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IN VITRO PRODUCTION OF VIRUS-FREE SWEET POTATO [*IPOMOEA BATATAS* (L.) LAM] BY MERISTEM CULTURE AND THERMOTHERAPY

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ABSTRACT: Viral disease is the major factor causing significant yield loss in sweet potato. Production of disease-free clones by tissue culture technique increases yield and income of farmers. Meristems from three varieties of sweet potato were cultured at different combinations of BAP, GA_3 and NAA in MS basal medium. Among the combinations, 1 mg/l BAP and 1 mg/l GA_3 with 0.01 mg/l NAA resulted in 66.67% shoot induction for Awassa-83 and Guntute while 63.33% shoot induction was obtained using 1 mg/l BAP, 2 mg/l GA_3 and 0.01 mg/l NAA for Awassa local. There was 100% sweet potato virus elimination from all the three varieties by meristem culture as observed by using NCM-ELISA technique. Shoot thermotherapy was done for Awassa-83 and Awassa local at 37°C for 31 days and 88.89% and 100% SPFMV and SPCSV virus elimination was achieved for the two varieties, respectively. Best shoot multiplication was obtained in MS medium containing 2 mg/l BAP for Awassa-83 (5.26 ± 0.02 shoots/explant) and Awassa local (5.12 ± 0.02 shoots/explant). For Guntute it was 2.48 ± 0.03 shoots/explant on 3 mg/l BAP. The best root length was 9.5 ± 0.10 cm, 9.68 ± 0.02 cm, and 11.03 ± 0.02 cm for Awassa-83, Awassa local and Guntute, respectively on growth regulators free ½ MS medium. The highest number of roots per shoot (6.34 ± 0.01) was obtained from Awassa-83 on 0.1 mg/l IBA. Acclimatizations were 100%, 91.11% and 90.10% for Guntute, Awassa-83 and Awassa local, respectively. This work indicates the practical applicability of plant tissue culture using meristem culture and thermotherapy to produce virus-free planting materials of sweet potato.

Key words/phrases: Ethiopia, meristem culture, sweet potato virus

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam) is an important staple food in tropical, subtropical and temperate regions. It is the seventh most important food crop in the world following wheat, rice, maize, potato, barely, and cassava (FAO, 2000). East Africa is the major producer (75%) and consumer of sweet potato in comparison to other parts of the African region (Gibson and Aritua, 2002). World's average sweet potato production is estimated to be 14.8 tones per hectare (t/ha) compared to 8 t/ha in Ethiopia (FAO, 2000). Even though sweet potato is a perennial crop, production as annual for its roots and vine is common practice. It is mostly propagated vegetatively either by vine cuttings or rooted shoots.

In Ethiopia, sweet potato is a widely cultivated root crop in eastern, southern and south-western parts. It grows with a minimum annual rainfall of 750–1000 mm. Sandy and fertile soil with high drainage capacity is optimum for its production (Assefa Tofa *et al.*, 2008). Production is mainly for food but also cultivated as animal feed (Tesfaye Kebede *et al.*, 2008). In Ethiopia, its production as root crop ranks third after *Enset* (*Ensete ventricosum* (W.) Cheesman) and potato (*Solanum tuberosum* L.), it is considered as one of the most important root crops among small scale resource poor farmers (Terefe Belehu, 2003).

The production of root crops in general and sweet potato in particular is limited by different factors among which yield loss caused by virus diseases is the major one. Virus diseases of sweet potato may cause yield loss of up to 100% though the exact figure depends on the type of the virus, cultivar, stage of infection and environmental conditions (Ngeve and Bouwkamp, 1991; Salazar and Fuentes, 2000). The use of storage root and

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vine cuttings as a method for vegetative propagation is the cause for the accumulation of viruses from generation to generation which could result in declining of root yield and loss of superior genotypes. In addition, aphids and whitefly vectors are the main causes of virus transmission (Moyer and Salazar, 1989; Gibson, 2004). Sweet potato virus disease (SPVD), a combination of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV), is the major cause for decline in sweet potato production in East Africa (Gibson *et al*., 1998; Mwanga, 2001).

The distribution and effect of ten sweet potato viruses, namely: Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato Latent Virus (SPLV), Sweet Potato Mild Mottle Virus (SPMMV), Sweet Potato Virus G (SPVG), Sweet Potato Mild Speckling Virus (SPMSV), Sweet Potato Chlorotic Fleck Virus (SPCFV), C-6 virus, Sweet Potato Chlorotic Stunt Virus (SPCSV), Sweet Potato Caulimo-Like Virus (SPCaLV) and *Cucumber Mosaic Virus* (CMV) have been studied and as a result antisera are already available against them (Ndunguru and Kapinga, 2007; Panta *et al*., 2007).

Most of sweet potato plants in southern and south-western parts of Ethiopia are infected by Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato Chlorotic Stunt Virus (SPCSV) and Sweet Potato Virus G (SPVG) with *SPFMV* being the most prevalent virus (Tewodros Tesfaye, 2010). Due to this problem of serious virus infection, Hawassa Agricultural Research Centre has already stopped distribution of sweet potato planting materials to farmers (Daniel Dauro, personal communication).

Eradication of viruses could result in more than double increase of yield (Ying and Davenport, 2004). Production and supply of virus-free planting material to farmers is, therefore, an essential step in countries like Ethiopia where virus disease is severely affecting sweet potato. Production of disease-free planting material by tissue culture techniques and dissemination of plantlets to farmers has been found to increase the income of farmers. It is also a means to improve the root quality. Tissue culture techniques such as meristem culture and shoot tip thermotherapy, are used for elimination of viruses in many crops (Lizarraga *et al*., 1992; Juan *et al.*, 2004; Ying and Davenport, 2004; Panta *et al*., 2006). Virus elimination was also done using high temperature treatment (34–37°C) in sweet potato (Panta *et al.,* 2006) and at 38°C in grapevine (Nicholas, 2006) and in rosaceous fruit trees (Spiegel *et al*., 1985) for one month. To this effect, in Ethiopia, development and application of tissue culture techniques for virus elimination is at its early stage. Hence, this work is one of the pioneer works in developing *in vitro* propagation protocol and virus elimination techniques.

The objective of this study was to determine the extent of virus elimination from three varieties of sweet potato: Awassa local, Awassa-83 and Guntute using meristem culture and shoot tip thermotherapy. Furthermore, the optimum concentrations of growth regulators for shoot induction, multiplication and rooting have been worked out for sweet potato virus elimination and also the efficiency of meristem culture and thermotherapy have been evaluated and compared.

Abbreviations: BAP, 6-Benzyl Amino Purine; GA_3, Gibberellic Acid; IBA, Indole Butyric Acid; NAA, α-Naphthalene Acetic Acid; NCM-ELISA, Nitrocellulose Membrane-Enzyme Linked Immunosorbent Assay.

MATERIALS AND METHODS

Plant materials

Three varieties of sweet potato, Awassa local, Awassa-83 and Guntute, were used for the study. These varieties were obtained from Hawassa Agricultural Research Centre, Southern Nations Nationalities and Peoples' Regional State (SNNPR), 273 km south of Addis Ababa, Ethiopia, in August 2008. Vine cuttings of about 25 cm long were planted and grown in pots in glasshouse at the College of Natural Sciences, Addis Ababa University.

Media preparation

MS basal medium (Murashige and Skoog, 1962) was used in this study. Stock solutions of MS macronutrients, micronutrients and vitamins were prepared and stored at -20°C until required for media preparation. Likewise, stock solutions of growth regulators were separately prepared and stored at +4°C for immediate use. To prepare the media, 100 ml/l macronutrients, 10 ml/l micronutrients, 10 ml/l vitamins from stock solutions with the addition of 30 g/l sucrose

were used. This was followed by the addition of required concentrations of growth regulators and the volume was set to the required level. Before the addition of 0.7% (w/v) agar, the components were mixed using magnetic stirrer and the pH was adjusted to 5.8 using either 1M NaOH or 1M HCL. The media were autoclaved at 121°C for 15 minutes and about 60 ml medium was dispensed into sterile Magenta boxes for multiplication and rooting and 40 ml into baby food jars for shoot-tip culture. In the same way, about 25 ml autoclaved medium was dispensed into 90 mm diameter Petri dishes for meristem culture.

Surface sterilization and shoot induction from meristem

About 1–2 cm long apical and axillary shoot tips were cut from virus infected (with SPFM and/or SPCSV) plants growing in glasshouse and put into tap water in 50 ml glass bottle. The shoots were washed three times with tap water and rinsed in 70% ethanol for about one minute. This was followed by sterilization with 10% (w/v) calcium hypochlorite containing 3–4 drops of Tween-20 for 15 minutes. Following sterilization with calcium hypochlorite, explants were washed three times in double distilled sterile water before meristem was excised under dissecting microscope. Sterile forceps were used to hold the shoot explants to a fixed position under dissecting microscope. Dissecting blade and syringe needle tips were used to remove leaf primordia and to isolate meristem, respectively (Fig. 1A). Experimental design was set to be 13x3 factorials, that is, 13 different combinations of growth regulators in the media were used to induce shoots for three different varieties. Growth regulators were one factor and genotype was another factor. Completely randomized design (CRD) was used for all the treatments. Thirteen different combinations of growth regulators (GA_3, NAA and BAP) were used for meristem cultures of the three varieties. Five meristems per Petri dish were cultured and each treatment had six replicates as experimental unit. All cultures were maintained at room temperature of 24 ± 2°C. The cultures were kept at light intensity of 40 µmol/m^{2}s and 12 h photoperiod. Subculturing was done every four weeks on the same fresh shoot initiation medium. The experiments were repeated at least once.

Thermotherapy

About 1–2 cm long apical and axillary shoots of Awassa-83 and Awassa local varieties that were growing in glass house were used in this experiment. The shoots were sterilized in the same way as used during meristem culture and trimmed to 0.5–1.0 cm. The lower end of the trimmed shoots were cut and cultured on MS medium containing 2 mg/l BAP and kept at 37°C for 31 days with photoperiod of 12 h. Twenty two shoots of Awassa local and 46 shoots of Awassa-83 were used for this experiment. The shoots were then transferred to multiplication medium (Fig. 2 A-D).

Shoot multiplication

Meristem cultures were left for at least eight weeks on the same culture medium until shoots were initiated and the initiated shoots were transferred to various shoot multiplication media to optimize shoot multiplication (Fig. 1 B and C). Shoots of all the three varieties were cultured on MS medium containing different concentrations of BAP for shoot multiplication. BAP concentrations used were 0.1, 1.0, 2.0, 3.0, 4.0 and 0.0 mg/l (control). Fifteen shoots (five shoots per Magenta box) were used for each of the clones in each treatment. The number of shoots produced per explant was recorded after four weeks for each of the BAP concentrations used.

Rooting and acclimatization

Shoots of 1.0 cm or more long were transferred to ½ MS medium (half macronutrients, micronutrients and vitamins) containing, 0.00, 0.01, or 0.1 mg/l IBA for rooting. Twenty five shoots derived from meristem and thermotherapy were used for each treatment. After seven days of incubation, mean length and mean number of roots in each treatment was recorded. Rooted shoots were transferred to moist soil in pots containing sand, red soil and compost in 2:1:1 ratio, respectively. Potted shoots were covered with porous polyethylene bags. The acclimatized plantlets were transferred to Wondo Genet Research Station by Hawassa Agricultural Research Centre to be propagated and distributed to farmers.

Fig. 1. Meristem isolation and dissection under dissecting microscope (A), 35 days old meristem culture of Awassa local producing shoot (B), Awassa-83 transferred to fresh medium for elongation (C), multiple shoot induction of Awassa local in 2 mg/l BAP added to MS medium (D), shoot multiplication with stunted growth of Guntute in 3 mg/l BAP added to MS medium (E) and multiple shoot growth of Awassa-83 in 2 mg/l BAP added to MS medium (F).

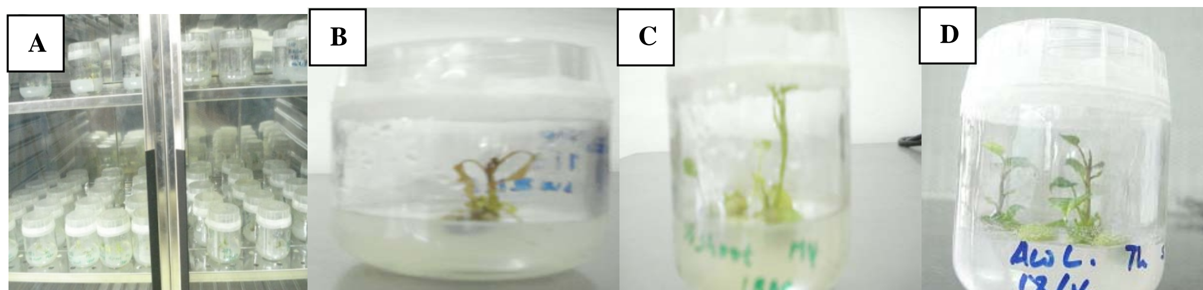

Fig. 2. Stages in shoot thermotherapy treatment: shoots at 37°C temperature (A), dead shoot as a result of high temperature (B), Awassa-83 shoot taken out of thermotherapy to be subcultured onto fresh multiplication media after a month long treatment (notice the elongated shoot despite the high temperature) (C) and well established shoots of Awassa local after thermotherapy on multiplication medium ready to be indexed for viruses (D).

Virus indexing

Polyclonal antibodies specific to ten of the most serious sweet potato viruses, Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato Latent Virus (SPLV), Sweet Potato Mild Mottle Virus (SPMMV), Sweet Potato Virus G (SPVG), Sweet Potato Mild Speckling Virus (SPMSV), Sweet Potato Chlorotic Fleck virus (SPCFV), C-6 virus, Sweet Potato Chlorotic Stunt Virus (SPCSV), Sweet Potato Caulimo-Like Virus (SPCaLV) and Cucumber Mosaic Virus (CMV) were used for virus indexing. Nitrocellulose Membrane-Enzyme Linked Immunosorbent Assay (NCM-ELISA) kit was used as described on the kit (Gibb and Padovan, 1993). The kit was purchased from International Potato Centre (CIP), Peru and generously provided by Dr Adane Abrham, Ethiopian Institute of Agricultural Research (EIAR) and Dr Settumba Mukasa, Makerere

University, Uganda. All mother plants used for meristem culture and thermotherapy were tested for all these ten viruses. Plantlets derived from meristem culture and thermotherapy were also tested to determine the efficiency of virus elimination of meristem culture and thermotherapy techniques. Sections of leaves about 1cm diameter were taken from lower, middle and upper part of the mother plants and plantlets for virus indexing. The presence or absence of the viruses was evaluated by visual observation of colour change.

Data analysis

Data were subjected to ANOVA using SAS software (version 9.2, 2010) for the differentiation of the effect of growth regulator combinations (CPGRs), variety (var) and variety growth regulator combinations interaction (var x CPGRs). Mean separation was done using Fisher's LSD test. For all data analysis, probability level of 0.05 ($p \leq 0.05$) was considered for statistical significance.

RESULTS

Shoot induction from meristem

The culture period for shoot induction varied among the three varieties. Four to six and eight weeks were for Guntute, and Awassa local and Awassa-83, respectively. Maximum shoot induction (66.67%) was obtained for the varieties Awassa-83 and Guntute in MS medium containing 1 mg/l BAP, 0.01 mg/l NAA and 1 mg/l GA_3. For the variety Awassa local, maximum shoot induction was 63.33% when GA_3 was raised to 2 mg/l (Table 1). For the variety Guntute, even though shoots were induced within 4–6 weeks, the growth was highly retarded. ANOVA showed that the effect of growth regulator combination, variety and variety × growth regulator combinations interaction was statistically significant ($p \leq 0.05$) on shoot induction from meristem. The effect of growth regulators is more significant on the rate of shoot induction than the effect of varieties (Table 2). Generally, there was no significant difference in the percentage of shoots induced between Awassa local and Awassa-83. However, Guntute is significantly different from these two varieties (Table 1).

Shoot multiplication

The highest mean number of shoots per explant was obtained from the medium containing 2 mg/l BAP for Awassa-83 (5.26 ± 0.02) and Awassa local (5.12 ± 0.02). The highest mean number of shoots produced per explant by Guntute was 2.48 ± 0.03 in the medium containing 3 mg/l BAP (Fig. 1E). Shoot multiplication and elongation was best when the concentration of BAP was 2 mg/l for the varieties Awassa local and Awassa-83 (Figs 1D and 1F). However, in all BAP concentrations used for shoot multiplication, shoot elongation was slow for Guntute. No significant difference was observed in the number of shoots produced per explant in medium containing 1 mg/l and 2 mg/l BAP for Guntute but there was significant difference in all the other BAP concentrations (0, 0.1, 3, or 4 mg/l BAP). However, Awassa local and Awassa-83 showed significant difference in rate of shoot multiplication in almost all media containing 0, 0.1, 1, 2, 3, or 4 mg/l BAP (Table 3). The effect of BAP was more significant in multiple shoot induction than the variety (Table 4).

Table 1. Percent of shoots induced by the three varieties of sweet potato using MS basal medium containing different growth regulators during meristem culture.

Growth regulator concentrations (mg/l)				Percent of shoots induced in each variety		
T	BAP	GA_3	NAA	Awl	A-83	Gun
1	0	0	0	0^{m}	0^{m}	0^{m}
2	0.1	1	0.01	30^{e}	13.33^{i}	33.33^{d}
3	0.25	1	0.01	9.67^{j}	10^{j}	16.67^{h}
4	0.75	1	0.01	0^{m}	0^{m}	26.67^{f}
5	0.5	1	0.05	0^{m}	0^{m}	6.67^{k}
6	1	1	0.05	0^{m}	0^{m}	0^{m}
7	1	1	0.01	33.33^{d}	66.67^{a}	66.67^{a}
8	1	2	0.01	63.33^{b}	30^{e}	16.67^{h}
9	1	2	0.05	0^{m}	0^{m}	6.67^{k}
10	1	3	0.01	0^{m}	0^{m}	3.33^{l}
11	2.5	1	0.01	0^{m}	0^{m}	36.67^{c}
12	2.5	2	0.01	10^{j}	10^{j}	20^{g}
13	2.5	1	0.05	0^{m}	0^{m}	10^{j}

Note: Numbers with different superscript, a-m are significantly different at 5% probability level. Results were recorded after 8 weeks. A-83 = Awassa-83, Awl. = Awassa local, Gun = Guntute, T = Treatment number.

Table 2. ANOVA **for the effect of three growth regulators combination (**BAP, NAA **and** GA_3**), variety and their interaction on shoot induction.**

Source	DF	Mean square	F-ratio
Variety	2	864.10256	674.00*
CPGRs	12	2515.20370	1961.86*
Variety x CPGRs	24	394.30627	307.56*
Error	78	1.28205	
Total	116	3774.8946	

*Significant at 5% probability level, DF= Degrees of freedom, CPGRs = Combination of plant growth regulators.

Table 3. Mean number of adventitious shoots produced using MS **medium with different concentrations of** BAP**, values given as mean ±** SD**.**

		Mean number of shoots/ explant		
T	BAP (mg/l)	Awassa-83	Awassa local	Guntute
1	0	0.00 ± 0.00^{m}	0.00 ± 0.00^{m}	0.00 ± 0.00^{m}
2	0.1	1.01 ± 0.01^{l}	1.06 ± 0.02^{kl}	1.13 ± 0.01^{k}
3	1	3.07 ± 0.02^{e}	3.07 ± 0.01^{e}	1.25 ± 0.01^{j}
4	2	5.26 ± 0.02^{a}	5.12 ± 0.02^{b}	1.25 ± 0.01^{j}
5	3	4.23 ± 0.19^{c}	3.94 ± 0.02^{d}	2.48 ± 0.03^{h}
6	4	2.93 ± 0.03^{f}	2.81 ± 0.01^{g}	1.38 ± 0.02^{i}

Note: Numbers with different superscript, a-m, are significantly different at 5% probability level, T = Treatment number. The results were recorded after a month.

Table 4. ANOVA **for the effect of** BAP**, variety and** BAP **x variety interaction on shoot multiplication.**

Source	DF	Mean square	F-ratio
Variety	2	12.82085	5162.76*
BAP	5	19.53851	7867.86*
BAP× Variety	10	2.18215	878.72*
Error	36	0.0024833	
Total	53	34.543993	

*Significant at 5% probability level, DF= Degrees of freedom.

Virus indexing

Among the ten tested viruses using NCM-ELISA technique on leaf tissues from mother plants of the three varieties, Awassa-83, Awassa local and Guntute, all mother plants were positive for either SPFMV or SPCSV or both but negative for the other eight tested viruses. However, when mersitem was isolated and cultured from these infected plants, all (100%) plantlets derived from meristem were found to be free from all ten viruses tested in this experiment as confirmed by the absence of colour in contrast to the purple colour observed in the positive controls (Fig. 3 A and B).

Thermotherapy

Awassa local with 54.55% shoot survival was more heat tolerant than Awassa-83 where only 34.78% of shoots survived (Fig. 2). During the 31 days thermotherapy treatment, 41.18 % of the shoots showed normal growth. Out of thermotherapy-treated shoots, 88.89% of Awassa-83 and 100% Awassa local shoots were free from any of the ten tested viruses (Table 5 and Fig. 3).

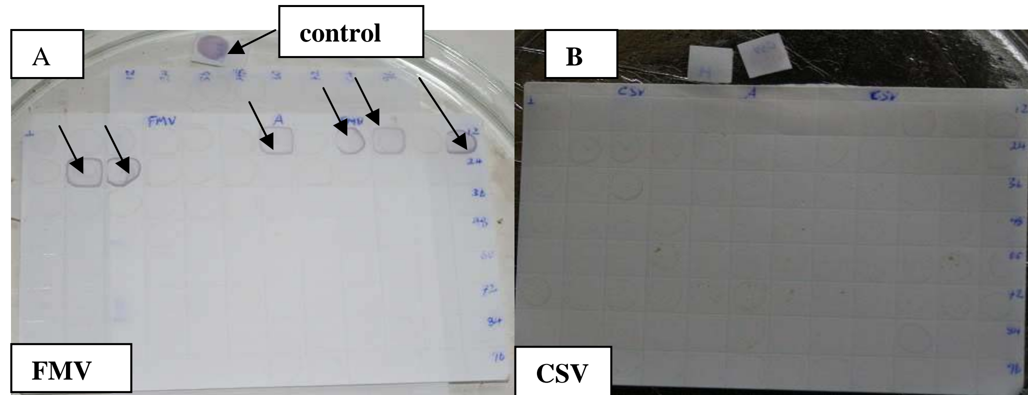

Fig. 3. NCM-ELISA virus detection for the presence of the tested viruses. Test for SPFMV (A) and SPCSV (B). The lighter the intensity of the purple colour the less concentrated virus titters (arrows show positive reactions).

Table 5. Summary of virus status of shoots derived from mother plants, meristem culture and shoot thermotherapy*.

	Mother plant				Meristem culture				Shoot thermotherapy			
Variety	#	N	P	%+	#	N	P	%-	#	N	P	%-
Awassa-83	2	0	2	100	9	9	0	100	9	8	1	88.89
Awassa l.	2	0	2	100	8	8	0	100	6	6	0	100
Guntute	2	0	2	100	6	6	0	100	0	0	0	0

*Awassa l. = Awassa local, N= Number of virus negatives, P = Number of virus positives, %+ = Percent of virus positive, %- = Percent of virus negative and # = Total number of clones tested. Virus positive samples were infected with either SPFMV or SPCSV or both but free from the other eight viruses.

Rooting and acclimatization

All the rooting media resulted in 100% rooting (Fig. 4 A-F). Among the three different IBA concentrations used, the highest number of roots per shoot was obtained in ½ MS medium containing 0.1 mg/l IBA for Awassa-83 (6.34 ± 0.01) followed by Awassa local (4.37 ± 0.03) whereas in the case of Guntute, rooting ability was not significantly different in all the three treatments. The best root length was 9.5 ± 0.10 cm, 9.68 ± 0.02 cm, and 11.03 ± 0.02 cm for Awassa-83, Awassa local and Guntute, respectively on growth regulators free MS medium (Table 6). Significant difference was found in the mean length of roots in all the three concentrations of IBA used among the three varieties except for Awassa-83 where the same number of roots (9.50±0.10) was obtained in ½ MS medium containing 0.01 mg/l IBA and without growth regulators. No significant difference was observed in mean number of roots in all the rooting media while the mean length of roots was significantly different in all the rooting media for Guntute. IBA and the interaction of variety with IBA significantly affected both mean root number and mean root length (Table 7).

After 25 days of hardening, 91.11%, 90.10% and 100% survival of Awassa-83, Awassa local and Guntute plantlets was observed, respectively (Fig. 4G). No aberrant plantlets were observed and all the clones were being propagated and were performing well at Wondo Genet Research field.

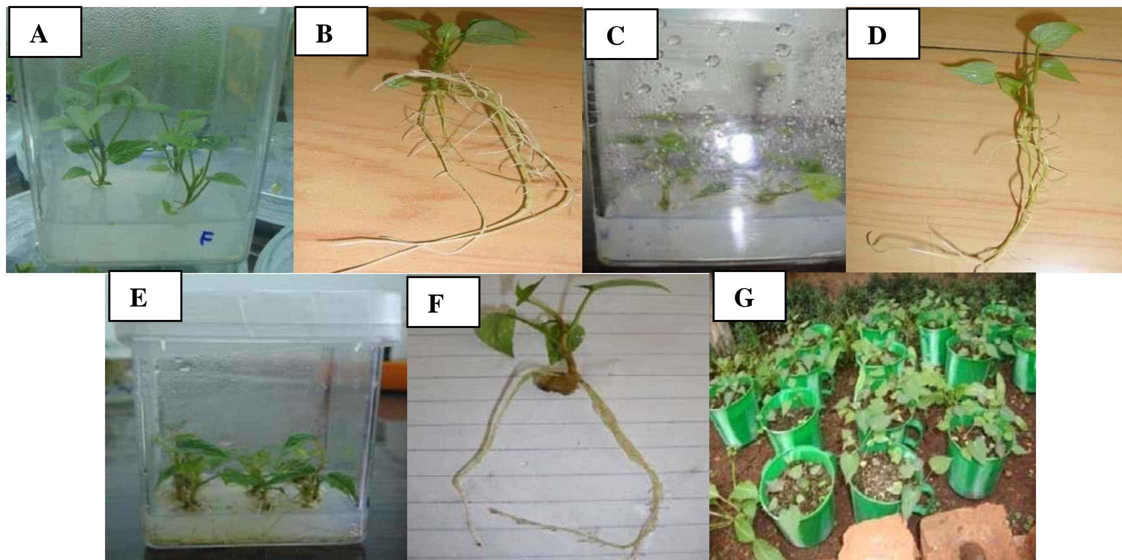

Fig. 4. Rooting and acclimatization: Awassa-83 shoots cultured on ½ MS medium without growth regulators (A), rooted shoot of Awassa-83 ready to be potted (B), Awassa local shoots cultured on ½ MS medium containing 0.01 mg/l IBA (C), rooted shoot of Awassa local ready to be potted (D), shoots of Guntute cultured on 0.1 mg/l IBA added to ½ MS medium (E), rooted shoot of Guntute ready to be potted (F) and shoots of the three varieties well established after acclimatization (G).

Table 6. Effect of different concentrations of IBA (mg/l) on root length and number, values given as ± SD. The results were recorded after a week.

		Mean length of roots (cm)			Mean number of main roots		
T	IBA (mg/l)	A-83	Awl.	Gun	A-83	Awl.	Gun
1	0	9.50±0.10[c]	9.68±0.02[b]	11.03±0.02[a]	3.14±0.01[d]	3.00±0.10[e]	2.93±0.01[ef]
2	0.01	9.50±0.10[c]	4.33±0.01[h]	6.20±0.10[f]	3.26±0.01[c]	2.67±0.01[g]	2.92±0.01[ef]
3	0.1	5.00±0.10[g]	7.07±0.01[e]	8.15±0.03[d]	6.34±0.01[a]	4.37±0.03[b]	2.90±0.10[f]

Note: Numbers with different superscript, a-h, are significantly different at 5% probability level. A-83 = Awassa-83, Awl. = Awassa local, Gun = Guntute, T = Treatment number.

Table 7. ANOVA for the effect of IBA, variety and IBA x variety interaction on main root number (A) and length (B).

Source	DF	Mean square	F-ratio
IBA (A)	2	7.2196	3022.16*
Variety (A)	2	4.1457	1735.41
IBA × Var (A)	4	2.5447	1065.22*
IBA (B)	2	33.9114	7284.08*
Variety (B)	2	4.820	1035.35
IBA × Var (B)	4	12.7613	2741*
Error	36	0.0070444	
Total	52	65.409744	

*Significant at 5% probability level, DF= Degrees of freedom, Var = Variety.

DISCUSSION

Slow shoot induction of sweet potato clones during mersitem culture and the fact that only one shoot is produced, is in agreement with previous studies (Frison and Ng, 1981). The recalcitrant nature of clone Guntute to elongation and multiplication was observed in previous studies on other sweet potato varieties (Liu *et al.*, 2001). Growth rate may be improved by using growth regulators free half strength MS medium for such stunted growth characteristics (Ozturk and Atar, 2004). Alternatively, GA_3 alone can also be used for shoot elongation. Gosukonda *et al.* (1995) found that different sweet potato varieties respond differently to *in vitro* shoot induction media. In our study, consistent with the work of Gosukonda *et al.* (1995) and Aloufa (2002), Awassa local was found to have unique optimum growth regulator concentrations for shoot induction by meristem culture unlike the other two varieties. In the case of Awassa-83 and Guntute, the optimum growth regulator combination was 1 mg/l BAP, 1 mg/l GA_3 and 0.01 mg/l NAA for meristem culture. Similar results were obtained by Ying and Davenport (2004). Hettiarachchi (1988) obtained optimum shoot induction in some varieties of sweet potato by meristem culture in MS medium containing 1mg/l 2, 4-D, 0.25 mg/l BA and 0.1 mg/l GA_3. In contrast to the above study, same concentration as that of Hettiarachchi (1988) and lower concentrations of 2, 4-D were also used in our study (data not shown here). However, in contrast to the above study, all the meristem explants in our study produced calli without inducing any shoot. Furthermore, subculturing of these calli did not produce any shoot. High amount of calli was observed when the NAA concentration was higher than 0.05 mg/l.

The success in eliminating viruses using meristem culture depends on the size of meristem explants. The smaller the size of the meristem (< 0.6 mm), the more likely to be free from the viruses (Lizarraga *et al.*, 1992; Juan *et al.*, 2004; Ying and Davenport, 2004; Panta *et al.*, 2006). Our results showed much higher shoot induction percentage than the work of Ying and Davenport (2004) who observed that even meristem with one primordium resulted in very low percentage of survival (8.5%). In the present study, 26.67% to 100% survival was observed using meristem size of 0.3–0.5 mm in diameter without leaf primordium. The use of meristem culture for plant virus elimination has been reported by many researchers such as Cha-um and Kirdmanee (2006) on sugarcane virus elimination and Tekalign Wondimu (2009) on sweet potato, using other varieties in Ethiopia, that agrees with our results. Our study showed that meristem culture is more effective in eliminating viruses than thermotherapy alone. The prevalence of SPVD and its effect on sweet potato production in East Africa, as described by Gibson *et al.* (1998) and Mwanga (2001) was also observed in Ethiopia in good agreement with the work of Tekalign Wondimu (2009). In the present study, the efficiency of virus elimination by shoot thermotherapy alone (though less efficient than meristem culture) is consistent with the work of Spiegel *et al.* (1985) who reported similar results in rosaceous fruits.

The recalcitrant nature of Guntute during shoot multiplication and elongation was an indication that exogenous growth regulator supplement inhibited elongation and multiple shoot formation. This result is in agreement with the work of Berrie (1984) who reported that synthetic cytokinins inhibit shoot elongation and multiplication at higher concentrations and with the works of Cutis and Barnes (1985) and Preece (1987) who pointed out that endogenous concentration of plant growth regulators could affect growth. Increasing the concentration of BAP resulted in large number of shoot buds in all the three varieties. This result is in agreement with the work of Subramaniam and Poobathy (2008) on banana. However, increasing the concentration of BAP to 3 mg/l in Awassa-83 and Awassa local and 4 mg/l in Guntute resulted in stunted growth of shoots. Such retarded growth of shoots at higher concentrations of BAP was also observed in *Ludwigia repens* (Ozturk and Atar, 2004). In all the three varieties, higher number of shoot buds per explants may be obtained when BAP is combined with one of the auxins than using BAP alone (Shende and Rai, 2005).

In vitro induced shoots are very delicate and prone to sudden environmental changes. Hence, rooting and acclimatization is required to enable shoots adapt to the natural environment (*ex vitro*). It was observed that acclimatization is

easily achieved once shoots are elongated and rooted under *in vitro* condition. Moreover, the necessity of appropriate root development under *in vitro* condition for successful *ex vitro* transplanting and growth of sweet potato shoots during acclimatization agrees with the work of Zobayed *et al*. (1999).

CONCLUSIONS AND RECOMMENDATIONS

In this study, best shoot induction from meristem explant was obtained from MS medium containing 1 mg/l BAP, 0.01 mg/l NAA and 1 mg/l GA_3 for Awassa-83 and Guntute while 1 mg/l BAP, 0.01 mg/l NAA and 2 mg/l GA_3 for Awassa local. Likewise, 2 mg/l BAP was best for Awassa-83 and Awassa local as 3 mg/l BAP was for Guntute in shoot multiplication. Both meristem culture and shoot thermotherapy resulted in successful elimination of SPVD from all the varieties as confirmed by NCM-ELISA. Shoots of Awassa-83 and Awassa local were best rooted in ½ strength MS medium containing 0.1mg/l IBA whereas it was on ½ MS medium containing 0.01 mg/l IBA for Guntute. There was no significant difference in all the three treatments for Guntute. During acclimatization, the percentage of survival of all the three varieties ranged from 90–100%.

This work indicates the importance of plant tissue culture using meristem culture and thermotherapy to produce virus-free planting materials of sweet potato. The cultivation of sweet potato requires limited agricultural input and the crop is drought tolerant. If the problem of viruses infecting the crop could be overcome, it may be beneficial as an alternative crop for low-income households inhabiting regions where mean annual rainfall is low. Virus-free sweet potato production could also open opportunities for investors who are interested in starch production, as disease-free plants will have higher productivity. In addition to *in vitro* propagation, genetic transformation should be sought to release virus resistant varieties.

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