of Explants	No. of survived explants	No. of died explants	% of survived explants	% of died explants
Kullufo	Moist red soil alone	13	7	6	53.85	46.15
	Sand soil alone	12	6	6	50	50
	Compost soil alone	15	11	4	73.33	26.67
	Mixture in 1:2:1	16	13	3	81.25	18.75
Tulla	Moist red soil alone	13	5	8	38.46	61.54
	Sand soil alone	12	5	7	41.67	58.33
	Compost soil alone	14	9	5	64.28	35.72
	Mixture in 1:2:1	17	12	5	70.59	29.41

No. = number and % = percent.

Figure 4: Acclimatization of *in vitro* derived sweet potato explants (A-D). where, (A) washed plantlet from agar medium; (B) the explant planted on cocupet tray for two weeks; (C) plantlet transferred into pot containing moist soil, (D) the acclamatized plant develop well green shoots.

CONCLUSION

This study provided optimal protocol for micro-propagation of sweet potato varieties through lateral bud culturing on MS basal medium supplemented with appropriate concentrations of different PGRs in sole or combination. This protocol can thus be utilized to micro-propagate the two sweet potato varieties to boost its production. However, the thesis findings are subject to more refinement of the protocol in the fulfillments.

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